Saif Hameed
Suriya Rehman *Editors*

Nanotechnology for Infectious Diseases

Springer

Nanotechnology for Infectious Diseases

Saif Hameed • Suriya Rehman
Editors

Nanotechnology for Infectious Diseases

Springer

Editors
Saif Hameed
Amity Institute of Biotechnology, Amity University Haryana
Gurugram, India

Suriya Rehman
Epidemic Disease Research
Imam Abdulrahman Bin Faisal University
Dammam, Saudi Arabia

ISBN 978-981-16-9189-8 ISBN 978-981-16-9190-4 (eBook)
https://doi.org/10.1007/978-981-16-9190-4

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Foreword by Dr. Shahid Jameel

The World Health Organization (WHO) examined data from 2000 to 2019 for its latest report on *Leading Causes of Death and Disability*. Non-communicable diseases increased globally, with heart diseases and stroke emerging as two of the biggest killers, deaths from HIV/AIDS and tuberculosis declined, and Alzheimer's is a growing problem. An estimated 30.6 million people died in 2019 of the top ten causes, of which 6.1 million were due to lower respiratory infections, diarrhoeal diseases, and other (infectious) neonatal conditions. However, if one were to consider Africa—the poorest of WHO administrative regions—the picture is very different. Infectious diseases contribute to six of the top ten causes of mortality with HIV/AIDS, malaria, and tuberculosis showing up in addition neonatal and lower respiratory infections. In the past 2 years, COVID-19 has added another dimension to the global infectious disease risk, with over 260 million confirmed cases and 5.2 million deaths—both likely to be undercounts.

Infectious organisms are complex and spread rapidly, especially in resource-limited settings, due to poor public health infrastructure. This underpins the need for inexpensive, easy-to-use, and sensitive diagnostics as well as treatment and prevention tools. Many challenges stand in the way of effective management of infectious diseases, including the paucity of safe and effective drugs. With increasing antimicrobial resistance (AMR), treating infections is becoming harder and more expensive, especially when new classes of antibiotics with novel targets are slow to the market compared to the emergence of resistance. Poor adherence to therapies and the need for sustained monitoring of patients remain important obstacles. Even in

India, which has a vibrant generics pharmaceutical industry and is dubbed 'Pharmacy of the World', high out-of-pocket (OOP) health expenditures hamper treatment, fuel poor adherence, and therapy failure. The 2021 Economic Survey notes that India has one of the highest levels of OOP expenditures on health that directly contribute to poverty. It is estimated that almost 39 million Indians, about the entire population of Canada, become impoverished annually due to high OOP health expenditures.

Detection, treatment, and prevention are the most vexing challenges for infectious diseases. Despite major advances in our understanding of infectious organisms, the diseases they cause and their pathogenesis, serious scientific challenges remain due to the complexity of several organisms that contribute to high disease burden. Population growth, increases in the density of humans, animals, and disease vectors, and environment changes allow the emergence of new infectious organisms with frightening frequency. These difficulties are further exacerbated by the fact that resource-poor regions of the world often face the most rapid spread of disease. This requires that inexpensive and easy-to-use diagnostics, devices, and medications are also developed and made available quickly and in an equitable manner. Nanotechnology has begun to address these challenges and holds promise, as outlined in the articles contained in this volume.

Nanotechnology involves the study and use of nanoparticles, which are traditionally defined as particles with a diameter of 1–100 nm, or about a thousand times less than the thickness of hair. Their size allows for unique properties, such as changes in electrochemical behaviour, magnetism, emission wavelength, and surface plasmon resonance not found in bulk material, and allows interesting applications. Nanosystems have been researched extensively for several decades, leading to the many clinical applications such as chemotherapeutics, anaesthetics, imaging agents, nutritional supplements, and others being approved by the United States Food and Drug Administration (US-FDA). It thus comes as no surprise that nanotechnology has also been evaluated to diagnose and treat infectious diseases. The past year has reminded us of the ongoing impact of nanotechnology in the form of successful mRNA, DNA, and protein vaccines for COVID-19. This includes a novel vaccine that uses the SARS-CoV-2 Spike protein assembled as a synthetic nanoparticle (from Novavax, USA).

To be successful in resource-limited regions of the world, diagnostic tests have to be low cost, robust, rapid, simple, and sensitive. As a platform, nanotechnology has fulfilled these needs. For example, gold nanoparticles have been used for visual detection owing to their unique property of changing colour due to shifts in surface plasmon resonance. Researchers at the Indian Institute of Technology, Guwahati, used aptamers specific to the *Plasmodium falciparum* lactate dehydrogenase enzyme (*Pf*LDH), a malaria biomarker to control the aggregation of gold nanoparticles such that presence of the biomarker turns these from red colour (free nanoparticles) to blue colour (aggregated nanoparticles). Since the gradient of colour change is proportional to the biomarker concentration, this can also be used as a quantitative test. A similar strategy has been used by researchers from Taiwan to develop a paper-based test to detect *Mycobacterium tuberculosis*, the causative agent for tuberculosis

(TB). Coupled with smartphones, such tests can be widely implemented with minimal on-site infrastructure. Nanoparticles have also been used to improve the sensitivity of traditional enzyme-linked immunosorbent assay (ELISA). A SiO_2:Eu particle was used as a fluorescence readout for detection of the HIV p24 antigen in minimal amounts of blood with about a 1000-fold increase in sensitivity. Similarly, a TB biomarker, lipoarabinomannan (LAM) was detected in urine samples with picogram sensitivity.

The treatment of high burden infectious diseases often faces challenges of patient compliance and rapid evolution of resistance, which requires patients to take multiple drugs for extended periods of time. Nanotechnology-based approaches seek to improve therapy by simplifying drug use and targeting them to pathogenic sites and reservoirs of infection. There are several promising preclinical studies, but the path to clinical translation is often not straightforward.

A lot of nanotechnology-based work is taking place on sustained systemic delivery of anti-infectives, especially for chronic infectious diseases such as HIV/AIDS and TB, which require long-term treatment. Developing systems that reduce dosing frequency and ease the dosing regimen are broadly classified into two categories—those that control drug release with a polymer or lipid excipient, and those that rely on slow dissolution of poorly soluble drug crystals. Polymer-based drug delivery systems such as poly(lactide-*co*-glycolide) (PLGA) and several others are examples of polymeric assemblies that are degraded by physiological esterases to release encapsulated drugs. Liposomal drug formulations have also shown good preclinical efficacy. For example, a liposomal formulation of amikacin, called MiKasomes, showed good tissue distribution and drug half-life in a rat model and positive results in early clinical trials for urinary tract infections. However, polymeric and liposomal formulations require large amounts of excipients, which increase injection volumes and sustained release systems can lead to prolonged exposure to sub-therapeutic drug concentrations that can fuel drug resistance.

Local delivery of therapeutics to epithelial surfaces holds promise as it is noninvasive and these sites are commonly used for the entry and residence of several pathogens. The advantages of using nanoformulations for local delivery include sustained delivery to reduce dosing frequency, enhanced drug uptake in cells to improve efficacy against intracellular pathogens, and protection of labile drugs from harsh physiological conditions such as low pH or enzymes. Possibly, the best example of this is the prophylactic vaginal administration of microbicides to protect against sexually transmitted pathogens such as HIV and herpes simplex virus (HSV). Starpharma's VivaGel is a dendrimer antimicrobial formulated as a mucoadhesive gel whose vaginal application has shown efficacy against bacterial vaginosis as well as HIV and HSV infections. The company is also using its patented dendrimer drug delivery platform (DEP®) to improve the pharmacological properties and efficacy of several existing cancer drugs such as Docetaxel, Cabazitaxel, and Irinotecan. Nanoparticle-based pulmonary drug delivery is another attractive route for the treatment of respiratory infections. Examples of this include good preclinical results with nanoparticle formulations of the antifungal drug Itraconazole against

Aspergillus fumigatus and liposomal formulations of Ciprofloxacin against *Francisella tularensis*. Biofilm-forming opportunistic pathogens such as *Staphylococcus aureus* and *Pseudomonas aeruginosa* are both implicated in lung and skin infections. Nanoparticle-based approaches are being deployed to break biofilms to improve the access and efficacy of existing drugs against these pathogens. This is especially useful for topical delivery to treat chronic non-healing wounds.

Targeted delivery to sites of infection is another important area of work with nanoparticle-based drug formulations. Passive targeting includes drug accumulation in macrophages that clear nanoparticles but are also sites for the residence of several intracellular bacterial (Mycobacteria), fungal (Aspergillus), and viral (HIV) pathogens. For targeted delivery to specific cell types and pathogens, a successful approach is to coat drug-loaded nanocarriers with ligands that bind receptors on the surface of infected host cells or pathogens. Overcoming resistance to anti-infectives is another important area of work. Secretion of drug-inactivating enzymes, reducing metabolic activity, deploying drug efflux pumps, forming biofilms, and developing an intracellular life cycle or being an obligate intracellular pathogen are some commonly used evasion strategies used by pathogens. There are several examples of nanocarrier systems being developed to overcome these microbial mechanisms of drug resistance.

Several nanotechnologies have advanced to preclinical and clinical applications for major infectious diseases—HIV/AIDS, TB, and malaria. Several anti-retroviral medications—Cabotegravir, Rilpivirine, and Dolutegravir, which have been formulated as injectable nanoparticles to reduce dosing frequency and target macrophages, are in preclinical and clinical testing. Others such as a Lopinavir-Ritonavir-Tenofovir as an injectable lipid nanoformulation and a Lopinavir-Efavirenz combination as orally delivered nanoparticles are also in preclinical testing. The WHO guidelines for treating drug-susceptible TB involve an oral regimen of four antibiotics taken daily for at least 6 months. This extended dosing regimen leads to poor compliance and the development of drug resistance. The TB drug combination of Rifampicin-Isoniazid-Pyrazinamide has been formulated as oral or nebulized nanoparticles to reduce dosing frequency and increase their bioavailability, while a five-drug combination, which also includes Ethambutol and Streptomycin, is formulated as oral polymer nanoparticles to target alveolar macrophages and bypass drug metabolism. All these combinations as well as aerosolized nanoparticles of the BCG vaccine are in preclinical testing in rodent models. Malaria continues to be a major problem for Africa, which harbours over 90% of global cases and deaths. Treatment is based largely on artemisinin-based combination therapy, but the drug is taken up poorly by RBCs, which house the parasite. Killing parasites requires prolonged treatment with high doses which lead to toxicity, compliance issues, and development of resistance. Several preclinical studies are evaluating chloroquine derivatives and artemisinin in combination with other drugs as injectable liposomes, nanoparticles, or microemulsions. A transmission blocking malaria vaccine candidate (Pfs25) in an injectable nanoliposomal formulation is also undergoing preclinical testing.

Infectious diseases are still a major cause of morbidity and mortality in large parts of the world. They can also emerge to become epidemics and pandemics that have a global reach, as exemplified by COVID-19, which has hit developed countries just as hard. Approaches that efficiently detect infections at minimal cost, simplify the use of drugs, and improve their efficacy will be needed to reduce the global burden. If the promise of innovative and cost-effective nanotechnologies is realized, individual patients as well as populations and health systems stand to benefit.

This volume on *Nanotechnology for Infectious Diseases* is both timely and strategically situated with the editors and most of the authors based in developing countries, which face the largest burden of infections. They address key aspects of the applications of nanotechnology to infectious diseases—detection, therapy, and prophylaxis. It is hoped that this volume will summarize new developments in the field and their impacts to the world in general, but resource-limited regions in particular.

OCIS and Green Templeton College, University of Oxford Shahid Jameel
Oxford, UK

Ashoka University
Sonipat, India

Foreword by Prof. Abdulhadi Baykal

The battle between the hosts and pathogens has been evolved since antiquity, and with passing time the microbes have changed their virulence machinery to combat host defence systems. Thus, adopting to multidisciplinary approach is the need of the hour to fight against infectious diseases. Nanotechnology is one such branch of science that has proven applications in wide ranges of scientific field.

I am happy to endorse the book entitled *Nanotechnology for Infectious Diseases* being published by Springer-Nature. The book carries the latest research and developments with focus on the recent advancements and modern trends in the field of nanotechnology applied against human infectious diseases. The book is an integrated approach to widely disseminate the current research topics and frontier areas of human infections around the globe. This book represents a collection of chapters authored by domain experts who have summarized their novel approaches using nanotechnology against major human pathogens, viz. bacteria, fungi, virus, etc. Each chapter provides an informative review on current nanotechnology-driven therapies to further defeat the infection process. The reference book will facilitate the students, faculty, research scholars, and policy professionals to gain the knowledge and plan their prospective research in the infectious disease research. The book could be an important addition in the human resource that will address the issue of infectious diseases for the betterment of human health nationally and internationally.

I appreciate the efforts taken up by both editors working in the research area of infectious diseases who have successfully managed to collect chapters from eminent

scientists working in diverse areas of infectious diseases and covering upon most of the apprehensible title points that provide completeness of the offering they made.

I wish this book will attract broad readership.

Abdulhadi Baykal

Nanomedicine Department, Institute for Research and Medical Consultations (IRMC),
Imam Abdulrahman Bin Faisal University (IAU)
Dammam, KSA

Preface

Infectious diseases have always been a thorn in human flesh since antiquity. Recently, the emergence of COVID-19 pandemic has made the entire world realize, yet again about the challenging effects of human pathogens not only on health sector but also on all facets of human life. Scientists are under immense pressure to counter the challenges associated and overburdened, finding out novel strategies to win the battle against the infectious microbes.

Nanotechnology is a multifaceted field that involves various disciplines of science and technology at nanoscale. The metallic nanoparticles like gold, silver, zinc, copper, etc. varying in size from 1 to 100 nm constitute the building material of nanotechnology. The application of nanoparticles has presented much advancement due to their significant characteristics such as shape, size, and biocompatibility. The utility of nanotechnology in today's medicine has potential edge, especially in infectious diseases. Research studies indicate the novel prospects of framing immune responses and combating severe infections using nanomaterials. Various studies have also proposed that the introduction of nanotechnology in vaccines and immunotherapy have a significant influence on public health.

This unique reference book covers major applications of naotechnologies in the field of human infectious diseases caused by predominant microbes (bacteria, fungi, protozoa, and virus). This book includes clearly structured sections discussing infectious diseases, nanomaterials, nano drug delivery systems, drug resistance, nanodiagnostic tools, and nanotherapeutics in gene therapy which are the pillars of nanotechnology. Resistance to antimicrobials is another one of the global concerns and in fact the reports issued by the World Health Organization (WHO) suggested that antibiotic resistance may lead to 300 million deaths by the year 2050. Under such circumstances, nanobiomedicine could be a potential approach for fighting the increasing rate of antibiotic resistance that we are currently encountering. Moreover, applications of alternative therapeutic nanomaterials and innovative diagnostic tools are also covered in the book.

Furthermore, each chapter layout has an introduction to the current state followed by the application of that technology and finishing with its prospects. The book is contributed by the eminent veterans and selected experts of international repute working in diverse areas of nanotechnology. It is their cumulative efforts that discuss cutting-edge research in their respective areas of infectious diseases dealing with

comprehensive description of recent developments and depicting the future leads. The research described in this book may further be extended to other pathogens, chronic and metabolic disorders. The book chapters are presented in a way accessible to the teachers, UG/PG students, research scholars, and health care workers including pathologist and clinicians worldwide. Overall, this book is an effort to update the understanding of applications of nanoparticles in the field of infectious diseases targeting human bacterial, fungal, viral, and parasitic pathogens. The book encompasses the broad topics to attract the diverse specialty of students, teachers, and researchers of nanotechnology, pharmacology, mycology, bacteriology, virology, pathology, biotechnology, and biomedical technology. The book has received contributions globally from prominent researchers in the field of nanotechnology and infectious diseases.

The editors are also thankful to their respective host institutions (Amity University Haryana, Gurugram, India, and the Institute for Research and Medical Consultations (IRMC), Imam Abdulrahman Bin Faisal University (IAU), Dammam, KSA) for their overall support to enhance their academic rigour. We are grateful to our esteemed contributors for their worthy and timely contributions during the tough times of global pandemic without which this compilation would not have become a ready reference for the researchers in this field. We take pride to thank Prof. Shahid Jameel, an eminent Indian virologist and Prof. Abdulhadi Baykal, a renowned nanotechnologist for endorsing this book by giving their valuable forewords. Patience and support from Springer-Nature during the book preparation is deeply acknowledged.

Gurugram, India Saif Hameed
Dammam, Saudi Arabia Suriya Rehman

Contents

About the Editors

Saif Hameed is currently working as an Associate Professor at Amity Institute of Biotechnology, Amity University Haryana (AUH). He did his bachelor's from the University of Delhi and master's from Jamia Hamdard. He completed his doctoral studies in Life Sciences from the Jawaharlal Nehru University. He was also a Visiting Scholar in Institut für Mikrobiologie, Heinrich-Heine-Universität, Düsseldorf, Germany. He received Young Scientist Award under the Fast Track Scheme from the Science and Engineering Research Board, Department of Science and Technology, New Delhi. Dr. Hameed is a life member of the International Society for Infectious Diseases (ISID), Association for Microbiologist (AMI), India, and the Society of Biological Chemists (SBC), India. Dr. Hameed is actively engaged in research in the field of infectious diseases, particularly on multidrug resistance (MDR), diagnostics in pathogenic fungi. He has published more than 65 research articles in peer-reviewed international journals and edited 7 books and contributed 25 book chapters.

Suriya Rehman is currently working as an Assistant Professor in the Department of Epidemic Diseases Research, Institute for Research and Medical Consultation, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia. She has obtained her Ph.D. in Microbial Toxicology from Jamia Hamdard (Hamdard University), New Delhi and the Council of Scientific and Industrial Research, IIIM Jammu/Srinagar, in 2010. She has extensive research experience in the field of infectious diseases, MDR, nanotechnology, drug development, and cancer therapeutics. She has published more than 70 peer-reviewed research articles in the international journals of high repute and has edited one book. She is a member of the editorial board of several international journals.

Part I

Infectious Diseases

A Holistic View of Human Infectious Diseases: Challenges and Opportunities

1

Uzma Ali, Syed Mehmood Ali, and Mehwish Hussain

Abstract

The infectious diseases are thought to be a constant confrontation between two separate worlds, the microbial world and human world. Over the centuries, the evolution of the microorganisms has posed major health concerns particularly for the developing nations and leading cause of morbidity and mortality among the vulnerable population. The understanding of the chain of infection and public health strategies to break it plays a significant role in prevention and control of these infectious diseases.

Although the battle against these infectious diseases was thought to be won by the developed nation through several preventive measures such as advent of vaccines, antibiotics, and public health reforms, but the emergence and re-emergence of novel pathogens are the new threats. These pathogens have the potential of frequent out breaks, extending beyond the borders and have become potential threat to the entire humanity. The unavailability of vaccines, non-judicial use of antibiotics and breech of public health interventions due to globalization, international trade and travelling, ecological and environmental changes along with zoonotic origin of pathogens are the major triggers. The introduction of newer technologies such as nanotechnology and nano chemistry has given new hopes to combat against these infectious diseases.

U. Ali (✉) · M. Hussain
Department of Public Health, College of Public Health, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia
e-mail: uasali@iau.edu.sa

S. M. Ali
Department of Biomedical Engineering, College of Engineering, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia

S. Hameed, S. Rehman (eds.), *Nanotechnology for Infectious Diseases*,
https://doi.org/10.1007/978-981-16-9190-4_1

Keywords

Infectious diseases · Chain of infection · Emerging diseases · Antibiotic resistance · Globalization · Nanotechnology

1.1 Overview

Infectious diseases are the illnesses that are caused by certain microorganisms like bacteria, viruses, fungi, and parasites. These microbes usually do not invade if a person's immune mechanism is fully functional and active, but only if these microbes overwhelm the immune system or the body system is compromised, may end up to infections (Alkharsah et al. 2019).

Over the centuries, infectious diseases are evidenced to be one of the major health concerns and reason behind morbidity and mortality globally, imposing substantial health and economic burden on societies and nations, especially the underdeveloped ones (Gu et al. 2021). Although a paradigm shift in the health status has been observed over the last few decades and biomedical advancement, improved sanitation awareness and adaptations, the increase in public health practices, and notable discoveries in immunology and microbiology aided surprisingly to the diminution of infectious diseases globally (Alkharsah et al. 2018). Some of the achievements of this infection diseases control efforts included the smallpox eradication and a good control over several childhood diseases such as polio, measles, and rubella that once claimed millions of lives and instigated suffering of tens of millions. Consequently, the attention of the industrialized nations, such as USA, was diverted towards the prevention and control of some chronic diseases like cancers and heart diseases.

However, according to World Health Organization Global health estimates, infectious diseases are still counted in the top ten leading causes of death. The three of these infectious diseases included diarrheal diseases, tuberculosis, and lower respiratory infections. Lower respiratory infections ranked as fourth fatal communicable disease around the globe and claimed 2.6 million lives in 2019, 460,000 deaths fewer than in 2000. Nevertheless, there observed substantial decline in number of deaths. Infectious diseases are the biggest threat for the people who resided in lower-income countries and are more likely to die from infectious diseases than non-infectious diseases. Despite the global decline, infectious diseases remained at the sixth ranked among the top ten fatal diseases in low-income countries (Gu et al. 2021). Infection causative organisms and pathogens persistently emerge and re-emerge, accentuating substantial epidemic challenges to public health epidemics (Fauci 2005) (Table 1.1).

The role of evolution has always been significant in infectious diseases (Chabas et al. 2018; Echaubard et al. 2018). This evolution is driven by an endless battle between disease-causing pathogens and the host. The pathogens have become strong enough to elude the defence mechanism of the host body (Hilleman 2004; Avondt et al. 2015; Bernard et al. 2018), become more drug resistant (Nathan and Cars 2014; Laxminarayan et al. 2016; Haldar et al. 2018), and develop the adaptation to host

Table 1.1 (Parry and Peterson 2020) Topmost devastating infectious diseases in the history of mankind

No	Infectious diseases	Species	Infectious agents
1	Covid 19 disease	Virus	*SAR-COV 19*
2	Smallpox	Virus	*Variola virus*
3	Plague	Bacteria	Bacteria carried by fleas
4	Malaria	Parasite	*Plasmodium species*
5	Influenza	Virus	*Influenza virus*
6	Tuberculosis	Acid fast bacilli	*Mycobacterium tuberculli*
7	HIV Aids	Virus	Human immunodeficiency virus
8	Cholera	Gram negative bacteria	*Vibrio cholerae*
9	Rabies	Virus	*Rabies virus*
10	Pneumonia	Bacteria/virus	*Streptococcal pneumoniae*
11	Infectious diarrhoea	Virus	*Rota virus*
12	Ebola	Virus	5 strains of *Ebola virus*
13	Variant Creutzfeldt-Jakob disease	Bacteria	*Bovine spongiform encephalopathy*
14	Middle East respiratory syndrome (MERS)	Virus	*MERS-CoV*
15	Dengue	Virus	*DENV 1,2,3,4*
16	Yellow Fever	Virus	*Flavivirus*
17	Hantavirus disease	Viruses	*Hantaviruses*
18	Anthrax fever	Bacteria	*Bacillus anthracis*
19	MRSA “superbug”	Bacteria	Methicillin-resistant *Staphylococcus aureus*
20	Pertussis	Bacteria	*Bordetella pertussis*
21	Tetanus	Bacteria	*Clostridium tetani*
22	Meningitis	Bacteria	*Neisseria meningitides*
23	Syphilis		
24	Severe acute respiratory syndrome	Virus	*SARS virus*
25	Leprosy	Bacteria	*Mycobacterium leprae*
26	Measles	Virus	*Rubeola/Measles virus*
27	Zika	Virus	*Flavivirus*

environment (Sperandio 2018), more virulent and infectious (Berngruber et al. 2013; Cressler et al. 2016; Geoghegan and Holmes 2018), and develop speedy transmission to the new hosts (McCallum et al. 2001; Antonovics et al. 2017). A better interpretation of the important evolutionary characteristics of infectious diseases including pathogenicity, infectiousness, and transmissibility may be helpful in efficient prevention and control strategies for infectious diseases (Gu et al. 2021).

The transmissibility or mode of transmission of pathogens in infectious diseases is a complex and diversified phenomenon and is central in terms of understanding disease epidemiology and biology. Despite this fact, evolution of transmission mode

has been less focused as compared to the other evolutionary consequences including evolution of virulence and host factors.

1.2 Chain of Infection

In order to understand this evolutionary process of transmissibility, it is important to know about chain of infection that elaborates the entire phenomena of disease transmission along with different modes of transmission of infection causing pathogens (Fatima et al. 2020).

The propagation of an infection within a group of people or community is defined as a "chain," and elucidates numerous interlinked phases that refer to how a pathogen spread about. To keep the pathogens from spreading, this chain is supposed to break through infection control and contact measures (Fig. 1.1).

The six links as chain relevant to the infection spread is defined as: "*Infectious agent (pathogen) Reservoir (the normal location of the pathogen) Portal of exit from the reservoir, Mode of transmission, Portal of entry into a host Susceptible*" (CDC).

1.2.1 Infectious Agents

These are the disease-causing pathogens and include microorganisms such as bacteria, parasites, viruses, fungi, and helminths. There are several determinants to the virulence or disease-causing capacity of these pathogens and included their numbers, potency, and capability to survive in host body along with immune status of the

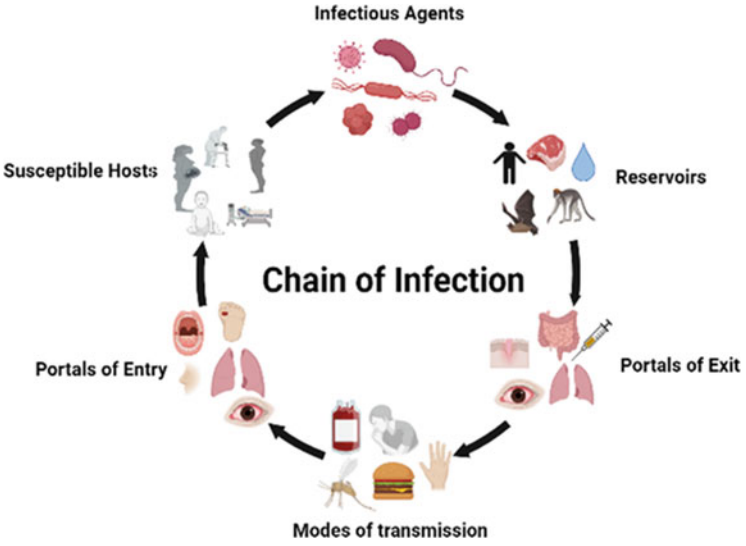

Fig. 1.1 Chain of infection demonstrating the six elements

susceptible host (Rehman et al. 2021a). For instance, the smallpox virus, although has been eradicated globally by active immunization, is highly virulent and can infect all the persons who come in contact despite well-established immune mechanism, whereas the tuberculosis causing tubercle bacilli invade only people with low immune system.

Talking about viruses, few can enter the body and survive for years until the appearance of the symports or systemic damages such as HIV virus and Hepatitis B and C viruses, on the contrary few viruses can spread and quickly reveal their presence including the influenza virus, Sar COV-19 and Rotaviruses (Khan et al. 2020; Rehman et al. 2020a).

1.2.2 Reservoir

Reservoirs are the sites anywhere in the environment where these disease-causing organisms live, thrive, and survive, since the reservoir provides a conducive environment for their growth and transmission (Fatima et al. 2021). These may include man, animals, arthropods, soil, plans, or combination of these. In case of human reservoir, the transmission of diseases is from person to person with no interference of intermediaries, such as diseases like HIV and other sexually transmitted disease, some respiratory diseases, measles, mumps, streptococcal diseases, etc.

Two forms of human reservoirs play significant role in disease transmission:

1. Cases: those who have symptoms of the diseases.
2. Carriers: person who appear either symptomatic or asymptomatic. Asymptomatic carries are those infected individuals who do not demonstrate symptoms, though unaffected, they can transmit the disease-causing pathogen to other persons. Symptomatic carriers state usually happen during incubation period, convalescent and post-convalescent period of a patient with a clinically recognizable disease condition such as influenza or any childhood disease.

The human beings are also subjected to contact the disease from animal reservoir origin and typically these diseases are transmitted within animal groups, but humans may present as incidental host. This condition is referred to as zoonosis, where under natural circumstances the disease is being contacted from vertebrae animals to men. Some of the emerging zoonosis includes brucellosis, anthrax, rabies, and some newly emerging zoonotic diseases such as Ebola, SARS, and Covid 19 (Rehman et al. 2020b).

The reservoirs from the inanimate or environmental sources include soil, water, faecal material, intravenous fluids, surgical and non-surgical devices, and instruments. Water supply may also be contaminated and become reservoir of microorganisms such as *Legionella pneumophila* causing outbreaks of legionnaires disease.

1.2.3 Modes of Escape

The pathogen leaves the reservoir by the pathway, portal of exit that corresponds usually to the site of pathogens localization. The portal of exit for respiratory disease pathogens such as *Mycobacterium tuberculosis* influenza, SARS, and Covid 19 viruses are respiratory tract, *Sarcoptes scabiei* in scabies resides in skin lesions, whereas *Vibrio cholera* exits through faeces. Conjunctival secretions are not only the reservoirs but also portal of exit for haemorrhagic conjunctivitis causing pathogen enterovirus 70. Some other examples include bloodborne agents, syphilis, rubella, toxoplasmosis that exit and cross the placenta from mother to foetus causing the diseases. The skin cuts and wounds are also reservoirs and portal of exit for Hepatitis B causing pathogens (Table 1.2).

1.2.4 Mode of Transmission

Once the infectious agent exits the reservoir through portal of exit, it requires a mode of transmission to be transmitted and enter itself to the body of host. There are several ways they can be transmitted from their natural reservoir to the susceptible host and classified into different modes of transmission.

1. Direct Transmission
   (a) Direct contact
   (b) Droplet transmission
2. Indirect transmission
   (a) Airborne transmission
   (b) Vehicle borne transmission
   (c) Vector borne transmission (may be mechanical or biological mode)

Table 1.2 Modes of escape of infectious agents from various body systems

Modes of escape	
Respiratory tract	Microorganisms leave from the throat secretions and nose from the body of infected person. These also escape by means of spray droplets when either just by breathing or sneezing, coughing, or talking.
Gastrointestinal tract	Body secretion such as passing stool or vomit is another mean for microorganisms' escape.
Skin	Skin lesions and wound drainage also cause microorganism to leave infected person body.
Blood	Blood transmission from one person to another person is also the cause of infection transfer.

1.2.5 Direct Contact

The most common and frequent mode of transmission of any healthcare associated infection is by contact which is comprised of direct and indirect contact. Direct transmission takes place when infectious causing agent travels from reservoir to a susceptible host either by direct spread or via droplet contact. It involves direct body surface contact, kissing, and sexual contact, example includes Infectious Mononucleosis (famously known Kissing disease) and gonorrhoea. Likewise, it can also happen by contacting with soil or vegetation that is harbouring infectious organism such as hookworm disease that spreads by direct contact with soil contamination.

1.2.5.1 Droplet Transmission

Droplet spread occurs when a spray with relatively large, short-range aerosols, primarily infectious agent laden, produced by coughing, sneezing, or even talking are propelled through the air. Droplet spread is categorized as direct since conduction is over only few feet by direct spray, in fact even before the droplets fall to the ground. Examples of such disease transmission are meningococcal and pertussis infection which are transmitted primarily via virus-laden fluid particles (i.e., aerosols and droplets) that are shaped in the respiratory tract of an infected patient and ejected from the nose and mouth during sneezing, coughing, talking, and breathing. They are then landed on person in contact, entering in the system of new person by contact with his/her nasal mucosa, conjunctivae, or mouth. These microorganisms are comparatively huge and can transport through shorter distances (up to 2 m/6 ft). Nevertheless, these infected droplets may stay on the surfaces for a longer time, therefore the surfaces that lie within the ranges of the sneezing/coughing person will require extra cleaning. That is why, there is a need for both droplet and contact precautions simultaneously. Respiratory syncytial virus (RSV), colds, influenza, and other some organisms causing pneumonia are the examples of microorganisms spread by droplet transmission.

Several competing factors are considered to play a role in this type of transmission such as evaporation, gravity, and inertia determine the destiny of these droplet particles. Smaller droplets evaporate faster than they settle. Thus, they form droplet nuclei that can become aerosolized, i.e., they stay airborne for hours, and may be carried over long distances. Large droplets, on the other hands, are supposed to usually settle down faster rather than these droplets evaporate and contaminate nearby exteriors (Mittal et al. 2020).

1.2.5.2 Indirect Transmission

This mode of transmission occurs due to transmission of infectious agent from their reservoir to the susceptible host through suspended air particles, vehicles (inanimate articles), or vectors (intermediate animates).

Airborne Transmission

Aerosols are small particles that are $\leq$5 μm in size and can immediately evaporate in the air. Thus, they leave behind **droplet nuclei** that are found to be light in weight and smaller enough to stay suspended for hours in the air (Klompas et al. 2020). Airborne transmission can take place when the remains of evaporated droplets suspended for long enough in the air and transferred to the respiratory tract of a vulnerable host. Measles infection in children is an example of airborne transmitted disease. Children who come into a doctor's clinic may contact the disease after another child with measles leave. Since, the measles virus remained suspended in the air, the chances of airborne infection transmission are high in this case. Data proposes that incipient coronavirus diseases have airborne spread possibility, additionally to more droplet transmission and direct contact (Santarpia et al. 2020).

Evidence is upsurging for the COVID-19 coronavirus infection that it can transfer through the air person-to-person, especially in poorly ventilated, enclosed spaces. It implies an infectious agent may remain communicable when suspended over long distances and long time in air (WHO).

Vehicle Borne Transmission

Vehicles are any inanimate intermediary articles that can transmit indirectly the pathogen between exit portal from the reservoir to the susceptible host. These vehicles may be water, biological products such as blood, and inanimate objects such as surgical instruments, handkerchiefs, or bed cloths. A vehicle may either act as a passive carrier of an infectious agent such as contaminated water or food may transmit hepatitis A virus, or the vehicle may offer a conducive environment in which the agent breeds, grows, or yields toxin, an example of which is an improperly canned food that provides a supporting environment for the production of botulinum toxin by *Clostridium botulinum*.

Vector Borne Transmission

Vectors are the animate intermediaries that are responsible for the infectious causing pathogens from the reservoir to the susceptible host. These vectors may either act as mechanical carrier for the pathogens such as flies carrying *Shigella* on their appendages, or the fleas carrying the plague causing agent *Yersinia pestis* in their gut. Additionally, these vectors act as biological transmission agent, few examples include the maturation of the causative agents like plasmodium in the blood of the mosquitoes that cause malaria and dengue fever (CDC).

1.2.6 Portal of Entry

The means through which a pathogen enters body of a vulnerable host is referred as portal of entry, which offer entrance to tissues in which the pathogen can reproduce, or a toxin may act. Generally, the infectious agent uses the same portal of entry to intrude the host body as the portal of exit where they leave the source, for example, the influenza virus leaves the host through respiratory tract and enters to another host

Table 1.3 (Kramer et al. 2006) Survival time of Microorganism on hard in animate surfaces

Micro organisms	Duration of survival (approximately)
Influenza virus	1–2 days
Escherichia coli	16 months
Corona virus	3 h
Clostridium difficile	3 months
MRSA	7 months
Mycobacterium tuberculosis	4 months
RSV	6 h
Norovirus	Till 7 days
Adenovirus	3 months

through the same respiratory tract. On the contrary, in case of gastroenteritis, the infectious agent leaves the host body through oral–faecal route and enters the body of new host through contaminated hands, food, or utensils (CDC) (Table 1.3).

1.2.7 Susceptible Host

The susceptible host is the final connection in chain of infection. There is an array of factors that determine the susceptibility of any host and include genetic or constitution, immunity specifications, and some general or nonspecific determinants like host own capacity that can provide resistance or limit the invasion and multiplication of pathogens. The factors that can increase the susceptibility of a host include malnutrition, alcoholism, certain drug therapies, and immune diseases. The immunity of a susceptible host can be enhanced through vaccination and immunization.

1.3 Emerging and Re-emerging Diseases

Over the last century, the trends of the infectious diseases especially in countries with established economies like North America and Europe have transformed with a substantial decline in mortality and morbidity and a rise in life expectancy.

This is attributed to "The Theory of epidemiological transition" which reflects a transition from an "age of pestilence and famine," where the pattern and causes of mortality were predominantly due to infectious diseases, especially among the youth population, to "era of manmade and degenerative illnesses," where the leading causes of morbidity and mortality are chronic diseases (Zhang et al. 2022). An estimate provided by Global Burden of Disease study reveals that infectious diseases contribute only 4.2% of all daily-adjusted life years lost (DALYs), although, the burden of chronic disease and neoplasias has mounted to 81% (Table 1.4).

Table 1.4 (Nii-Trebi 2017) Examples of emerging infectious diseases

Diseases	Infectious agents	Year emerged	Contributing factors
Lassa fever	Arenaviridae Family of virus	1969	Urbanization and certain factors causing favourable conditions for the rodents
Ebola Haemorrhagia fever	Filoviridae Family	1977	Natural reservoir unknown, also has nosocomial transmission
Legionella disease	*Legionella pneumophila*	1977	Plumbing and cooling systems
Lyme disease	*Borrelia burgdorferi*	1982	Reforestation near residential areas, favouring the growth of tick vector and deer
Haemolytic uremic syndrome	*Escherichia coli*	1982	Production of food at mass scale
AIDS	Human immunodeficiency virus	1983	Mass migration to urban areas, international travelling, transfusion of unscreened blood, drug use via intravenous route, multiple sex partners
Gastric ulcers	*Helicobacter pylori*	1983	Newly recognized bacteria
Cholera	*Vibrio cholerae 0139*	1992	New strains of bacteria evolution and enhanced virulence and environmental survival
Pandemic influenza	Orthomyxoviridae	Novel viral strains emerging periodically	Duck, birds, pig agricultaur (possibly)
Severe acute respiratory syndrome coronavirus	SARS CoV-1	2003	Wild animals are sold as food in the local market in Guangdong, China (probably)
Middle East respiratory syndrome	MERS-CoV	2012	Infected dromedary camels
Covid-19	SARS COV-2	2019	Bat reservoir (probably) emerging from Wuhan, China

About five to six decades back, the mankind began to believe that long going human battle against infectious diseases are almost over and an array of public health measures and introduction of vaccines and biomedical advancements had enabled them to conquer the war against these diseases (Rehman et al. 2019a, b, 2021b). Nevertheless, the emergence or re-emergence of infectious diseases has posed new challenges and burden on health care system globally, for both developed and underdeveloped nations (McArthur 2019).

Emerging infectious diseases are defined as either to be previously unknown infections or newly identified infections, posing threats and challenges to the public health at both local and international levels equally. They present some other characteristics of emerging diseases as well, such as these diseases have not been shown among the humans previously, and if occurred before, then affected only limited number of populations in isolated places examples include AIDS and Ebola haemorrhagic fever (CDC; Petersen et al. 2018).

While it is still under consideration if these emerging diseases are really novel for the humans or they were present but not recognized or diagnosed over years, but there is no doubt about it that most of them are considered as a cause of closer contact of humans with their reservoirs in nature, resulted from successful transfer of the infectious agent to man from animal across the species barrier (Morens and Fauci 2013). The proceedings of the tenth International Conference on Emerging Infectious diseases revealed that they account for at least 15% of all human pathogens. Other estimates delineated that out of nearly 400 known emerging pathogens, 25% happen to be human pathogens (Woolhouse et al. 2001).

Other studies report that nearly 175 species are associated with emerging diseases. Among them nearly 75% are of zoonotic origin, where an animal receptacle incubates the disease-causing organism with random or incidental transmission into humans (Slingenbergh et al. 2004). These diseases may originate from contaminated food, water, or airborne, but most of these are thought to have zoonotic or synoptic in their origin (CDC International Conference on Emerging Infectious Diseases 2018).

One of the most ideal examples of a recently emerged infectious disease is The AIDS pandemic: and its unprecedented rapid spread over the past few decades worldwide has put this up one of the noteworthy pandemic public health concerns of recent time (Chareonsook et al. 1999). At the turn of the century, globally, over 36 million of the population are in pined with this disease including 1.7 million of children younger than 15 years of age, with a disparity of significantly higher number of victims living in developing countries particularly in the Southern and Central parts Continent Africa, (Frieden et al. 2014). Globally, over 20 million people perished after the emergence of the pandemic. HIV infection/AIDS has become the leading cause of death among persons from 25 to 44 years of age in the USA (World Health Organization 2004).

One of the major reasons for recent failure to overcome other infectious diseases is the AIDS pandemic, especially in developing countries. Since the developing countries are more vulnerable to AIDS pandemic, it was observed that they also lost their priority over the control of other more common infectious diseases (Vignier and Bouchaud 2018; Jacob 2020).

The entire world is currently being hit by unprecedented pandemic wave of a novel strain of Corona virus named as, Coronavirus disease 2019 (COVID-19) (World Health Organization. Coronavirus disease (COVID-2019) situation reports 2020). Some of the emerging infectious disease that have been proven to be highly infectious and almost more than dozen "new" diseases have been identified as shown

in Table 1.4. Undoubtedly, the struggle has not been accomplished (Fauci and Morens 2012).

1.3.1 Re-emerging Infectious Diseases

Re-emerging infectious diseases are those that were once considered as major health concerns globally in a certain country or region, and then the incidence decreased dramatically or controlled by health measure, nevertheless are once again becoming health concerns among substantial percentage of the population (malaria and tuberculosis are examples). Many infectious disease specialists consider re-emerging diseases as a subclass of emerging diseases.

Re-emerging infectious diseases often come back in the face of epidemic proportions. A dramatic increase in the tuberculosis worldwide is due to its strong association with HIV infection; cholera has been re-emerged into regions, countries, and continents where it had earlier controlled, and the major reason behind is the poor sanitation and water contamination; dengue or "breakbone" fever has begun to show up in urban areas where mosquito control programmes are not been implemented vigorously (Morens and Fauci 2020) (Table 1.5).

Table 1.5 Examples of Re-emerging infectious diseases (Nii-Trebi 2017)

Diseases	Causative infectious agents	Contributing factors
Cryptosporidiosis	*Cryptosporidium parvum*	Water supply inadequate control, increased international travelling
Diphtheria	*Corynebacterium diphtheriae*	Lack of immunization program due to political crisis
Meningitis	*Group A Streptococcus*	Causes unknown
Rabies	*Rhabdovirus group*	Breech of public health measures, ecological changes
Malaria	*Protozoa, Plasmodium species*	Resistance from conventional drug, in efficient vector control program
Pertussis (whooping cough)	*Bordetella pertussis*	Reluctance to get vaccinated due to safety fears, reduction in vaccine efficacy
Schistosomiasis (helminth)	*Schistosoma species*	Environmental and ecological changes
Measles	*Morbillivirus*	Lack of vaccination or failure to deliver second dose
Tuberculosis	*Mycobacterium tuberculosis*	Multidrug resistance, poor sanitation, increased immunocompromised population
Yellow fever	*Flavivirus group*	Urbanization, increase in resistant insecticide

1.3.2 Factors Contributing to Emergence or Re-emergence of Infectious Diseases

The emergence and re-emergence of infectious diseases may be attributed to and influenced by multiple determinants that are interconnected to each other and include not only socio-economic and political factors, but also physical, ecological, and environmental factors along with behavioural, genetic and biological factors. Moreover, several other factors were also identified by Chareonsook et al. that contributed significantly to the emergence of infectious diseases. Few of these include changes in human demography and behaviour, massive increase in human population along with poor sanitation practices, fast and intense travelling, advancement of science, technology and industrialization, and microbial resistance to antibiotics and adaptation, less political will and failure of public health measure are adequate enough factors for the emergence and re-emergence of infectious diseases (Chareonsook et al. 1999).

1.3.3 Globalization, Trade, and Travelling

The world has become a global village and the advent of twenty-first century has brought about an unprecedented era of infectious disease with high frequency and speed, and like humans no geo-political boundaries are left for these infectious agents as well (Mackey and Liang 2012; Frieden et al. 2014). Few of the most recent pandemics are of great global health concerns such as Ebola virus disease (EVD), severe acute respiratory syndrome (SARS), and the most currents COVID-19. The damages and loses caused by these newly emerging diseases have clearly revealed that the world is still not ready to handle them and in upcoming time, human is becoming more vulnerable to new pathogens. The phenomena attributed to globalization, increase in global trade, and other ecological determinants hypothetically influence burden of many critical human infections (Daszak et al. 2008).

It is quite evidence that a massive increase in international travelling had been responsible for the transportation of the diseases as well, few examples can be clearly seen as the dengue fever and malaria have been transported to France, Italy, and other European countries by travellers and tourists. The most recent example is of Covid-19, as it emerged in China and taken to the whole world in not time, reason being the massive travelling (Delisle et al. 2015; Jacob 2020). However, it is an undeniable fact that the trade and travelling have become essential for mankind, some preventive measures such as awareness campaigns among travellers and tourists and travel restriction are paramount to flatten the curve and minimize the rising threat to the public health concerns (Chinazzi et al. 2020).

1.3.4 Environmental and Ecological Changes

Over the past few decades, global environmental and ecological modifications such as climate change with extreme weathers, depletion of ozone layer, issues in fresh water supplies layer, ecosystem variation due to deforestation and loss of biodiversity, urbanization and food producing stress to fulfil human demands have threatened the world. All these environmental and ecological disruption are attributed to human activities (Hautier et al. 2015; World Health Organization Global environmental change 2020). The changes in environment, agricultural, and food handling practices apparently increase the contact chances of human and the microorganism carrying reservoirs that were previously not known by humans, and therefore the transmission as well. Climate changes also contribute to breeding, growth, and propagation of some pathogens and according to study on climate variation, infectious diseases have revealed that vector borne, food and waterborne disease-causing organisms are highly affected by climatic changes (Galvani et al. 2016; Semenza and Ebi 2019).

1.3.5 Human-Reservoir (Wild Animal) Interface

There are a variety of instances that have increased the human and wild animal interface and so increase the chances of transmission of the novel microorganism that were previously not known by humans, such as hunting or having them as domestic animals. There has been long time evidence that this narrowing of gap between human and domestic animal has contributed to pathogens and parasite transmission, one example includes occurrence of Taenia tapeworms in humans.

1.3.6 Adaptations and Changes of Microbial Agents/Antibiotic Resistance

Another significant reason especially for the re-emergences of the many infectious disease that were not considered any more a threat for the public health is antimicrobial or antibiotic resistance. The viruses, parasites, bacteria, and fungi, like any other living things are evolving and have become resistant to base line antibiotics, therefore posed a critical health concern globally (Harrison et al. 1998; Boucher et al. 2009; Spellberg et al. 2008). Approximately an estimation of 700,000 deaths per year have been reported globally due to antimicrobial resistance and by 2050, may be the reason behind significant reduction in gross domestic product by almost 3.8% (World Bank 2016). The antibiotic resistant bacteria emergence as a consequence of antimicrobial ubiquity everywhere in the environment is a lesson to learn about the evolution of these microorganisms. Although this resistance is predominantly attributed to inappropriate and undesirable use of antibiotics, but the pathogens also have the capability to acquire the antimicrobial resistant genes from other non-pathogenic variants in the surrounding environment. Judicial,

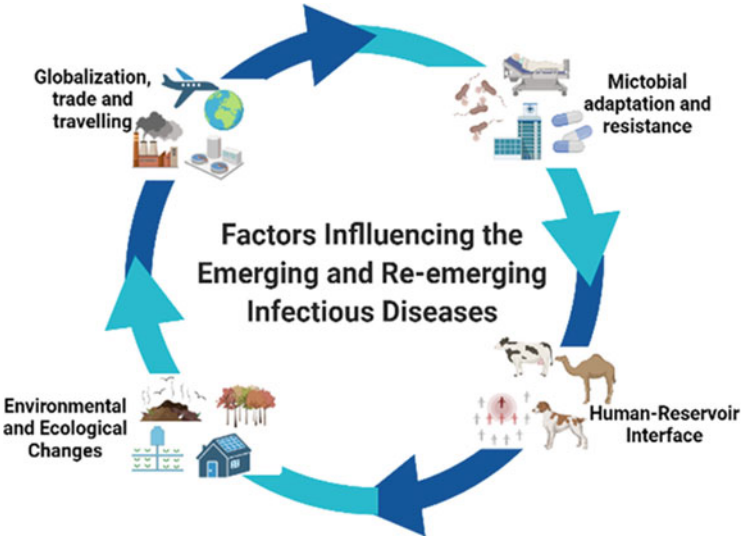

Fig. 1.2 Factors influencing the emerging and re-emerging diseases

evident based and needful use of antibiotics is the main stay to avoid and bar the emergence of antimicrobial resistant pathogens.

Perhaps in the recent scenarios, the most important recent viral pandemics caused by influenza virus that keeps on mutating and causing frequent epidemics world-wide. The novel Covid-19 virus is another example of emerging disease, that keep on becoming evolving and new variants are being observed and puzzled the whole world with high morbidity and mortality (Cui et al. 2019) (Fig. 1.2).

1.4 Interventions for Infectious Disease Control and Prevention

There are three major tools that are being used as weaponry for the control and prevention of infectious disease.

1. Antibiotics
2. Vaccines
3. Public health interventions for prevention and control

1.4.1 Antimicrobials/Antibiotics

The role and value of antibiotic are undeniable. Penicillin was the first antibiotic given to a young lady who was admitted with high grade fever history of almost a month and diagnosed with haemolytic streptococcal bacteraemia. Starting from

March 14, 1942, intravenous penicillin was given to her and recovered and survived till 90 years of age. This advent and breakthrough of successful antibiotic invention were supposed to be a miracle for the mankind healing, but emergence antimicrobial resistance has shadowed down and threatened it. Judicial and evident based use of antibiotics is the only remedy to combat this emergence of antimicrobial resistance (Azam et al. 2020). Despite this, antiviral development and enhancement, especially for HIV and Hepatitis B and C virus, have been quite impressive in terms of its ability to cure, skills of the pharmaceutical industry, and speedy progress towards recovery for the patients (Samji et al. 2013).

1.4.2 Vaccines

The role and impact of vaccines are really remarkable. Vaccines invention has miraculously aided not only to control but eradication of some highly infectious and devastating diseases, one example of which is smallpox. According to a report made available by CDC, a comparison of the vaccine preventive diseases in USA before 2013 after the advent of vaccines was really surprising. It showed 100% eradication of poliomyelitis and diphtheria, while 99.9% decrease in mumps and rubella, 99.2% and 88% decline in invasive type B *Haemophilus influenza* and pertussis, respectively (Hughes and Tartasky 1996). Another report revealed that the globally almost more that 100 million people's lives have been saved with the advent and application of various vaccines and these number could be even higher if the global access is enhanced, people become more aware through public health campaigns and introduction of new vaccines for emerging infectious diseases (van Panhuis et al. 2013).

1.4.3 Public Health Priority for Infection Control

The cost that is associated with the infection control programs made both by developed and underdeveloped countries is whooping and helped massively for the control of infectious diseases. According to a recent analysis made by CDC, annual spending on five major nosocomial or hospital acquired infections in USA is over $130,000. This disease included central line bacteraemia, ventilation associated pneumonia, catheter associated infections, surgical site infections, and *Clostridium difficile* infections (Zimlichman et al. 2013).

Moreover, the frequent surge of epidemics and pandemics of emerging and re-emerging diseases has put extraordinary burden on economies. Therefore, the prevention and control of the infectious diseases need a rational allocation and judicial utilization of resources, specially where the budget constraints have put the developing countries under threats (Krause et al. 2018). In any situation, the selection of appropriate prevention, control and treatment plan, and strategy depends upon the pathogen, or the clinical disease needed to be controlled (Krause 2008).

1.5 New Strategies and Hopes

In this era of globalization and advancement, the humans will remain in threats of old and emerging both kinds of infectious causing species, and therefore will have to utilize all of their creative strengths and mastery to combat this threat by finding newer and novel solutions; so new scientific technologies are desirable for this new world. The evolution of scientific research and technology in the current time is focused more to apprehend the phenomena of pathogens at micro and even nano levels, instead of searching their global utterance of complexity (Almessiere et al. 2020a; Qureshi et al. 2021; Rehman et al. 2021c; Nahvi et al. 2021). Although the efforts and discoveries in the field of epidemiology, public health and environment research are unparalleled, it is quite appreciable even at smaller scale, this emerging field has put impact in this battle against infectious diseases (Lewnard and Reingold 2019). The novel advancement in the field of micro and nano technology has provided a hope to solve some of the critical challenges to humanity as explained in previous sections. Certain significant methodological, scientific, and technological advancements are discussed which will probably enhance and influence out understanding about infectious diseases.

1.5.1 Nanotechnology and Nanobiology

Recent years carried incredible advancement at an ever-lower level of the life scale, i.e., at the level of the nanometre. Nanotechnology is a technique that enables to manipulate the matter at near atomic level (nearly one billion times smaller) for the development of structures, materials, and devices (Akhtar et al. 2020; Baig et al. 2020; Rehman et al. 2020c). Therefore, the search of new discoveries in the field of drug delivery and discovery is getting smaller. For discovery and delivery of the drug is moving smaller, it is no longer remain a dream to target the tumour vessels using this technology and multiple novel biologically active nanostructures are being established (Ravinayagam and Rehman 2020; Rehman et al. 2019a, b, 2020d, 2021d; Alahmari et al. 2021; Almessiere et al. 2020a, b; Al-Jameel et al. 2021).

1.5.2 Gene Sequencing to Inform Infection Control

The conventional techniques for the diagnosis and control of infectious diseases have been considerably improved by using gene sequencing techniques. There are several studies and researches going on replace the conventional studies on microorganisms by gene sequencing technique, for example, studies from United Kingdom have essentially negated conventional thinking about the epidemiology of *C. difficile* infection. Also, this technology seems to disprove some contemporary concepts about *Staphylococcus aureus* mode of transmission (Price et al. 2014). This technique is also proven to be a useful tool for the investigation of hospital and community outbreak of *Klebsiella pneumoniae* carbapenemase (Snitkin et al. 2014).

Apparently, it is cleared that this technology will be regarded as an infection control standard as it gets faster and cheaper (Won et al. 2011).

1.5.3 Data Handling and Simulation Systems

The collected data in the fields of life sciences and microbiology do not allow the advanced hypotheses and experimental conditions to be implemented in tests any longer. The advancement in the field of virtual technology and experimentation has now become an integral section of laboratory-based investigation as now we can observe in case of publication of an artificial cell, referred as the e-cell. This e-cell model comprised of 495 reaction rules along with 22 RNA encoding genes and 102 protein encoding genes. All the reactions are initiated in parallel, as the simulation is initiated. When simulation initiates, all reactions are initiated in parallel, with a gap window that is used to monitor the expression of genes. This method can be help in artificial testing of several conditions and therefore, thus letting concentration on critical real experiments.

1.5.4 Healthcare Reforms

The new healthcare system should be more focussed on the development of the expertise for addressing the infection control techniques and reforms for the resistant nosocomial infections and the high cost associated with them. Still, the payment system and current structure are not prioritized as a good fit for skills to prevent and control the infectious diseases. These nosocomial infections may later on become a threat for the emergence of resistance microorganisms and outbreak at hospital and community levels (Pronovost et al. 2018).

1.6 Conclusion

In conclusion, it is obvious that scientific evolutions have brought to us multiple lifesaving medicines, vaccines, diagnostic, and therapeutic tools, there seems no reason to believe that these solely can overcome ever rising than before threats of infectious diseases. The frequent emergence and re-emergence of more devastating infectious diseases are epiphenomena of existence of mankind and its interaction with nature and one another. With the growth in size of human population, the complexities also growing and so are the opportunities for the infectious agents to emerge with in the ecological and environmental niches that are created by us. There is more vigorous need to develop and implement a global strategy to deal with these threats and must start to think sincerely to develop a fruitful harmony with nature, or else must be always ready for new surprises.

References

Akhtar S, Rehman S, Asiri SM, Khan FA, Baig U, Hakeem AS, Gondal MA (2020) Evaluation of bioactivities of zinc oxide, cadmium sulfide and cadmium sulfide loaded zinc oxide nanostructured materials prepared by nanosecond pulsed laser. Mater Sci Eng C 116:111156

Alahmari F, Rehman S, Almessiere M, Khan FA, Slimani Y, Baykal A (2021) Synthesis of Ni0.5Co0.5-xCdxFe1.78Nd0.02O4 ($x \leq 0.25$) nanofibers by using electrospinning technique induce anti-cancer and anti-bacterial activities. J Biomol Struct Dyn 39(9):3186–3193

Al-Jameel SS, Rehman S, Almessiere MA, Khan FA, Slimani Y, Al-Saleh NS, Manikandan A, Al-Suhaimi EA, Baykal A (2021) Anti-microbial and anti-cancer activities of Mn0.5Zn0.5DyxFe2-xO4 ($x \leq 0.1$) nanoparticles. Artif Cells Nanomed Biotechnol 49(1): 493–499

Alkharsah KR, Rehman S, Alkhamis F, Alnimr A, Diab A, Al-Ali AK (2018) Comparative and molecular analysis of MRSA isolates from infection sites and carrier colonization sites. Ann Clin Microbiol Antimicrob 17(1):1–1

Alkharsah KR, Rehman S, Alnimr A, Diab A, Hawwari A, Tokajian S (2019) Molecular typing of MRSA isolates by spa and PFGE. J King Saud Univ Sci 31(4):999–1004

Almessiere MA, Slimani Y, Rehman S, Khan FA, Güngüneş ÇD, Güner S, Shirsath SE, Baykal A (2020a) Magnetic properties, anticancer and antibacterial effectiveness of sonochemically produced Ce3+/Dy3+ co-activated Mn-Zn nanospinel ferrites. Arab J Chem 13(10):7403–7417

Almessiere MA, Slimani Y, Rehman S, Khan FA, Polat EG, Sadaqat A, Shirsath SE, Baykal A (2020b) Synthesis of Dy-Y co-substituted manganese-zinc spinel nanoferrites induced anti-bacterial and anti-cancer activities: comparison between sonochemical and sol-gel auto-combustion methods. Mater Sci Eng C 116:111186

Antonovics J, Wilson AJ, Forbes MR et al (2017) The evolution of transmission mode. Philos Trans Biol Sci 372:20160083

Avondt KV, van Sorge NM, Meyaard L (2015) Bacterial immune evasion through manipulation of host inhibitory immune signaling. PLoS Pathog 11:e1004644

Azam M, Kumar V, Siddiqui K, Jan AT, Sabir JS, Rather IA, Rehman S, Haq QM (2020) Pharmaceutical disposal facilitates the mobilization of resistance determinants among microbiota of polluted environment. Saudi Pharm J 28(12):1626–1634

Baig U, Gondal MA, Rehman S, Akhtar S (2020) Facile synthesis, characterization of nano-tungsten trioxide decorated with silver nanoparticles and their antibacterial activity against water-borne gram-negative pathogens. Appl Nanosci 10(3):851–860

Bernard Q, Smith AA, Yang X (2018) Plasticity in early immune evasion strategies of a bacterial pathogen. Proc Natl Acad Sci U S A 115:E3788–E3797

Berngruber TW, Froissart R, Choisy M, Gandon S (2013) Evolution of virulence in emerging epidemics. PLoS Pathog 9:e1003209

Boucher HW, Talbot GH, Bradley JS (2009) Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clin Infect Dis 48:1–12

CDC International Conference on Emerging Infectious Diseases (2018). https://www.cdc.gov/iceid/index.html. Accessed 3 Aug 2018

Chabas H, Lion S, Nicot A (2018) Evolutionary emergence of infectious diseases in heterogeneous host populations. PLoS Biol 16:e2006738

Chareonsook O, Foy HM, Teeraratkul A, Silarug N (1999) Changing epidemiology of dengue hemorrhagic fever in Thailand. Epidemiol Infect 122:161–166

Chinazzi M, Davis JT, Ajelli M (2020) The effect of travel restrictions on the spread of the 2019 novel coronavirus (COVID-19) outbreak. Science 368:395–400

Cressler CE, Mcleod DV, Rozins C, Van Den Hoogen J, Day T (2016) The adaptive evolution of virulence: a review of theoretical predictions and empirical tests. Parasitology 143:915–930

Cui J, Li F, Shi Z-L (2019) Origin and evolution of pathogenic coronaviruses. Nat Rev Microbiol 17 (3):181–192. https://doi.org/10.1038/s41579-018-0118-9
Daszak P, Jones KE, Gittleman JL (2008) Global trends in emerging infectious diseases. Nature (London) 451:990–993
Delisle E, Rousseau C, Broche B (2015) Chikungunya outbreak in Montpellier, France, September to October 2014. Euro Surveill 20:8–13
Echaubard P, Rudge JW, Lefevre T (2018) Evolutionary perspectives on human infectious diseases: challenges, advances, and promises. Evol Appl 11:383–393
Fatima Z, Kumar B, Rehman S, Hameed S (2020) Retrograde signaling: a novel antifungal drug target. In: New and future developments in microbial biotechnology and bioengineering. Elsevier, Amsterdam, pp 219–226
Fatima Z, Kumari P, Rehman S, Hameed S (2021) Virulence traits of Candida spp.: an overview. In: Recent trends in mycological research, pp 439–455
Fauci AS (2005) Emerging and reemerging infectious diseases: the perpetual challenge. Acad Med 80:1079–1085
Fauci AS, Morens DM (2012) The perpetual challenge of infectious diseases. N Engl J Med 366: 454–461
Frieden TR, Tappero JW, Dowell SF, Hien NT, Guillaume FD, Aceng JR (2014) Safer countries through global health security. Lancet (British edition) 383:764–766
Galvani AP, Bauch CT, Anand M, Singer BH, Levin SA (2016) Human–environment interactions in population and ecosystem health. Proc Natl Acad Sci U S A 113:14502–14506
Geoghegan JL, Holmes EC (2018) The phylogenomics of evolving virus virulence. Nat Rev Genet 19:756–769
Gu J, Zhou Z, Wang Y (2021) Editorial: Evolutionary mechanisms of infectious diseases. Front Microbiol 12:667561
Haldar K, Bhattacharjee S, Safeukui I (2018) Drug resistance in Plasmodium. Nat Rev Microbiol 16:156–170
Hilleman MR (2004) Strategies and mechanisms for host and pathogen survival in acute and persistent viral infections. Proc Natl Acad Sci U S A 101:14560–14566
Hughes DL, Tartasky D (1996) Implementation of a flu immunization program for homebound elders: a graduate student practicum. Geriatr Nurs 17:217–221
https://www.who.int/hiv/epi-update2005_en.pdf
Institute of Medicine (US) Forum on Emerging Infections, Harrison PF, Lederberg J (1998) Antimicrobial resistance: issues and options: workshop report. National Academy Press, Washington, DC
Jacob E (2020) Coronavirus: trois premiers cas confirmes en France, deux d'entre eux vont bien. https://www.lefigaro.fr/sciences/coronavirus-trois-premiers-cas-confirmes-en-france-20200124 . Accessed 26 Jul 2020
Khan S, Tomburoglu H, Hassanein SE, Rehman S, Bozkurt A, Cevik E, Abdel-Ghany S, Nabi G, Ali A, Sabit H (2020) Coronavirus diseases 2019: current biological situation and potential therapeutic perspective. Eur J Pharmacol 886:173447
Klompas M, Baker MA, Rhee C (2020) Airborne transmission of SARS-CoV-2 theoretical considerations and available evidence. JAMA 324:441–442
Kramer A, Schwebke I, Kampf G (2006) How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. BMC Infect Dis 6(1). https://doi.org/10.1186/1471-2334-6-130
Krause G (2008) How can infectious diseases be prioritized in public health? EMBO Rep 9(S1). https://doi.org/10.1038/embor.2008.76
Krause G, Working Grp Prioritization, Working Group on Prioritization at Robert Koch Institute (2018) How can infectious diseases be prioritized in public health? A standardized prioritization scheme for discussion. EMBO Rep 9:S22–S27
Laxminarayan R, Sridhar D, Blaser M, Wang M, Woolhouse M (2016) Achieving global targets for antimicrobial resistance. Science 353:874–875

Lewnard JA, Reingold AL (2019) Emerging challenges and opportunities in infectious disease epidemiology. Am J Epidemiol 188:873–882
Mackey TK, Liang BA (2012) Lessons from SARS and H1N1/A: employing a WHO-WTO forum to promote optimal economic-public health pandemic response. J Public Health Policy 33:119–130
McArthur DB (2019) Emerging infectious diseases. Nurs Clin North Am 54(2):297–311
McCallum H, Barlow N, Hone J (2001) How should pathogen transmission be modelled? Trends Ecol Evol (Amsterdam) 16:295–300
Mittal R, Ni R, Seo J (2020) The flow physics of COVID-19. J Fluid Mech 894. https://doi.org/10.1017/jfm.2020.330
Morens DM, Fauci AS (2013) Emerging infectious diseases: threats to human health and global stability. PLoS Pathog 9:e1003467
Morens DM, Fauci AS (2020) Emerging pandemic diseases: how we got to COVID-19. Cell 183:837
Nahvi I, Belkahla S, Asiri SM, Rehman S (2021) Overview and prospectus of algal biogenesis of nanoparticles. In: Microbial nanotechnology: green synthesis and applications. Springer, Singapore, pp 121–134
Nathan C, Cars O (2014) Antibiotic resistance—problems, progress, and prospects. N Engl J Med 371:1761–1763
Nii-Trebi NI (2017) Emerging and neglected infectious diseases: insights, advances, and challenges. Biomed Res Int 2017:5245021. https://doi.org/10.1155/2017/5245021
Parry W, Peterson E (2020) Devastating infectious diseases. https://www.livescience.com/13694-devastating-infectious-diseases-smallpox-plague.html. Accessed 25 Sep 2021
Petersen E, Petrosillo N, Koopmans M (2018) Emerging infections—an increasingly important topic: review by the Emerging Infections Task Force. Clin Microbiol Infect 24:369–375
Price JR, Golubchik T, Cole K (2014) Whole-genome sequencing shows that patient-to-patient transmission rarely accounts for acquisition of Staphylococcus aureus in an intensive care unit. Arch Clin Infect Dis 58:609–618
Pronovost PJ, Armstrong CM, Demski R, Peterson RR, Rothman PB (2018) Next level of board accountability in health care quality. J Health Organ Manag 32(1):2–8. https://doi.org/10.1108/JHOM-09-2017-0238
Qureshi A, Blaisi NI, Abbas AA, Khan NA, Rehman S (2021) Prospectus and development of microbes mediated synthesis of nanoparticles. In: Microbial nanotechnology: green synthesis and applications. Springer, Singapore, pp 1–15
Ravinayagam V, Rehman S (2020) Zeolitic imidazolate framework-8 (ZIF-8) doped TiZSM-5 and mesoporous carbon for antibacterial characterization. Saudi J Biol Sci 27(7):1726–1736
Rehman S, Al Salem Z, Al Jindan R, Hameed S (2019a) Microbial natural products: exploiting microbes against drug-resistant bugs. In: Pathogenicity and drug resistance of human pathogens. Springer, Singapore, pp 393–404
Rehman S, Almessiere MA, Tashkandi N, Baykal A, Slimani Y, Jermy R, Ravinayagam V, Yaman C (2019b) Fabrication of spinel cobalt ferrite (CoFe2O4) nanoparticles with unique earth element cerium and neodymium for anticandidal activities. Chem Select 4(48):14329–14334
Rehman S, Majeed T, Ansari MA, Ali U, Sabit H, Al-Suhaimi EA (2020a) Current scenario of COVID-19 in pediatric age group and physiology of immune and thymus response. Saudi J Biol Sci 27(10):2567–2573
Rehman S, Almessiere MA, Khan FA, Korkmaz AD, Tashkandi N, Slimani Y, Baykal A (2020b) Synthesis and biological characterization of Mn0.5Zn0.5EuxDyxFe1.8-2xO4 nanoparticles by sonochemical approach. Mater Sci Eng C 109:110534
Rehman S, Majeed T, Ansari MA, Al-Suhaimi EA (2020c) Syndrome resembling Kawasaki disease in COVID-19 asymptomatic children. J Infect Public Health 13(12):1830–1832
Rehman S, Asiri SM, Khan FA, Jermy BR, Ravinayagam V, Alsalem Z, Al Jindan R, Qurashi A (2020d) Anticandidal and in vitro anti-proliferative activity of sonochemically synthesized indium tin oxide nanoparticles. Sci Rep 10(1):1–9

Rehman S, Ravinayagam V, Nahvi I, Aldossary H, Al-Shammari M, Amiri MS, Kishore U, Al-Suhaimi EA (2021a) Immunity, sex hormones, and environmental factors as determinants of COVID-19 disparity in women. Front Immunol 12:680845

Rehman S, Ansari MA, Al-Dossary HA, Fatima Z, Hameed S, Ahmad W, Ali A (2021b) Current perspectives on mycosynthesis of nanoparticles and their biomedical application. In: Modeling and control of drug delivery systems. Academic, New York, pp 301–311

Rehman S, Almessiere MA, Al-Jameel SS, Ali U, Slimani Y, Taskhandi N, Al-Saleh NS, Manikandan A, Khan FA, Al-Suhaimi E, Baykal A (2021c) Designing of Co0.5Ni0.5GaxFe2-xO4 ($0.0 \leq x \leq 1.0$) microspheres via hydrothermal approach and their selective inhibition on the growth of cancerous and fungal cells. Pharmaceutics 13(7):962

Rehman S, Almessiere MA, Al-Suhaimi E, Hussain M, Yousuf Bari M, Mehmood Ali S, Al-Jameel SS, Slimani Y, Khan FA, Baykal A (2021d) Ultrasonic synthesis and biomedical application of Mn0.5Zn0.5ErxYxFe2− 2xO4 nanoparticles. Biomolecules 11(5):703

Samji H, Cescon A, Hogg RS, Modur SP, Althoff KN, Buchacz K, Burchell AN, Cohen M, Gebo KA, Gill MJ, Justice A, Kirk G, Klein MB, Korthuis PT, Martin J, Napravnik S, Rourke SB, Sterling TR, Silverberg MJ, Deeks S, Jacobson LP, Bosch RJ, Kitahata MM, Goedert JJ, Moore R, Gange SJ, Okulicz JF (2013) Closing the gap: increases in life expectancy among treated HIV-positive individuals in the United States and Canada. PLoS One 8(12):e81355. https://doi.org/10.1371/journal.pone.0081355

Santarpia JL, Rivera DN, Herrera VL (2020) Aerosol and surface contamination of SARS-CoV-2 observed in quarantine and isolation care. Sci Rep 2020:12732

Semenza JC, Ebi KL (2019) Climate change impact on migration, travel, travel destinations and the tourism industry. J Travel Med 26:taz026

Slingenbergh J, Gilbert M, De Balogh K, Wint W (2004) Ecological sources of zoonotic diseases. Rev Sci Tech 23:467–484

Snitkin ES, Zelazny AM, Thomas PJ (2014) Tracking a hospital outbreak of carbapenem-resistant Klebsiella pneumoniae with whole-genome sequencing. Sci Transl Med 4:148ra116

Spellberg B, Guidos R, Gilbert D (2008) The epidemic of antibiotic-resistant infections: a call to action for the medical community from the Infectious Diseases Society of America. Clin Infect Dis 46:155–164

Sperandio V (2018) Pathogens' adaptation to the human host. Proc Natl Acad Sci U S A 115:9342–9343

van Panhuis WG, Grefenstette J, Jung SY (2013) Contagious diseases in the United States from 1888 to the present. N Engl J Med 369:2152–2158

Vignier N, Bouchaud O (2018) Travel, migration and emerging infectious diseases. EJIFCC 29:175–179

WHO (2004). https://www.who.int/whr/2004/en/report04_en.pdf. Accessed 22 Sep 2021

Won SY, Munoz-Price LS, Lolans K (2011) Emergence and rapid regional spread of Klebsiella pneumoniae Carbapenemase—producing Enterobacteriaceae. Clin Infect Dis 53:532–540

Woolhouse MEJ, Taylor LH, Haydon DT (2001) Population biology of multihost pathogens. Science 292:1109–1112

World Bank (2016) Drug-resistant infections: a threat to our economic future (discussion draft). World Bank, Washington, DC

World Health Organization (2020) Global environmental change. https://www.who.int/globalchange/environment/en/. Accessed 26 Jul 2021

Zhang JF, Rehman S, Alghamdi T, Sheikh FA, Hassan MS, Amna T (2022) Bionanotechnology approaches to combat biofilms and drug resistance. In: Innovative approaches for nanobiotechnology in healthcare systems. IGI Global, Hershey, PA, pp 230–248

Zimlichman E, Henderson D, Tamir O (2013) Health care–associated infections: a meta-analysis of costs and financial impact on the US Health Care System. JAMA Intern Med 173:2039–2046

Application of Nanotechnology in the Treatment of Infectious Diseases: An Overview

2

Ifeanyi Elibe Mba and Emeka Innocent Nweze

Abstract

Despite the advances in science, infectious diseases remain a major driver of morbidity and mortality worldwide. The recent outbreak of severe acute respiratory syndrome coronavirus (SARS-CoV-2) has questioned whether the current global health system can offer the needed protection against an array of infectious disease threats. Furthermore, it has re-ignited the urgency to develop and deploy countermeasures to control infectious diseases. To add to the above-established issues is the rapid emergence of antimicrobial resistance (AMR) infections. However, evidence is emerging that the field of nanotechnology is beginning to address all the challenges presented by diverse infectious pathogens. Nanotechnology approaches are increasingly and effectively being developed to design new therapeutic biomaterials, develop new vaccines and as drug delivery systems for the treatment of infectious diseases. This chapter discusses the recent preclinical and clinical nanotechnology progress and advances towards several infectious diseases. First, we highlight the application of nanotechnology to the development and improvement of new and existing treatment modalities against bacteria, fungi, and viruses. Then, we further summarize the various nanotechnology approaches, illustrating the progress on the application of nanotechnology against infectious diseases. Finally, we ponder over the challenges in translating nanotechnology from the laboratory to the clinic.

Keywords

Nanotechnology · Nanomaterials · Nanoparticles · Infectious diseases · Bacteria · Fungi · Viruses · Nanovaccines · Drug delivery system · Vaccines

I. E. Mba · E. I. Nweze (✉)
Department of Microbiology, University of Nigeria, Nsukka, Nigeria
e-mail: emeka.nweze@unn.edu.ng

S. Hameed, S. Rehman (eds.), *Nanotechnology for Infectious Diseases*,
https://doi.org/10.1007/978-981-16-9190-4_2

2.1 Introduction

Infectious diseases are a major contributor to the global disease burden. Lower respiratory tract infections, diarrhoea, tuberculosis (TB), HIV infection, and malaria are associated with high mortality rates, especially in developing nations with low SDI. The lack of clinical trial studies related to these infectious diseases compared to conditions like cardiovascular diseases and cancer even make it more challenging. In developing countries alone, microbial infections are responsible for millions of deaths annually (Roth et al. 2018), and antimicrobial resistance infection persists as the most challenging (CDC 2019). Different microbes cause malaria, TB, and HIV/AIDS. However, these pathogens present similar vaccination challenges. In each of these health concerns, the immunological response mechanism does not generally lead to protective immunity. Thus, despite several years of intense research, a vaccine against HIV, malaria, and TB has not been successfully developed. The inability to develop a vaccine against this deadly viral agent is due to several reasons, as will be seen later.

Moreover, clinical trials for most critical infectious diseases are lacking compared to other non-infectious conditions (cardiovascular diseases and cancer). As already mentioned, resistance to antimicrobial agents is at the centre of the whole issue. Even in most developed nations, antimicrobial resistance is still a major public health problem. Biofilm formation is one thing that encourages development of resistance. Infections associated with biofilm often pose a huge treatment difficulty in addition to a high mortality rate (Khatoon et al. 2018; Fleming and Rumbaugh 2018). In chronic infections, biofilms play a crucial role, and tactics that overcome biofilm formation may be crucial in treating chronic infections. Currently, the unavailability of safe and effective drugs makes it difficult to manage infectious diseases. Furthermore, the emergence of multidrug resistance microbes makes treatment even more challenging and difficult (Munita and Arias 2016).

The need to identify a practical and more impactful strategy to curtail the proliferation of infectious diseases cannot be overemphasized, and the use of nanoparticles in the management of infections is a promising strategy. The past few decades have witnessed an upsurge in research relating to the clinical application of nanotechnology. Nanotechnology has shown promises against several bacterial infections (Mba and Nweze 2021), fungal infections (Mba and Nweze 2020; Kischkel et al. 2020a, b), and viral infections (Mba et al. 2021; Vazquez-Munoz and Lopez-Ribot 2020). With this rapidly emerging therapeutic option, the world's main sources of morbidity and mortality will be substantially impacted. Furthermore, for the detection and treatment of a variety of infectious diseases, nanotechnology has shown good potentials. It has been extensively evaluated to treat infectious diseases, especially those resistant to the available antimicrobial agents. Thus, the field of nanotechnology is well-positioned to tackle the multidrug resistance crisis and transform both detection and treatment of several infectious diseases.

Also, the field of nanotechnology offers potential paths to uniquely engineer vaccines that trigger immune responses different from that of natural infection. The development of an effective vaccine may require engaging both humoral and cellular

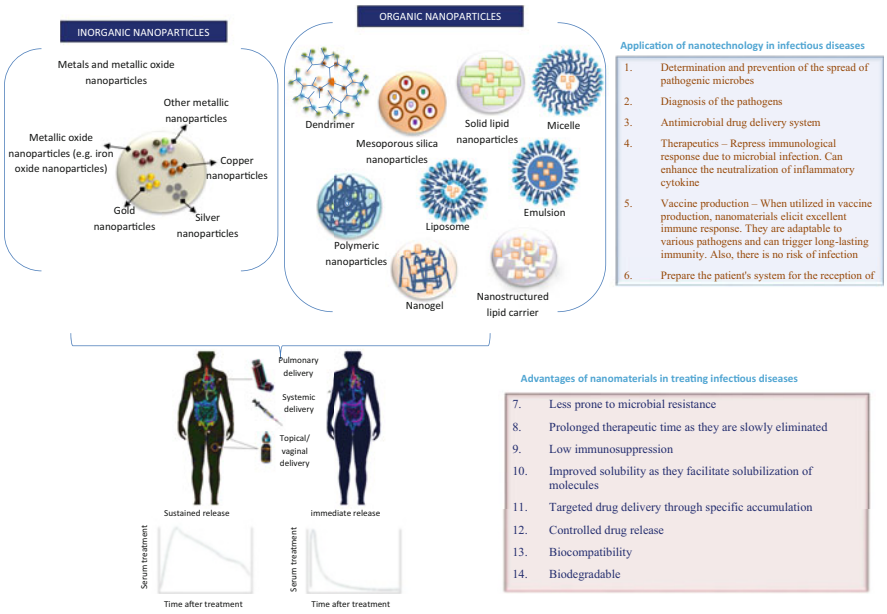

Fig. 2.1 Applications of nanomaterials against infectious diseases

immune systems. Most of these limitations may be overcome by utilizing nanomaterial-based vaccines, and nanomaterials that can carry multiple antigens are crucial in this regard. In addition, these nanomaterials can provide adjuvants that are necessary to fine-tune the immunological response. The multifunctionality of nanomaterials and the ability to be specifically engineered for a given function make them potential candidates.

Generally, nanomaterials can be classified into two groups: organic—polymeric, liposomes, micelles, and ferritin, and inorganic—metal and metal oxide nanoparticles. They have all been successfully utilized to treat various infectious diseases (Anselmo and Mitragotri 2016) and offer several advantages and clinical applications (Fig. 2.1). Therefore, the field of nanotechnology has the potential to reposition the detection and treatment of several infectious diseases. The opportunities presented by nanotechnology in clinical settings are endless. Here, we critically but concisely discussed the ongoing impact of nanotechnology in the treatment of infectious diseases, especially multidrug resistance ones. Also, preclinical and clinical progress on nanomaterials were discussed. Finally, we evaluated the available challenges to translating these technologies from the laboratory to the clinic and present key avenues for future studies.

2.2 Nano Formulations for the Treatment of Bacterial Infections

Nanomaterials have been successfully used against several bacterial pathogens, especially the ESKAPE group (*Enterococcus*, *Staphylococcus*, *Klebsiella*, *Acinetobacter*, *Pseudomonas*, *Enterobacter*), as they represent the greatest public health threat as reported by World Health Organization (WHO). However, they are also associated with a high mortality rate. The antibacterial mechanism of nanoparticles against bacteria is shown in Fig. 2.2. In a study by Sarwar et al. (2017), ZnONps were able to disrupt the structure of cholera toxin, preventing its interaction with the receptors in the erythrocytes. A recent investigation also shows that AgNps were highly effective against multidrug-resistant *S. aureus*, *P. aeruginosa*, and *E. coli* (Huq 2020). Nanomaterials have also shown promises against *Mycobacterium tuberculosis*. Recent in vitro studies have demonstrated that AgNps (Tăbăran et al. 2020), SeNps (Estevez et al. 2020), and TiO_2 (Ramalingam

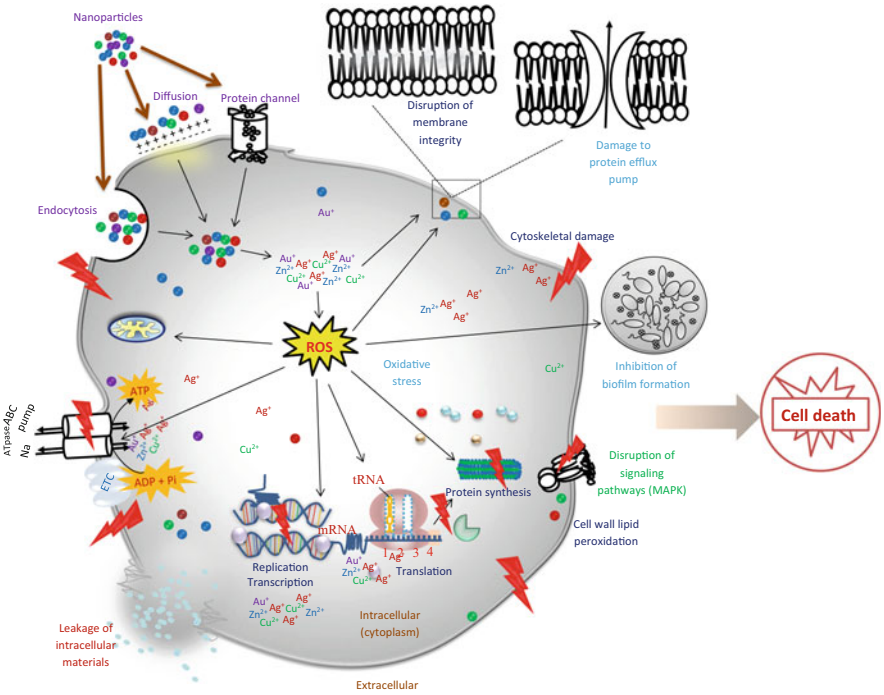

Fig. 2.2 Mechanisms of nanoparticles activity against bacteria. (1) The nanoparticles first attach and penetrate the bacterial membrane. (2) There is usually the release of ions that trigger ROS generation and oxidative stress initiation. (3) The generation of ROS affects several signalling pathways and also disrupts the biofilm-forming ability of the cell, (4) the ions also interact with DNA leading to cell death, (5) the released ions and the ROS also affect membrane structural integrity, which subsequently can lead to cell apoptosis. (6) The ions and ROS also affect other important cellular processes. (Reproduced from Mba and Nweze 2021)

et al. 2019) were effective against *M. tuberculosis*. However, the mechanism of action remains to be properly elucidated. Other recent studies showing the effect of nanoparticles against different bacteria are available (Liao et al. 2019; Vazquez-Munoz et al. 2019; Tariq et al. 2020; Ansari et al. 2020).

2.3 Nano Formulations for the Treatment of Fungal Infections

To date, treatment modalities for most fungal infections are still limited. The mortality rate resulting from systemic fungal infections is increasing daily due to the increased number of immunocompromised individuals (Lockhart and Guarner 2019). The increasing mortality is also linked to the failure of most conventional antifungal agents (azoles, polyenes, echinocandins), their high cost and the associated toxicity (Brunet et al. 2018). Antifungal drugs can also interact with other drugs increasing treatment difficulties (Brunet et al. 2018; Bicanic 2014). Moreover, the development of resistance by major pathogenic fungal species is also another issue. Sadly, there is no licensed vaccine or prophylactic preparation to treat human systemic fungal infections (Travassos and Taborda 2017). Several obstacles have stalled the development of fungal vaccines. The lack of clear-cut appropriate adjuvants, lack of adequate formulation, high cost of development, and lack of market interest are major issues in vaccines development. Moreover, the fact that most major pathogenic fungi affect mainly immunocompromised individuals has also contributed to hindering the development of effective fungal vaccines (Cassone and Casadevall 2012; Travassos and Taborda 2017).

The field of nanotechnology has been seen as an innovative technology with the potential to bypass those obstacles and enable the production of efficient fungal vaccines. Nanomaterials have been used as antifungal drug delivery agents and as immunostimulant adjuvants in vaccine formulation (Zhao et al. 2014a, b). *Candida* happens to be the most implicated human fungal pathogen (Mba and Nweze 2020) and has received the most attention. Thus, the antifungal activity of nanoparticles, as explained using *Candida* spp., is through several mechanisms (Fig. 2.3) (Mba and Nweze 2020). The potential of nano formulations to disrupt the EPS biofilm matrix has been reported (Gondim et al. 2018; Yang et al. 2019). AuNPs have been shown to reduce the biofilm-forming ability of *C. albicans* and increase photodynamic therapy's effectiveness (Sherwani et al. 2015; Maliszewska et al. 2017). The effect of AgNps and other metallic nanoparticles against *C. albicans* has also been studied in many recent investigations (Khatoon et al. 2019; Golipour et al. 2019; Abo-Shama et al. 2020; Munoz-Escobar and Reyes-Lopez 2020).

Fernandes et al. (2019) encapsulated farnesol and miconazole with chitosan nanoparticles. The result showed that farnesol-containing chitosan nanoparticles were able to reduce pathogenicity in a murine model of vulvovaginal candidiasis. The nano formulation also inhibited the growth of hyphae and also showed no toxicity in fibroblasts. The efficacy of chitosan containing AmB (PLGA-CHI-AmB) against *Candida* spp. was reported in a study by Ludwig et al. (2018), while Jansook et al. (2018) showed that AmB-SLNs-NLCs nano formulation was

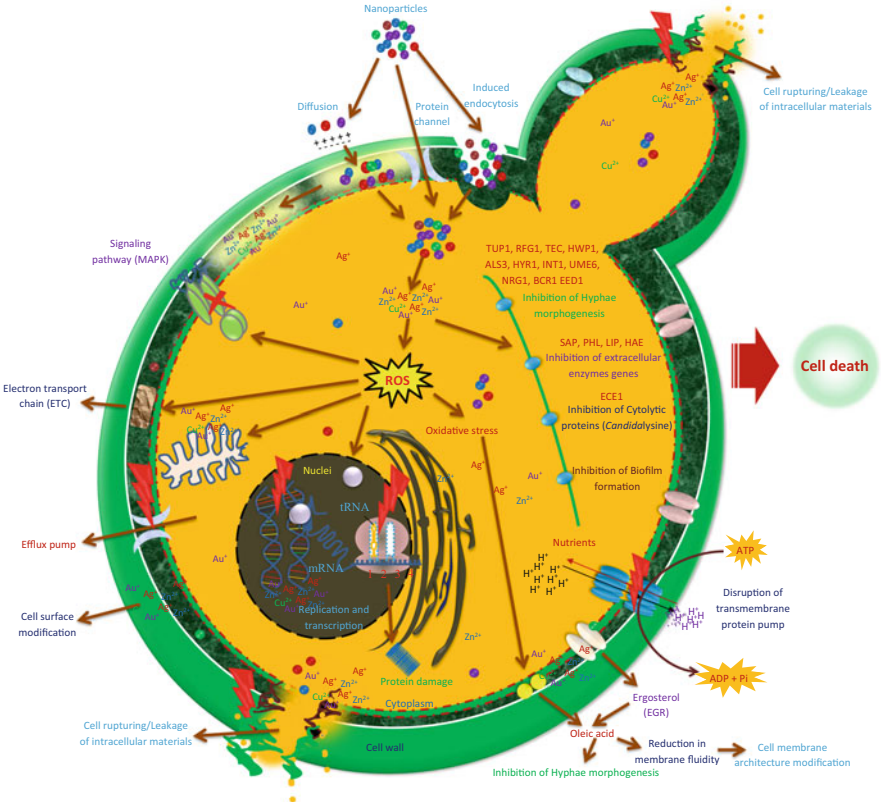

Fig. 2.3 Mechanism of anti-candida activity of nanomaterials. (a) Attachment of nanoparticle to cell wall, (b) Invasion/penetration into cell membrane, (c) Production of metallic ions, (d) Change in cell membrane conformation, architecture, and integrity, (e) Disruption of cell wall and modification of cell surface, (f) Impairment of transport activity, (g) Cell rupturing and leakage of intracellular materials, (h) Reduction and alteration in membrane fluidity and temperature sensing, (i) Alteration in cellular ergosterol (ERG) content, (j) Alteration in fatty acid composition (Oleic acid) crucial for yeast to hyphal transition, (k) Inhibition of extracellular hydrolytic enzyme (proteinase, phospholipase, hemolysin, and lipase) production, (l) Inhibition of genes crucial for morphogenesis and other key cellular processes, (m) Disruption of β-glucan synthase, (n) Production of reactive oxygen species (ROS), (o) Inhibition of biofilm formation, (p) Disruption of electron transport chain (ETC), (q) Disruption of cellular, signaling, and metabolic pathways, (r) Disruption of mitochondrial membrane, (s) DNA damage, (t) Interrupt H^+-ATPase mediated proton pumping, (u) Formation of pit and holes and inhibition of budding process. (Reproduced from Mba and Nweze 2020)

more effective against *C. albicans* than AmB or Fungizone® free from nanomaterial. Nano formulations have also been used as antigen delivery vehicles for *C. albicans* (Knotigová et al. 2015; Carneiro et al. 2016) and *Paracoccidioides brasiliensis* (Jannuzzi et al. 2018) vaccine. Chitosan-silver-copper nanocomposite has also been shown to have substantial activity against *C. albicans* (Ashrafi et al. 2020).

In addition, nanotechnology has been utilized to treat *Aspergillus fumigatus* (Yang et al. 2018; Siopi et al. 2019; Khames et al. 2019), *Cryptococcus neoformans* (Lu et al. 2019; Domingues Bianchin et al. 2019), *P. brasiliensis*, and *P. lutzii* (Singulani et al. 2018; Stewart et al. 2018). Other studies on the anti-candida activity of nanomaterials are also available (Spadari et al. 2019; Domingues Bianchin et al. 2019; Carbone et al. 2020; Kischkel et al. 2020a, b).

2.4 Application of Nanotechnology Against Viral Infections

Viral entry into the cell involves the interaction between the receptors on the host cell and the proteins on the viral surface. Nanomaterials can effectively inhibit viral entry by interfering with the interaction between the receptors and the host cell. They can block the viral surface proteins. For example, the HIV gp120 glycoprotein is essential for HIV to bind to the CD4+ T cells (Wilen et al. 2012). Studies by Vijayakumar and Ganesan (2012) show that AuNPs coated with polyethylene glycol and SiNPs modified with -OH and -NH_2 groups successfully blocked the gp120 on the HIV-1 surface. AgNPs have a broad-spectrum antiviral effect as they can interfere with the viral gp120 in both cell-free and cell-attached viruses, inhibiting viral binding and entry into the cell (Lara et al. 2010). AgNPs also interfere with the entry of herpes simplex virus-1 (HSV-1), HSV-2, and human parainfluenza virus 3 (HPIV-3) via its interaction with the sulfhydryl group on viral glycoproteins (Gaikward et al. 2013). In addition, nanomaterials can also block cell membrane receptors preventing the entry of the virus into the cell (Dong et al. 2017).

Another mechanism of antiviral activity of nanomaterials is by inhibition of viral replication via interaction with viral proteins or viral genome. It can also be achieved by making the environment unconducive for viral replication (Luther et al. 2020). Graphene oxide-silver nanocomposites were also reported to prevent viral entry and activate the antiviral innate immunological response (Du et al. 2018).

The fact that most viruses can hijack host cell machinery, resulting in the activation of the MEK/ERK cascade, which promotes the replication of viruses (Bonjardim 2017), greatly impede the development of an effective antiviral agent. This area demands further investigation.

Different nanomaterials can also directly inactivate different viruses via a different approach. For enveloped viruses, damage to the envelopes is usually via photocatalytic oxidation or physical damage. For example, a study by Banerjee et al. (2012) shows that there is an interaction between carbon nanotube attached to photoactivating protoporphyrin IX and influenza virus H3N2. This interaction leads to the destabilization of the viral envelope by ROS. Nanomaterials can also inactivate viruses by damaging the viral capsid. This is achieved by photocatalytic oxidation and protein degradation. This mechanism has been illustrated using TiO_2NPs (Syngouna and Chrysikopoulos 2017), Au/CuS core-shell nanoparticle (Broglie et al. 2015), and other nanomaterials (Huy et al. 2017). The antiviral potential of graphene-based nanomaterials against viruses was recently summarized in a review by Seifi and Kamali (2021).

Furthermore, studies by (Velthuis et al. 2010; Ghaffari 2019) showed that ZnONps were effective against H1N1 and SARS-CoV-2, respectively. More recent investigations showed that surfaces coated with nanoparticles showed antiviral activity against SARS-CoV-2 (Lea 2020a, b). According to the study, these materials produce ions that damage SARS-CoV-2. The insertion of nanomaterials into SARS-CoV-2 to neutralize the particles is another strategic therapeutic means as these materials can help to control the multiplication of the virus in the host cell. The encapsulation of the SARS-CoV-2 with nanomaterials has also been shown to prevent viral-host cell interaction and subsequent attachment (Tyagi et al. 2020). In addition, nanomaterials can also be used to deliver antiviral drugs. In recent investigations, nanovesicles carrying ACE2 protein to compete with the host cells for attachment to SARS-CoV-2 have been promising (Rao et al. 2019, 2020a).

2.5 Nanotechnology in Drug Delivery Targeting Infectious Diseases

Available drug delivery systems using nanotechnology include liposomes, micelles, nanostructured lipid carriers (NLCs), polymeric NPs, solid lipid NPs (SLNs), nanocapsules, and nanotubes. Others are dendrimers, nanogels, quantum dots, emulsions, vesicles, and cyclodextrin-based systems (Fig. 2.1). They have all shown antimicrobial potentials as they have been utilized to increase drug bioavailability and facilitate drug delivery of most antimicrobial agents (Zhang et al. 2015, 2018a, b, 2020; Kupferschmidt and Cohen 2020). In severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), nanovesicles have been successfully employed for membrane-coated nanoparticles. This has been used in neutralizing cytokines (Zhang et al. 2018a, b; Rao et al. 2020a, b). They offer the advantage of slow drug release and delivery to the target cells. In addition, engineered liposomes, nanosponges, and exosomes have shown antibacterial potential (Hu et al. 2013; Keller et al. 2020).

Generally, nanocarriers that can deliver drugs for a prolonged period have been the subject of intense research. Some nanocarriers control drug release with an excipient, while some depend on the slow dissolution of poorly soluble drug crystals in interstitial fluid. Encapsulation of drugs in liposomes is an excellent strategy for sustained drug release. The need for many excipients to control drug release is a major setback of most polymeric and liposomal formulations. The development of aqueous dispersions of nano-milled drug crystals could be a crucial means to circumvent this issue (Williams et al. 2015).

There are different routes of drug delivery. Local delivery of drugs across epithelial surfaces holds great promise since the epithelial surfaces are common residents and sites of microbial entry. This approach enhances on-target drug exposure although its utility is limited for invasive infections. Encapsulating drugs in nanocarriers offers the following advantages: (1) Reduction in dosing frequency due to sustained drug delivery, (2) enhance drug release in the presence of certain inducers and provide temporal control on drug exposure, (3) enhance cellular drug

intake, (4) increase in drug efficacy, (5) offer protection to liable drugs during adverse environmental conditions (e.g., presence of certain enzymes or low pH). Dye-loaded PLGA nanoparticles have been effective in the delivery of drugs across the vaginal tract (Cu et al. 2011) despite several barriers that impede drug delivery across the vaginal tract (Leal et al. 2017). The administration of nanoparticles embedded in films is another crucial tactic to ensure drug retention administered via the vaginal tract (Cunha-Reis et al. 2016).

Pulmonary delivery is an alternative but attractive route since it enables greater on-target drug exposure. One major setback with this approach is that drug formulations that cannot pass through the mucous and extracellular matrix (ECM) layers are often associated with low pulmonary bioavailability due to rapid deactivation by enzymes, drug sequestration, and elimination by coughing (Leal et al. 2017). In comparison with oral or intravenous administration, pulmonary administration has greater local drug bioavailability, as seen in some studies (Vaughn et al. 2006; Alvarez et al. 2007). Topical drug delivery is another approach for drug delivery although it shares therapeutic targets with pulmonary delivery. Compared with unloaded nanoparticles and the control group, nitric oxide releasing nanoparticles could decrease overall bacterial infection in murine skin infection models of *S. aureus* and MRSA biofilms (Martinez et al. 2009). Over the years, appreciable efforts have been made in engineering a system that allows targeted drug delivery to infection sites. This becomes necessary due to low drug tissue penetration (Sarathy et al. 2016), distribution of drugs into toxicity sites (Huth et al. 2011), and elimination of the normal microbiota (Tedijanto et al. 2018). Targeted drug delivery has been made possible through three ways: (1) absorption of the serum protein on the nanocarriers surface on systemic injection, and subsequent phagocytosis by macrophages (Löbenberg et al. 1998), (2) increase vascular permeability at the infected sites—this is due to the activation of kallikrein—kinin pathways and inflammation (Siegel et al. 2017), (3) coating nanocarriers with ligands that bind to infected tissues (Azade et al. 2014; Chono et al. 2008).

It is important to emphasize that during nanocarriers circulation, drug molecules released are subject to non-selective distribution. Thus, it is very important to design a system that prevents the scenario whereby no drug is released in the systemic circulation, and suddenly all the content is released on pathogen encountering. A successful attempt was made by Xiong et al. (2012) in developing such a system. Not minding the efforts so far, it is important to emphasize that host enzymes may act to trigger drug release. Thus, the specificity of drug release in most cases is still elusive. Moreover, it is difficult to avoid the release of drugs at non-target sites. This is partly because of the lack of specificity in nanocarriers' biodistribution that typically precedes triggering. Not minding the route of administration, it is important to properly understand if all the drug targeting approaches are applicable if the nanocarriers are administered through the clinically relevant routes.

2.6 Nanomaterials Vaccines for Infectious Diseases

Several benefits of nanotechnology can be leveraged to increase the therapeutic potentials of formulated vaccines (Zhou et al. 2020). For appropriate immune stimulation, it is important to co-deliver antigenic materials with an immunostimulatory adjuvant. The adjuvant can be incorporated into the core of the nanoparticle (Darrah et al. 2020). It can also be functionalized into the surface of the nanomaterial. However, the nanomaterial itself can act as a stimulus (Lienhardt et al. 2016).

The nanomaterials-based vaccine may help improve vaccines targeting multiple epitopes by providing biomaterials whose design can be systematically optimized. It can also offer different pathways for engagement in cellular and humoral immunity (Fries et al. 2021). However, despite the recent advances in nanomedicine, several critical challenges persist in developing effective nanovaccines against certain infectious diseases: (1) Ability of pathogens to undergo mutation, (2) Host-microbiota regulatory influence (Hagan et al. 2019), (3) Decrease in antibody response with time (Iwasaki and Yang 2020) and antibody-dependent enhancement of several infections, (4) Production of suboptimal antibodies which only neutralize a small fraction of different microbial strains (Xu et al. 2018), (5) Inability to trigger adequate somatic hypermutation in antibody-producing B cells, (6) Inadequate T-cell response, (7) Safety measures remain an issue.

For the development of vaccines, nanomaterials that can carry several antigens are of high interest. Besides, they can act as adjuvants needed to fine-tune the immune system and elicit the required immune responses against several infectious agents. Protein-based nanoparticles have been employed in designing several vaccines effective in treating most infectious diseases (Babapoor et al. 2011; Kaba et al. 2012). However, non-protein-based vaccine utilizing polymeric nanomaterials such as chitosan, polyesters (PLGA, polyamides like gamma polyglutamic acid (γ-PGA)) is becoming appealing to the most researcher. PLGA has been employed to encapsulate and co-deliver several protein antigens and adjuvants in a prolonged fashion (Rostami et al. 2017). The abovementioned approaches are essential to engineering vaccines that can adequately shape the immunological response far above the response triggered during natural infection.

Polymeric nanoparticles (γ-PGA, chitosan, dendrimers) have shown promise to deliver DNA vaccines adequately. In this approach, the delivered genetic material increased the immunogenicity and half-life. It induces the expression of antigens in the cell, causes an increase in immunological response at mucosal sites, and presents greater resistance to several infectious challenges (Feng et al. 2013; Chahal et al. 2016). Delivery systems utilizing liposomes, just like polymeric nanocarriers, allow for the combination and co-delivery of several antigens and adjuvants. In addition, it allows antigens to be displayed on the membrane surface.

Interestingly, these approaches allow the adjustment of several parameters like charge, size, and number of antigen copies. It also enables the adjustment of several other physical factors that might influence trafficking and the triggered immune responses. These properties are highly beneficial to infectious diseases (e.g., malaria,

HIV, and tuberculosis) whose immune protective mechanisms have not been adequately elucidated. Liposomes have been utilized in designing several vaccines (Pauthner et al. 2017; Van Der Meeren et al. 2018). In a study by Ingale et al. (2016), Env trimers formed an array on liposomes. The formation of the array leads to the activation of B cells and the production of neutralizing antibodies against HIV strains. Liposomal nanomaterials can also incorporate multiple antigens (Huang et al. 2018). It also offers the benefit to tune and study vesicle stability effects (Tokatlian et al. 2018).

In summary, there has been an increase in the number of nanomaterials with potentials as new vaccines over the years. Based on several emerging evidence, they might offer protection against several infectious agents. However, specific questions and issues are still elusive. How the different nanomaterials, both in single, in combination with different adjuvants, proteins, and antibodies, can be efficiently sorted out remains elusive. A multifactorial experimental design may be necessary to sort through the different delivery considerations and dosing regimens efficiently. Recent advances in antigen design and engineering of nanomaterials have raised the hope of possible vaccine formulation against the more critical diseases like HIV, tuberculosis, and even malaria. However, the non-specific localization of the antigen within lymph node compartments persists as a critical issue. Moreover, monitoring lymph node response against infectious diseases coupled with other issues still remains challenging. The readers are directed to other chapters of this book discussing the potentials of utilizing nanotechnology in vaccine formulation against infectious diseases.

2.7 Nanomaterials in Photothermal Therapy Against Infectious Pathogens

Photothermal antibacterial (PTA) and photodynamic antibacterial (PDA) are promising phototherapy against drug-resistant pathogens. In PDA, the photosensitizers generate toxic ROS (singlet oxygen, hydroxyl radicals, and superoxides) to kill pathogens upon exposure to light of a specific wavelength (Feng et al. 2018). Antimicrobial photodynamic therapy has been applied in the treatment of several infections, although this approach depends on light fluence, the PS concentration, and the treatment duration (Ullah et al. 2018). Therefore, antimicrobial light therapy can be used alone or combined with a photosensitizer (PS) for improved therapeutic potential. When PS is excited with light of a specific wavelength, an exciting triple-state is attained. The PS in an excited state can transfer electrons or energy to biomolecules or molecular oxygen. This electron transfer can lead to ROS or singlet oxygen radical's formation, disrupting membrane permeability, enhancing drug penetration, and can cause cell death (Mai et al. 2017; Ullah et al. 2018; Yu et al. 2020).

Several efforts have been made to improve the therapeutic potential of PSs in recent years. For improvement of the PS delivery, the use of co-administered nanoparticles to enable the PS to gain access across the membrane or for a

synergistic ROS response leading to antimicrobial activity is crucial. However, one major limitation of photosensitizers is that they are prone to aggregation-induced reduction inefficiency. Generally, for PDT, the generation of ROS may stop after light irradiation is halted. This will allow the re-growth of un-killed bacteria. However, some researchers have made attempts to enhance the availability of PS by functionalizing PS with other molecules (e.g., efflux pump inhibitors, galactose, amino acids, EDTA, potassium iodide, etc.) (Wozniak and Grinholc 2018; Hu et al. 2018).

Zhang et al. (2019) complexed nano-graphene oxide (NGO)-based near-infrared (NIR) photothermal antimicrobial agent with biocompatible bovine serum albumin (BSA) nanoparticles and aggregation-induced emission fluorogen (AIEgen) having daylight-induced ROS generating features for dual-mode photothermal and photodynamic synergistic therapy against amoxicillin (AMO)-resistant *E. coli* and *S. aureus*. NGO-BSA-AIE showed >99% antibacterial activity against amoxicillin-resistant *E. coli* and *S. aureus* when daylight and NIR laser were co-irradiated. NGO-BSA-AIE also showed excellent firmness and amenable biocompatibility. Moreover, there was the generation of ROS and the display of fluorescence images for bacterial tracking by AIEgen under daylight irradiation. Thus, phototherapy can be switched on/off by external light irradiation to destroy bacteria (Tan et al. 2016; Yuan et al. 2018).

Over the years, photothermal materials that can generate heat when irradiated by external light has received considerable attention (Gao et al. 2018). This ability enhances their bacteriostatic potentials. A Thermo-Responsive-Inspired Drug-Delivery Nano-Transporter (TRIDENT) was able to eradicate bacteria, according to an investigation by Qing et al. (2019). The efficient antibacterial activity of the nanostructure was linked to the integrated fluorescence monitoring and synergistic chemo-photothermal killing. It was evident that the rise in temperature produced by NIR melts the nano transporter through a mechanism involving a phase change. There was also reversible destruction of bacterial cell membranes facilitating imipenem permeation. The overall events interfere with the cell wall's biosynthesis, causing the bacteria's abrupt death. Notably, while low doses of imipenem-encapsulated TRIDENT killed MRSA, imipenem alone showed little effect on the same organism. Thus, the MDR bacteria can be proficiently destroyed via a synergistic antibiotic photothermal strategy with a relatively small antibiotic dosage, finally hastening the recuperation of the infected skin (Qing et al. 2019).

Compared with other stimulus-responsive nanostructures, thermo-responsive nanostructures (TRN) are highly suitable for controlled drug release. This is due to their large latent heat of fusion in addition to their ability to reversibly transit from solid to liquid over a small range of temperature (Xue et al. 2018). They are also highly biocompatible. Also, there is no leakage of the encapsulated drugs. It is also easy to incorporate several active molecules into the nano transporter. There is also a quick release of the encapsulated drug when exposed to NIR, in addition to the synergistic antimicrobial activity that involves photothermal therapy.

Carbon nanoparticles-poly-pyrrole nanocomposite (C-PPY), when irradiated with an 808 nm laser, was shown to destroy *P. aeruginosa* (Behzadpour et al.

2019). According to the report, there was a significant decrease in bacterial cell viability. In a study by Jia et al. (2017), graphene-based nanocomposites (GO-IO-CS) irradiated with NIR for 10 min were highly effective in killing *S. aureus*, *E. coli*, and destruction of bacterial biofilm. The super-paramagnetic properties of the GO-IO-CS (chitosan-functionalized magnetic graphene oxide) help improve photothermal sterilization efficiency, thus enhancing bacterial aggregation. It was also recently shown that the NIR laser irradiation of silica-coated gold-silver nanocages (Au-Ag@SiO2 NCs) eliminated *E. faecium*, *S. aureus*, *K. pneumonia*, *A. baumannii*, *P. aeruginosa*, and *Enterobacter* spp. (ESKAPE) pathogens compared to Au-Ag NCs alone (Wu et al. 2019).

Gold nanoclusters coated with DNase (AuNCs-DNase) under 808 nm NIR laser irradiation were able to disrupt the biofilm and kill gram-positive and gram-negative bacteria (Xie et al. 2020). Zhang et al. (2019) combined MoS2 silver nanocomposites with polydopamine (PDA). This MoS2-coated PDA was again coupled with PEG-SH and IgG of anti-protein A. The study provided evidence of antibody-functionalized MoS_2 (MoS2@PDA-PEG/IgG) nanosheets for the targeted photothermal therapy (PTT) of *S. aureus* infection. Pezzi et al. (2019) showed that highly localized targeting with a plasmonic-based heating therapy could be possible by appropriately functionalizing the surface of nanoparticles using covalently linked antibodies. Thus, providing evidence that the use of chemically functionalized and correctly manipulated nanomaterials could offer an easy route for treating bacterial infections.

2.8 Application of Nanodiamonds in Infectious Diseases

Nanodiamond is a new class of nanomaterials in the carbon family with a size smaller than 100 nm. This class of nanoparticle was discovered in 1963 but re-discovered in 1983 (Mironov et al. 2004). However, they were commercialized in the USA in 1988 (Mochalin et al. 2011; Lai and Barnard 2011). Their unique properties (size, shape, versatility, low cytotoxicity, biocompatibility, and manufacturability) make them excellent candidates as therapeutic agents, drug delivery systems (Kuthati et al. 2017), and other biomedical applications (Ansari et al. 2016; Chipaux et al. 2018; Pham et al. 2017). Nanodiamond detonation is widely used in the biomedical field. They can act as immune potentiators, adjuvants, and co-adjuvants. They can stimulate the pro-inflammatory or anti-inflammatory signalling pathways (Zhao et al. 2014a, b) and can be used to deliver the antibodies and enhance the host immune response (Xiang et al. 2006). Nanodiamonds oxidized with strong acid have an outstanding high affinity for proteins. They can easily form stable conjugates in different conditions through physical absorption (Pham et al. 2013). This property is likely due to the intrinsic hydrophobicity of nanodiamonds (Pham et al. 2013). Nanodiamonds are effective vaccine enhancers. In a study by Pham et al. (2017), H7 nanodiamonds vaccines produced a more robust antibody response than trimeric H7. However, one area of concern is the non-specific binding of the nanodiamonds. They can attach to the red blood cell (RBC) and remain in

circulation in the blood without being excited (Chipaux et al. 2018). Moreover, studies have shown that they can accumulate in the lungs, liver, spleen, kidneys, and bone (Tsai et al. 2016), whether they are useful or detrimental is a question yet to be adequately understood.

The intrinsic bactericidal activity of nanodiamonds has been demonstrated (Wehling et al. 2014; Jira et al. 2018). Following exposure of *E. coli* to nanodiamonds in a study by Wehling et al. (2014), there was almost 100% disruption of the cells after only 15 min of exposure. The ingestion of the nanodiamond by *E. coli* causes the disruption and damage of the cell, and an association exists between cell death and oxygen level. In the study by Szunerits et al. (2016), nanodiamonds inhibit the formation of biofilm among *S. aureus*.

Studies are available on the synergistic combination of nanodiamonds with antimicrobial agents such as aflatoxin B1 (Puzyr et al. 2007), vancomycin and tetracycline (Giammarco et al. 2016), polymyxin B (Mochalin et al. 2013). Nanodiamonds conjugated with amoxicillin also demonstrated bactericidal activity against *E. coli*. The antiviral potential of nanodiamonds was also demonstrated by Baron et al. (2016). The result shows that the nanomaterial has the ability to bind to viruses like hepatitis B and C. These nanomaterials can also be conjugated with anti-HIV drug with the potential to reduce the viral load.

However, biodistribution and toxicity remain critical issues in the utilization in clinical practice. They can remain in circulation in the blood over a long period. They can also accumulate in the liver and lung tissues, thus limiting their potential (Sangiao et al. 2019). However, studies have shown that their inclusion in microgels can remove any unspecific reaction.

2.9 Synergistic Antimicrobial Potentials of Nanomaterials/Nanocomposite in Combination with Other Antimicrobial Agents Against Infectious Diseases

When nanomaterials are conjugated or coated with other antimicrobial agents, their activities are usually enhanced. This strategy could be important in eliminating microbes resistant to antibiotics. The synergistic combination is essential because if a microbe is resistant to one antimicrobial agent, the other agent could facilitate its elimination. Moreover, nanomaterial could enhance antibiotic access to microbial cell walls acting as a carrier of the antibiotics, enabling it to bypass the microbial defence mechanisms. On the other hand, the antibiotic can enhance the cell wall penetration of the nanomaterial. Thus, to increase the chances of overcoming drug resistance mechanisms, different antimicrobials can be packaged within the same nanoparticle instead of using only one drug. Schiffelers et al. (2001) evaluated the efficacy of gentamicin and ceftazidime in combination and as single agents when formulated as free-drug solutions and as liposomes.

Using rat unilateral lung infection model with drug-resistant and drug-sensitive *K. pneumonia*, it was evident that the system was able to treat drug-resistant

infection at a low dose. In addition, there was also a dramatic reduction in the number of administration events needed to generate the desired efficacy.

A very recent investigation demonstrated that AuNps conjugated with ampicillin/amoxicillin exhibit an appreciable antimicrobial activity (Mohamed 2020). Also, AgO_2Nps conjugated with ceftriaxone showed significant synergistic activity against *E. coli* (Sajjad et al. 2019).

Also, a synergistic combination of two or three different nanomaterials is another efficient, viable way of utilizing nanomaterials in the treatment of infections. For example, it was demonstrated by Cobos et al. (2020) that AgNps coated with graphene oxide nanocomposites was able to disrupt microbial cells. In addition, Ag-Au/ZnO nanostructure synthesized from *Justicia adhatoda* plant extract showed good antimicrobial activity against *E. coli* and *S. aureus* (Pandiyan et al. 2019). Other studies are also available reporting the successful synergistic combination of nanoparticles with other nanomaterials for tackling microbial cells (Bankier et al. 2019; Abo-Shama et al. 2020; Saravanakumar et al. 2020).

2.10 Challenges and Future Perspectives

Nanotherapeutic approaches targeting physiochemical and biopharmaceutical features of pathogenic microbes will solve most treatment issues against infectious diseases. The low bioavailability of some drugs encountered in most therapeutic interventions could be ameliorated using nanotechnology (Bhardwaj and Tiwari 2020; Hosny and Sherif 2020). In addition, nanomaterials can penetrate the cell barriers due to their small size (Lembo and Cavalli 2020)—this gives them the ability to be utilized as drug delivery agents. Also, their large surface areas are another reason for their high therapeutic efficiency. However, several challenges need to be overcome before nanotechnology will be fully integrated into medicines.

The inability of nanomaterial to penetrate the mucous barriers and the ability to be degraded in the gut are some of the issues (Li et al. 2010). The interaction of the nanomaterials with biological fluids is also an issue (Sanvicens and Marco 2008). Thus, nanoparticles interaction with biological fluids is an area demanding urgent attention. In addition, despite the progress made so far in nanotechnology, safety concern remains a lingering issue. Cytotoxicity and undesirable side effects raise a lot of questions regarding their use as agents against infectious diseases. Also, the inability of the renal system to clear the nanomaterial can lead to cytotoxicity (Ochekpe et al. 2009). It is important to note that the design of nanoparticles, method of synthesis, and the reducing and capping agent used during the synthesis greatly influence the toxicity of nanomaterials (Chen et al. 2009). In vivo and in vitro studies of these nanomaterials will help to understand their utility in the biological system fully.

Therefore, the need for a thorough assessment of the safety of these products and even devices utilizing nanotechnology cannot be overemphasized. Thus, an understanding of the biodistribution and pharmacokinetics of nanomaterials are needed. In addition, the response of the host cells to nanomaterials needs to be thoroughly

evaluated. The establishment of a validated methodology for assessing the exposure to nanomaterials and detection of potentials hazards remains an area demanding more attention. The unavailability of standard experimental procedures has led to differences in results relating to the toxicity of nanomaterials (Tao 2018). Variations in experimental techniques, routes of drug administration, and the administered dose are pending issues requiring a regional and regulatory body, as Mba and Nweze (2021) suggested.

Therefore, validated guidelines and regulatory standards need to be established before the field of nanomedicine will be completely embraced and integrated into all therapeutic interventions. In addition, it will make it possible for nanotechnology to become an integral approach in the quest for a solution against several infectious pathogens, especially those of urgent priority and in response to possible outbreaks of diseases in the future. However, the search for biomaterials that can increase nanoparticles' efficacy without increasing the nanomaterial concentration should be intensified. Optimization, scale-up processes considering good manufacturing practices are crucial while also considering the cost of production. Furthermore, the size of the nanoparticle greatly influences its biodistribution and antimicrobial activity. Thus, the design of nanomaterials should focus on synthesizing particles with a size less than 200 nm.

Also, to improve the therapeutic potential of the already available conventional drugs, these drugs can be impregnated with nanoparticles. Moreover, nanoparticles can be combined with antibodies and other functional biomaterials to improve therapeutic outcomes and reduce the issues of resistance, which is an already public health burden. The combination can also help to reduce drug dosage, thus reducing cytotoxicity and increasing stability and bioavailability. Also, the combination will make it possible to use nanomaterials as adjuvants to already available biomaterials. Therefore, nanoparticle design that can support synthesizing nanoparticles that can conjugate effectively at a low dose with other biomaterials will be highly appreciated in nanomedicine.

Furthermore, more insights are still needed regarding nanomaterials' antimicrobial mechanisms as the mechanisms of action of different nanotechnology approaches remain to be completely elucidated. Sadly, despite the progress made so far in nanomedicine, reports are emerging of microbes developing resistance to nanomaterials (Wang et al. 2018; Zhang et al. 2018a, b). Therefore, attention should be adequately given to this area to avoid microbial resistance common in the use of conventional drugs. More studies focusing on the preclinical and clinical trials of nanomaterials against different infectious pathogens are also urgently needed. Table 2.1 summarizes different preclinical and clinical nanotechnologies for the treatment of several infectious diseases.

Table 2.1 Few selected studies on preclinical and clinical nanotechnologies for treating infectious diseases

Infectious pathogen	Nanomaterial/nanotechnology	Drug	Type of test	Features	Reference
SARS-CoV-2	Liposome	Lactoferrin	–	Pulmonary targeted Inhibit ROS generation Decrease viral replication	NCT04475120
SARS-CoV-2	Inhaled nanoparticle	Remdesivir	–	Accessible administration Lower systemic toxicity Pulmonary targeted	NCT04480333
HIV	PLGA	Bictegravir	In vivo	Prolonged intracellular retention Lower toxicity Long acting	Mandal et al. (2019)
HIV	PLGA	Tenofovir alafenamide, Elvitegravir, Emtricitabine	In vivo	Slow and sustained drug release Decrease viral load Limited system clearance	Mandal et al. (2018)
HIV	Injectable nanoparticles	Rilpivirine	In vivo	Reduce dosing frequency	Phase III NCT02951052
Tuberculosis	Solid lipid nanoparticles (SLNs) and nanostructured lipid carrier (NLCs)	Rifampicin, Isoniazid	In vivo and in vivo	Improved stability Prolonged encapsulation and release Good bioavailability	Banerjee et al. (2018)
Malaria	Injectable nanoparticles	Artemether, Lumefantrine	In vivo (rodents)	Good bioavailability Reduce dosing frequency	Parashar et al. (2016)

(continued)

Table 2.1 (continued)

Infectious pathogen	Nanomaterial/nanotechnology	Drug	Type of test	Features	Reference
C. albicans	Solid lipid nanoparticles (SLNs) and nanostructured lipid carrier (NLCs) Polymeric nanoparticles	Farnesol/Miconazole	In vitro and in vivo (mice)	Enhance solubility Prolonged release Reduced toxicity	Jansook et al. (2018) Fernandes et al. (2019)

2.11 Conclusion

It is becoming evident that in no distance time, nanotechnology will innovate the field of medicine by improving available therapies and or introducing new therapies with a general outcome of significant improvement in clinical care. However, efforts should be made to address all the challenges mentioned earlier. Research should be intensified to increase the efficacy of different combinations and engineer nanomaterials that can prevent, control, and treat several infectious diseases, especially those of high epidemiological relevance. Furthermore, toxicity, bioavailability, and stability of nanomaterials on humans should be given more attention. Therefore, more studies on cytotoxicity and biocompatibility are needed to fully translate and integrate nanotechnology into clinical practice. Importantly, the place of multidisciplinary research-driven efforts becomes indispensable to achieve this goal.

References

Abo-Shama UH, El-Gendy H, Mousa WS, Hamouda RA, Yousuf WE, Hetta HF, Abdeen EE (2020) Synergistic and antagonistic effects of metal nanoparticles in combination with antibiotics against some reference strains of pathogenic microorganisms. Infect Drug Resist 13:351–362

Alvarez CA et al (2007) Aerosolized nanostructured itraconazole as prophylaxis against invasive pulmonary aspergillosis. J Infect 55:68–74

Ansari SA, Satar R, Jafri MA, Rasool M, Ahmad W, Kashif Zaidi S (2016) Role of nanodiamonds in drug delivery and stem cell therapy. Iran J Biotechnol 14:130–141

Ansari MA, Murali M, Prasad D, Alzohairy MA, Almatroudi A, Almomary MN et al (2020) *Cinnamomum verum* bark extract mediated green synthesis of ZnO nanoparticles and their antibacterial potentiality. Biomolecules 10:336

Anselmo AC, Mitragotri S (2016) Nanoparticles in the clinic. Bioeng Transl Med 1(1):10–29

Ashrafi M, Bayat M, Mortazavi P, Hashemi SJ, Meimandipour A (2020) Antimicrobial effect of chitosan-silver-copper nanocomposite on *Candida albicans*. J Nanostruct Chem 10:87–95

Azade AK, Rajaram MVS, Schlesinger LS (2014) Exploitation of the macrophage mannose receptor (CD206) in infectious disease diagnostics and therapeutics. J Cytol Mol Biol 1: 1000003

Babapoor S et al (2011) A novel vaccine using nanoparticle platform to present immunogenic M2e against avian influenza infection. Influenza Res Treat 2011:1–12

Banerjee I, Douaisi MP, Mondal D, Kane RS (2012) Light-activated nanotube porphyrin conjugates as effective antiviral agents. Nanotechnology 23(10):105101

Banerjee S, Roy S, Bhaumik KN, Kshetrapal P, Pillal J (2018) Comparative study of oral lipid nanoparticle formulations (LNFs) for chemical stabilization of antitubercular drugs: physicochemical and cellular evaluation. Artif Cells Nanomed Biotechnol 46(1):540–558

Bankier C, Matharu RK, Cheong YK, Ren GG, Cloutman-Green E, Ciric L (2019) Synergistic antibacterial effects of metallic nanoparticle combinations. Sci Rep 9:16074

Baron AV, Osipov NV, Yashchenko SV, Kokotukha YA, Baron IJ, Puzyr AP, Olkhovskiy IA et al (2016) Adsorption of viral particles from the blood plasma of patients with viral hepatitis on nanodiamonds. Dokl Biochem Biophys 469(2):244–246

Broglie JJ, Alston B, Yang C, Ma L, Adcock AF, Chen W et al (2015) Antiviral activity of gold/copper sulphide core/shell nanoparticles against human norovirus virus-like particles. PLoS One 10(10):e0141050

Behzadpour N, Sattarahmady N, Akbari N (2019) Antimicrobial photothermal treatment of Pseudomonas aeruginosa by a carbon nanoparticles-polypyrrole nanocomposite. J Biomed Phys Eng 9(6):661–672

Bhardwaj S, Tiwari A (2020) Highlights on evidence-based treatment strategies for COVID-19: a review. Lett Appl NanoBiosci 3:1359–1371

Bicanic TA (2014) Systemic fungal infections. Medicine 42:26–30

Bonjardim CA (2017) Viral exploitation of the MEK/ERK pathway - a tale of vaccinia virus and other viruses. Virology 507:267–275

Brunet K, Alanio A, Lortholary O, Rammaert B (2018) Reactivation of dormant/latent fungal infection. J Infect 77:463–468

Carbone C, Fuochi V, Zielinska A, Musumeci T, Souto EB, Bonaccorso A, Pugalia C, Petronio GP, Furneri PM (2020) Dual drugs delivery in solid nanoparticles for the treatment of *Candida albicans* mycosis. Colloids Surf B Biointerfaces 186:110705

Carneiro C, Correia A, Lima T, Vilanova M, Pais C, Gomes AC et al (2016) Protective effect of antigen delivery using monoolein-based liposomes in experimental hematogenously disseminated candidiasis. Acta Biomater 39:133–145

Cassone A, Casadevall A (2012) Recent progress in vaccines against fungal diseases. Curr Opin Microbiol 15:427–433

CDC (2019) Antimicrobial resistance threats in the United States, 2019. U.S. Department of Health and Human Services, CDC, Atlanta, GA. https://doi.org/10.15620/cdc:82532

Chahal JS et al (2016) Dendrimer-RNA nanoparticles generate protective immunity against lethal Ebola, H1N1 influenza, and Toxoplasma gondii challenges with a single dose. Proc Natl Acad Sci U S A 113:E4133–E4142

Chen Y-S, Hung Y-C, Liau I, Huang GS (2009) Assessment of the in vivo toxicity of gold nanoparticles. Nanoscale Res Lett 4:858

Chipaux M, van der Laan K, Hemelaar SR, Hasani M, Zheng T, Schirhagl R (2018) Nanodiamonds and their applications in cells. Small 14(24):1704263

Chono S, Tanino T, Seki T, Morimoto K (2008) Efficient drug targeting to rat alveolar macrophages by pulmonary administration of ciprofloxacin incorporated into mannosylated liposomes for treatment of respiratory intracellular parasitic infections. J Control Release 127:50–58

Cobos M, De-La-Pinta I, Quindos G, Fernandez MJ, Fernendez MD (2020) Graphene oxide-silver nanoparticle nanohybrids: synthesis, characterization and antimicrobial properties. Nanomaterial 10:376

Cu Y, Booth CJ, Saltzman WM (2011) In vivo distribution of surface-modified PLGA nanoparticles following intravaginal delivery. J Control Release 156:258–264

Cunha-Reis C et al (2016) Nanoparticles-in-film for the combined vaginal delivery of anti-HIV microbicide drugs. J Control Release 243:43–53

Darrah PA et al (2020) Prevention of tuberculosis in macaques after intravenous BCG immunization. Nature 577:95–102

Domingues Bianchin M, Borowicz SM, da Rosa Monte Machado G, Pippi B, Stanisçuaski Guterres S, Raffin Pohlmann A et al (2019) Lipid core nanoparticles as a broad strategy to reverse fluconazole resistance in multiple *Candida* species. Colloids Surf B Biointerfaces 175: 523–529

Dong X, Moyer MM, Yang F, Sun YP, Yang L (2017) Carbon Dots' antiviral functions against Noroviruses. Sci Rep 7(1):519

Du T, Lu J, Liu L, Dong N, Fang L, Xiao S, Han H (2018) Antiviral activity of graphene oxide-silver nanocomposites by preventing viral entry and activation of the antiviral innate immune response. ACS Appl Biomater 1(5):1286–1293

Estevez H, Palacios A, Gil D, Anguita J, Vallet-Regi M, González B, Prados-Rosales R, Luque-Garcia JL (2020) Antimycobacterial effect of selenium nanoparticles on Mycobacterium tuberculosis. Front Microbiol 11:800

Feng G et al (2013) Enhanced immune response and protective effects of nano-chitosan-based DNA vaccine encoding T cell epitopes of Esat-6 and FL against *Mycobacterium tuberculosis* infection. PLoS One 8:e61135

Feng Z, Liu X, Tan L, Cui Z, Yang X, Li Z, Zheng Y, Yeung KWK, Wu S (2018) Electrophoretic deposited stable chitosan@MoS2 coating with rapid in situ bacteria-killing ability under dual-light irradiation. Small 14:e1704347

Fernandes Costa A, Evangelista Araujo D, Santos Cabral M, Teles Brito I, de Menezes B, Leite L, Pereira M et al (2019) Development, characterization, and in vitro-in vivo evaluation of polymeric nanoparticles containing miconazole and farnesol for treatment of vulvovaginal candidiasis. Med Mycol 57:52–62

Fleming D, Rumbaugh K (2018) The consequences of biofilm dispersal on the host. Sci Rep 8: 10738

Fries CN, Curvino EJ, Chen J-L, Permar SR, Fouda GG, Collier JH (2021) Advances in nanomaterial vaccine strategies to address infectious diseases impacting global health. Nat Nanotechnol 16:385–398

Gaikward S, Ingle A, Gade A, Rai M, Falanga A, Incoronato N et al (2013) Antiviral activity of mycosynthesized silver nanoparticles against herpes simplex virus and human parainfluenza virus type 3. Int J Nanomedicine 8:4303–4314

Gao DY, Ji X, Wang JL, Wang YT, Li DL, Liu YB, Chang KW, Qu JL, Zheng J, Yuan Z (2018) Engineering a protein-based nanoplatform as an antibacterial agent for light activated dual-modal photothermal and photodynamic therapy of infection in both the NIR I and II windows. J Mater Chem B 6:732–739

Ghaffari H (2019) Inhibition of H1N1 influenza virus infection by zinc oxide nanoparticles: another emerging application of nanomedicine. J Biomed Sci 26:70

Giammarco J, Mochalin VN, Haeckel J, Gogotsi Y (2016) The adsorption of tetracycline and vancomycin onto nanodiamond with controlled release. J Colloid Interface Sci 468:253–261

Golipour F, Habibipour R, Moradihaghgou L (2019) Investigating effects of superparamagnetic iron oxide nanoparticles on *Candida albicans* biofilm formation. Med Lab J 13(6):44–50

Gondim BLC, Castellano LRC, de Castro RD, Machado G, Carlo HL, Valença AMG et al (2018) Effect of chitosan nanoparticles on the inhibition of Candida spp. biofilm on denture base surface. Arch Oral Biol 94:99–107

Hagan T et al (2019) Antibiotics-driven gut microbiome perturbation alters immunity to vaccines in humans. Cell 178:1313–1328.e1313

Hosny NM, Sherif Y (2020) Molecular docking study on some isonicotinoyl hydrazide derivatives as potential inhibitors of COVID-19. Lett Appl NanoBiosci 3:1217–1224

Hu CMJ, Fang RH, Copp J et al (2013) A biomimetic nanosponge that absorbs pore-forming toxins. Nat Nanotechnol 8(5):336–340

Hu X, Huang YY, Wang Y, Wang X, Hamblin MR (2018) Antimicrobial photodynamic therapy to control clinically relevant biofilm infections. Front Microbiol 9:1–24

Huang WC et al (2018) A malaria vaccine adjuvant based on recombinant antigen binding to liposomes. Nat Nanotechnol 13:1174–1181

Huq MA (2020) Green synthesis of silver nanoparticles using *Pseudoduganella eburnean* MAHUQ-39 and their antimicrobial mechanisms investigation against drug resistant human pathogens. Int J Mol Sci 21:1510

Huth ME, Ricci AJ, Cheng AG (2011) Mechanisms of aminoglycoside ototoxicity and targets of hair cell protection. Int J Otolaryngol 2011:937861

Huy TQ, Thanh HNT, Thuy NT, Chung PV, Hung PN, Le AT et al (2017) Cytotoxicity and antiviral activity of electrochemical - synthesized silver nanoparticles against poliovirus. J Virol Methods 241:52–57

Ingale J et al (2016) High-density array of well-ordered HIV-1 spikes on synthetic liposomal nanoparticles efficiently activate B cells. Cell Rep 15:1986–1999

Iwasaki A, Yang Y (2020) The potential danger of suboptimal antibody responses in COVID-19. Nat Rev Immunol 20:339–341

Jannuzzi GP, Souza N, Françoso KS, Pereira RH, Santos RP, Kaihami GH et al (2018) Therapeutic treatment with scFv–PLGA nanoparticles decreases pulmonary fungal load in a murine model of paracoccidioidomycosis. Microbes Infect 20:48–56

Jansook P, Pichayakorn W, Ritthidej GC (2018) Amphotericin B-loaded solid lipid nanoparticles (SLNs) and nanostructured lipid carrier (NLCs): effect of drug loading and biopharmaceutical characterizations. Drug Dev Ind Pharm 44:1693–1700

Jia X, Rong IA, Yang R, Wang C (2017) Versatile graphene-based photothermal nanocomposites for effectively capturing and killing bacteria, and for destroying bacterial biofilms. J Mater Chem B 5(13):2459–2467

Jira J, Rezek B, Kriha V, Artemenko A, Matolínová I, Skakalova V, Stenclova P, Kromka A (2018) Inhibition of E. coli growth by nanodiamond and graphene oxide enhanced by Luria-Bertani medium. Nanomaterials 8:140

Kaba SA et al (2012) Protective antibody and CD8+ T-cell responses to the Plasmodium falciparum circumsporozoite protein induced by a nanoparticle vaccine. PLoS One 7:e48304

Keller MD, Ching KL, Liang FX et al (2020) Decoy exosomes provide protection against bacterial toxins. Nature 579(7798):260–264

Khames A, Khaleel MA, El-Badawy MF, El-Nezhawy AOH (2019) Natamycin solid lipid nanoparticles - sustained ocular delivery system of higher corneal penetration against deep fungal keratitis: preparation and optimization. Int J Nanomedicine 14:2515–2531

Khatoon Z, Mc Tiernan CD, Suuronen EJ, Mah T-F, Alarcon EI (2018) Bacterial biofilm formation on implantable devices and approaches to its treatment and prevention. Heliyon 4(12):e01067

Khatoon N, Sharma Y, Sardar M, Manzoor N (2019) Mode of action and anti-*Candida* activity of *Artemisia annua* mediated-synthesized silver nanoparticles. J Mycol Med 29(3):201–209

Kischkel B, Castilho PF, de Oliveira KMP, de Bruschi ML, Svidzinski TIE et al (2020a) Silver nanoparticles stabilized with propolis shows reduced toxicity and potential activity against fungal infections. Future Microbiol 15:521–539

Kischkel B, Rossi SA, Santos Junior SR, Nosanchuk JD, Travassos LR, Taborda CP (2020b) Therapies and vaccines based on nanoparticles for the treatment of systemic fungal infections. Front Cell Infect Microbiol 10:463

Knotigová PT, Zyka D, Mašek J, Kovalová A, Krupka M, Bartheldyová E et al (2015) Molecular adjuvants based on nonpyrogenic lipophilic derivatives of norAbuMDP/GMDP formulated in nanoliposomes: stimulation of innate and adaptive immunity. Pharm Res 32:1186–1199

Kupferschmidt K, Cohen J (2020) Race to find COVID-19 treatments accelerates. Science 367(6485):1412–1413

Kuthati Y, Kankala RK, Busa P, Lin SX, Deng JP, Mou CY, Lee CH (2017) Phototherapeutic spectrum expansion through synergistic effect of mesoporous silica trio-nanohybrids against antibiotic-resistant gram-negative bacterium. J Photochem Photobiol B 169:124–133

Lai L, Barnard AS (2011) Modeling the thermostability of surface functionalisation by oxygen, hydroxyl, and water on nanodiamonds. Nanoscale 3:2566–2575

Lara HH, Ayala-Nunez NV, Ixtepan-Turrent L, Rodriguez-Padilla C (2010) Mode of antiviral action of silver nanoparticles against HIV-1. J Nanobiotechnol 8:1

Lea R (2020a) Graphene-based masks launched to combat COVID-19 [Internet]. AZoNano, UK. https://www.azonano.com/news.aspx?newsID=37431. Accessed 27 Mar 2021

Lea R (2020b) The development of a new anti-COVID-19 nanocoating [Internet]. AZoNano, UK. https://www.azonano.com/news.aspx?newsID=37294. Accessed 27 Mar 2021

Leal J, Smyth HDC, Ghosh D (2017) Physicochemical properties of mucus and their impact on transmucosal drug delivery. Int J Pharm 532:555–572

Lembo D, Cavalli R (2020) Nanoparticulate delivery systems for antiviral drugs. Antivir Chem Chemother 21:53–70

Li WR, Xie XB, Shi QS et al (2010) Antibacterial activity and mechanism of silver nanoparticles on Escherichia coli. Appl Microbiol Biotechnol 85(4):1115–1122

Liao S, Zhang Y, Pan X, Zhu F, Jiang G, Liu Q, Cheng Z, Dai G, Wu F, Wang L, Chen L (2019) Antibacterial activity and mechanism of silver nanoparticles against multidrug-resistant *Pseudomonas aeruginosa*. Int J Nanomedicine 14:1469–1487

Lienhardt C et al (2016) Translational research for tuberculosis elimination: priorities, challenges, and actions. PLos Med 13:e1001965

Löbenberg R, Araujo L, von Briesen H, Rodgers E, Kreuter J (1998) Body distribution of azidothymidine bound to hexyl-cyanoacrylate nanoparticles after i.v. injection to rats. J Control Release 50:21–30

Lockhart SR, Guarner J (2019) Emerging and reemerging fungal infections. Semin Diagn Pathol 36(3):177–181

Lu R, Hollingsworth C, Qiu J, Wang A, Hughes E, Xin X et al (2019) Efficacy of oral encochleated amphotericin b in a mouse model of cryptococcal meningoencephalitis. MBio 10:e00724–e00719. https://doi.org/10.1128/mBio.00724-19

Ludwig DB, de Camargo LEA, Khalil NM, Auler ME, Mainardes RM (2018) Antifungal activity of chitosan-coated poly(lactic-co-glycolic) acid nanoparticles containing Amphotericin B. Mycopathologia 183:659–668

Luther DC, Huang R, Jeon T, Zhang X, Lee YW, Nagaraj H et al (2020) Delivery of drugs, proteins, and nucleic acids using inorganic nanoparticles. Adv Drug Deliv Rev. https://doi.org/10.1016/j.addr.2020.06.020

Mai B, Gao Y, Li M, Wang X, Zhang K, Liu Q et al (2017) Photodynamic antimicrobial chemotherapy for Staphylococcus aureus and multidrug-resistant bacterial burn infection in vitro and in vivo. Int J Nanomedicine 12:5915–5931. https://doi.org/10.2147/IJN.S138185

Maliszewska I, Lisiak B, Popko K, Matczyszyn K (2017) Enhancement of the efficacy of photodynamic inactivation of Candida albicans with the use of biogenic gold nanoparticles. Photochem Photobiol 93:1081–1090

Mandal S, Kang G, Prathipati PK, Fan W, Li Q, Destache CJ (2018) Long-acting parenteral combination antiretroviral loaded nanodrug delivery system to treat chronic HIV-1 infection: a humanized mouse model study. Antivir Res 156:85–91

Mandal S, Prathipati PK, Belshan M, Destache CJ (2019) A potential long-acting bictegravir loaded nano-drug delivery system for HIV-1 infection: a proof-of-concept study. Antivir Res 167:83–88

Martinez LR et al (2009) Antimicrobial and healing efficacy of sustained release nitric oxide nanoparticles against *Staphylococcus aureus* skin infection. J Invest Dermatol 129:2463–2469

Mba IE, Nweze EI (2020) The use of nanoparticles as alternative therapeutic agents against *Candida* infections: an up-to-date overview and future perspectives. World J Microbiol Biotechnol 36:163

Mba IE, Nweze EI (2021) Nanoparticles as therapeutic options for treating multidrug-resistant bacteria: research progress, challenges, and prospects. World J Microbiol Biotechnol 37:108

Mba IE, Sharndama HC, Osondu-Chuka GO, Okeke OP (2021) Immunobiology and nanotherapeutics of severe acute respiratory syndrome 2 (SARS-CoV-2): a current update. Infect Dis. https://doi.org/10.1080/23744235.2021.1916071

Mironov VA, Sergienko OV, Nastasiak IN, Danilenko VN (2004) Biogenesis and regulation of biosynthesis of erythromycins in Saccharopolyspora erythraea: a review. Prikl Biokhim Mikrobiol 40:613–624

Mochalin VN, Shenderova O, Ho D, Gogotsi Y (2011) The properties and applications of nanodiamonds. Nat Nanotechnol 7:11–23

Mochalin VN, Pentecost A, Li XM, Neitzel I, Nelson M, Wei C, He T, Guo F, Gogotsi Y (2013) Adsorption of drugs on nanodiamond: toward development of a drug delivery platform. Mol Pharm 10:3728–3735

Mohamed MA (2020) Myco-engineered gold nanoparticles from *Jahnula aquatica* coated with ampicillin/amoxicillin and their antibacterial and anticancer activity against cancer cells. Biotechnol Lett 42:151–170

Munita JM, Arias CA (2016) Mechanisms of antibiotics resistance. Microbiol Spectr 4:2

Munoz-Escobar A, Reyes-Lopez SY (2020) Antifungal susceptibility of *Candida* species to copper oxide nanoparticles on polycaprolactone fibers (PCL-CuONps). PLoS One 15(2):e0228864

Ochekpe NA, Olorunfemi PO, Ngwuluka NC et al (2009) Nanotechnology and drug delivery part 2: nanostructures for drug delivery. Trop J Pharm Res 8(3):275–287

Pandiyan N, Murugesan B, Arumugam M, Sonamuthu J, Samayanan S, Mahalingam S (2019) Ionic liquid. A greener templating agent with *Justicia adhatida* plant extract assisted green synthesis of morphologically improved Ag-Au/ZnO nanostructures and its antibacterial and anticancer activities. J Photochem Photobiol B Biol 198:111559

Parashar D, Aditya NP, Murthy RSR (2016) Development of artemether and lumefantrine co-loaded nanostructure lipid carriers: physicochemical characterization and in vivo antimalarial activity. Drug Deliv 23(1):123–129

Pauthner M et al (2017) Elicitation of robust tier 2 neutralizing antibody responses in nonhuman primates by HIV envelope trimer immunization using optimized approaches. Immunity 46: 1073–1088.e6

Pezzi L, Pane A, Annesi F, Losso MA, Guglielmelli A, Umeton C, De Sio L (2019) Antimicrobial effects of chemically functionalized and/or photo-heated nanoparticles. Materials 12:1078

Pham MD, Yu SS, Han CC, Chan SI (2013) Improved mass spectrometric analysis of membrane proteins based on rapid and versatile sample preparation on nanodiamond particles. Anal Chem 85:6748–6755

Pham NB, Ho TT, Nguyen GT, Le TT, Le NT, Chang HC, Pham MD, Conrad U, Chu HH (2017) Nanodiamond enhances immune responses in mice against recombinant ha/h7n9 protein. J Nanobiotechnol 15:69

Puzyr AP, Purtov KV, Shenderova OA, Luo M, Brenner DW, Bondar VS (2007) The adsorption of aflatoxin b1 by detonation-synthesis nanodiamonds. Dokl Biochem Biophys 417:299–301

Qing G, Zhao X, Gong N, Chen J, Li X, Gan Y, Wang Y, Zhang Z, Zhang Y, Guo W, Luo Y, Liang X-J (2019) Thermo-responsive triple-function nanotransporter for efficient chemo-photothermal therapy of multidrug-resistant bacterial infection. Nat Commun 10:4336

Ramalingam V, Sundaramahalingam S, Rajaram R (2019) Size dependent antimycobacterial activity of titanium oxide nanoparticles against Mycobacterium tuberculosis. J Mater Chem B 7:4338–4346

Rao L, Wang W, Meng Q-F et al (2019) A biomimetic nanodecoy traps Zika virus to prevent viral infection and fetal microcephaly development. Nano Lett 19(4):2215–2222

Rao L, Tian R, Chen X (2020a) Cell-membrane-mimicking nanodecoys against infectious diseases. ACS Nano 14(3):2569–2574

Rao L, Xia S, Xu W et al (2020b) Decoy nanoparticles protect against COVID-19 by concurrently adsorbing viruses and inflammatory cytokines. Proc Natl Acad Sci U S A 117(44):27141–27147

Rostami H, Ebtekar M, Ardestani MS, Yazdi MH, Mahdavi M (2017) Co-utilization of a TLR5 agonist and nano-formulation of HIV-1 vaccine candidate leads to increased vaccine immunogenicity and decreased immunogenic dose: a preliminary study. Immunol Lett 187:19–26

Roth GA et al (2018) Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980–2017: a systematic analysis for the global burden of disease study 2017. Lancet 392:1736–1788

Sajjad S, Uzair B, Shaukat A, Jamshed M, Leghari SAK, Ismail M, Mansoor Q (2019) Synergistic evaluation of AgO2 nanoparticles with ceftriaxone against CTXM and blaSHV genes positive ESBL producing clinical strains of uropathogenic *E. coli*. IET Nanobiotechnol 13(4):435–440

Sangiao ET, Holban AM, Gestal MC (2019) Applications of nanodiamonds in the detection and therapy of infectious diseases. Materials 12:1639

Sanvicens N, Marco MP (2008) Multifunctional nanoparticles—properties and prospects for their use in human medicine. Trends Biotechnol 26(8):425–433

Sarathy JP et al (2016) Prediction of drug penetration in tuberculosis lesions. ACS Infect Dis 2:552–563

Saravanakumar K, Jeevithan E, Hu X, Chelliah R, Oh D-H, Wang M-H (2020) Enhanced anti-lung carcinoma and anti-biofilm activity of fungal molecules conjugated with β-D-glucan from barley. J Photochem Photobiol B 203:11728

Sarwar S, Ali A, Pal M, Chakrabarti P (2017) Zinc oxide nanoparticles provide anti-cholera activity by disrupting the interaction of cholera toxin with the human GM1 receptor. J Biol Chem 292: 18303–18311

Schiffelers RM et al (2001) In vivo synergistic interaction of liposome-coencapsulated gentamicin and ceftazidime. J Pharmacol Exp Ther 298:369–375

Seifi T, Kamali AR (2021) Antiviral performance of graphene-based materials with emphasis on COVID-19: a review. Med Drug Discov 25:100099

Sherwani MA, Tufail S, Khan AA, Owais M (2015) Gold nanoparticle-photosensitizer conjugate based photodynamic inactivation of biofilm producing cells: potential for treatment of C. albicans infection in BALB/c mice. PLoS One 10:e0131684

Siegel RA, Kirtane AR, Panyam J (2017) Assessing the benefits of drug delivery by nanocarriers: a partico/pharmacokinetic framework. IEEE Trans Biomed Eng 64:2176–2185

Singulani J, Scorzoni L, Lourencetti NMS, Oliveira LR, Conçolaro RS, da Silva PB et al (2018) Potential of the association of dodecyl gallate with nanostructured lipid system as a treatment for paracoccidioidomycosis: in vitro and in vivo efficacy and toxicity. Int J Pharm 547:630–636

Siopi M, Mouton JW, Pournaras S, Meletiadis J (2019) In vitro and in vivo exposure-effect relationship of liposomal amphotericin b against Aspergillus fumigatus. Antimicrob Agents Chemother 63:e02673–e02618

Spadari CC, de Bastiani FWM, Lopes LB, Ishida K (2019) Alginate nanoparticles as non-toxic delivery system for miltefosine in the treatment of candidiasis and cryptococcosis. Int J Nanomedicine 14:5187–5199

Stewart ER, Eldridge ML, McHardy I, Cohen SH, Thompson GR (2018) Liposomal amphotericin B as monotherapy in relapsed coccidioidal meningitis. Mycopathologia 183:619–622

Syngouna VI, Chrysikopoulos CV (2017) Inactivation of MS2 bacteriophage by titanium dioxide nanoparticles in the presence of quartz sand with and without ambient light. J Colloid Interface Sci 497:117–125

Szunerits S, Barras A, Boukherroub R (2016) Antibacterial applications of nanodiamonds. Int J Environ Res Public Health 13:413

Tăbăran A-F, Matea CT, Mocan T, Tăbăran A, Mihaiu M, Iancu C, Mocan L (2020) Silver nanoparticles for the therapy of tuberculosis. Int J Nanomedicine 15:2231–2258

Tan X, Wang J, Pang X, Liu L, Sun Q, You Q, Tan F, Li N (2016) Indocyanine green loaded silver nanoparticle@polyaniline core/shell theranostic nanocomposites for photoacoustic/near-infrared fluorescence imaging-guided and single-light-triggered photothermal and photodynamic therapy. ACS Appl Mater Interfaces 8:34991–35003

Tao C (2018) Antimicrobial activity and toxicity of gold nanoparticles: research, challenges and prospects. Lett Appl Microbiol 67:537–543

Tariq F, Ahmed N, Afzal M, Khan MAU, Zeshan B (2020) Synthesis, characterization and antimicrobial activity of *Bacillus subtilis*-derived silver nanoparticles against multi drug resistant bacteria. Jundishapur J Microbiol 13(5):e91934

Tedijanto C, Olesen SW, Grad YH, Lipsitch M (2018) Estimating the proportion of bystander selection for antibiotic resistance among potentially pathogenic bacterial flora. Proc Natl Acad Sci U S A 115:E11988–E11995

Tokatlian T et al (2018) Enhancing humoral responses against HIV envelope trimers via nanoparticle delivery with stabilized synthetic liposomes. Sci Rep 8:16527

Travassos LR, Taborda CP (2017) Linear epitopes of Paracoccidioides brasiliensis and other fungal agents of human systemic mycoses as vaccine candidates. Front Immunol 8:224

Tsai LW, Lin YC, Perevedentseva E, Lugovtsov A, Priezzhev A, Cheng CL (2016) Nanodiamonds for medical applications: interaction with blood in vitro and in vivo. Int J Mol Sci 17(7):1111

Tyagi PK, Tyagi S, Kumar A et al (2020) Contribution of nanotechnology in the fight against COVID 19. Bio Res Appl Chem 11(1):8233–8241

Ullah A, Zhang Y, Iqbal Z, Zhang Y, Wang D, Chen J et al (2018) Household light source for potent photo-dynamic antimicrobial effect and wound healing in an infective animal model. Biomed Opt Express 9:1006–1019
Van Der Meeren O et al (2018) Phase 2b controlled trial of M72/AS01 EVaccine to prevent tuberculosis. N Engl J Med 379:1621–1634
Vaughn JM et al (2006) Single dose and multiple dose studies of itraconazole nanoparticles. Eur J Pharm Biopharm 63:95–102
Vazquez-Munoz R, Lopez-Ribot JL (2020) Nanotechnology as an alternative to reduce the spread of COVID-19. Challenges 11:15
Vazquez-Munoz R, Meza-Villezcas A, Fournier PGJ, Soria-Castro E, Juarez-Moreno K, Gallego-Hernandez AL et al (2019) Enhancement of antibiotics antimicrobial activity due to the silver nanoparticles impact on the cell membrane. PLoS One 14(11):e0224904
Velthuis AJW, Van-den-Worm SHE, Sims AC et al (2010) Zn2þ inhibits coronavirus and arterivirus RNA polymerase activity in vitro and zinc ionophores block the replication of these viruses in cell culture. PLoS Pathog 6(11):e1001176
Vijayakumar S, Ganesan S (2012) Gold nanoparticles as an HIV entry inhibitor. Curr HIV Res 10(8):643–646
Wang X, Yang F, Zhao J, Xu Y, Mao D, Zhu X, Luo Y, Alvarez P (2018) Bacterial exposure to ZnO nanoparticles facilitates horizontal transfer of antibiotic resistance genes. NanoImpact 10:61–67
Wehling J, Dringen R, Zare RN, Maas M, Rezwan K (2014) Bactericidal activity of partially oxidized nanodiamonds. ACS Nano 8:6475–6483
Wei G, Yang G, Wang Y, Jiang H, Fu Y, Yue G, Ju R (2020) Phototherapy-based combination strategies for bacterial infection treatment. Theranostics 10(26):12241–12262
Wilen CB, Tilton JC, Doms RW (2012) HIV: cell binding and entry. Cold Spring Harb Perspect Med 2(8):a006866
Williams PE, Crauwels HM, Basstanie ED (2015) Formulation and pharmacology of long-acting rilpivirine. Curr Opin HIV AIDS 10:233–238
Wozniak A, Grinholc M (2018) Combined antimicrobial activity of photodynamic inactivation and antimicrobials-state of the art. Front Microbiol 9:930
Wu S, Li A, Zhao X, Zhang C, Yu B, Zhao N, Xu F-J (2019) Silica coated gold-silver nanocages as photothermal antibacterial agents for combined anti-infective therapy. ACS Appl Mater Interfaces 11(19):17177–17183
Xiang SD, Scholzen A, Minigo G, David C, Apostolopoulos V, Mottram PL, Plebanski M (2006) Pathogen recognition and development of particulate vaccines: does size matter? Methods 40:1–9
Xie Y, Zheng W, Jiang X (2020) Near-infrared light-activated phototherapy by gold nanoclusters for dispersing biofilms. ACS Appl Mater Interfaces 12(8):9041–9049
Xiong M-H et al (2012) Bacteria-responsive multifunctional nanogel for targeted antibiotic delivery. Adv Mater 24:6175–6180
Xu K et al (2018) Epitope-based vaccine design yields fusion peptide-directed antibodies that neutralize diverse strains of HIV-1. Nat Med 24:857–867
Xue J, Zhu C, Li J, Li H, Xia Y (2018) Integration of phase-change materials with electrospun fibers for promoting neurite outgrowth under controlled release. Adv Funct Mater 28:1705563
Yang C, Xue B, Song W, Kan B, Zhang D, Yu H et al (2018) Reducing the toxicity of amphotericin B by encapsulation using methoxy poly(ethylene glycol)-b-poly(l-glutamic acid-co-l-phenylalanine). Biomater Sci 6:2189–2196
Yang M, Du K, Hou Y, Xie S, Dong Y, Li D et al (2019) Synergistic antifungal effect of Amphotericin B-loaded poly(lactic-co-glycolic acid) nanoparticles and ultrasound against Candida albicans biofilms. Antimicrob Agents Chemother 63:e02022–e02018. https://doi.org/10.1128/AAC.02022-18
Yu Z, Li Q, Wang J, Yu Y, Wang Y, Zhou Q, Li P (2020) Reactive oxygen species-related nanoparticle toxicity in the biomedical field. Res Lett 15:115

Yuan L, Lei T, Zhenduo C, Xianjin Y, Yufeng Z, Kelvin Y, Kwok W, Paul CK, Wu S (2018) Rapid sterilization and accelerated wound healing using Zn^{2+} and graphene oxide modified g-C_3N_4 under dual light irradiation. Adv Funct Mater 28:1800299

Zhang P, Chen Y, Zeng Y et al (2015) Virus-mimetic nanovesicles as a versatile antigen-delivery system. Proc Natl Acad Sci U S A 112(45):E6129–EE613

Zhang P, Zhang L, Qin Z et al (2018a) Genetically engineered liposome-like nanovesicles as active targeted transport platform. Adv Mater 30:170535

Zhang Y, Gu AZ, Xie S, Li X, Cen T, Li D, Cgen J (2018b) Nanometal oxides induce antimicrobial resistance via radical-mediated mutagenesis. Environ Int 121:1162–1171

Zhang Y, Xiu W, Gan S, Shan J, Ren S, Yuwen L, Weng L, Teng Z, Wang L (2019) Antibody-functionalized mos2 nanosheets for targeted photothermal therapy of Staphylococcus aureus focal infection. Front Bioeng Biotechnol 7:218

Zhang Q, Honko A, Zhou J et al (2020) Cellular nanosponges inhibit SARS-CoV-2 infectivity. Nano Lett 20(7):5570–5574

Zhao L, Seth A, Wibowo N, Zhao C-X, Mitter N, Yu C et al (2014a) Nanoparticle vaccines. Vaccine 32(136):327–337

Zhao L, Xu Y-H, Akasaka T, Abe S, Komatsu N, Watari F, Chen X (2014b) Polyglycerol-coated nanodiamond as a macrophage-evading platform for selective drug delivery in cancer cells. Biomaterials 35:5393–5406

Zhou J, Kroll AV, Holay M, Fang RH, Zhang L (2020) Biomimetic nanotechnology towards personalized vaccines. Adv Mater 32(13):e1901255

3 Understanding the Pharmacology and Pharmacotherapeutics for Infectious Diseases

Nishtha Agrawal, Indu Singh, Madhu Khanna, Gagan Dhawan, Pradeep Kumar, and Uma Dhawan

Abstract

Infectious diseases are caused by living microorganisms such as bacteria, virus, parasite, and fungi that infect millions of people around the globe. These infectious diseases have been responsible for frequent outbreaks, sometimes culminating into epidemic or pandemic, the most recent one being the on-going COVID-19 pandemic caused by SARS-CoV-2. The major challenge posed by these infectious agents is the increasing cases of drug resistance and mutations (mainly in viruses). Another issue is the non-targeted approach of the conventional therapeutic agents which may lead to cytotoxic side-effects, low bioavailability, and the development of drug resistance. Hence, to overcome these

N. Agrawal
Department of Virology (a Unit of Department of Microbiology), Vallabhbhai Patel Chest Institute, University of Delhi, New Delhi, India

Department of Biomedical Science, Acharya Narendra Dev College, University of Delhi, New Delhi, India

I. Singh · G. Dhawan
Department of Biomedical Science, Acharya Narendra Dev College, University of Delhi, New Delhi, India

M. Khanna
Department of Virology (a Unit of Department of Microbiology), Vallabhbhai Patel Chest Institute, University of Delhi, New Delhi, India

P. Kumar
CSIR-Institute of Genomics and Integrative Biology, New Delhi, India

U. Dhawan (✉)
Department of Biomedical Science, Bhaskaracharya College of Applied Sciences, University of Delhi, New Delhi, India
e-mail: uma.dhawan@bcas.du.ac.in

S. Hameed, S. Rehman (eds.), *Nanotechnology for Infectious Diseases*,
https://doi.org/10.1007/978-981-16-9190-4_3

shortcomings a target-based approach has been adopted in drug designing that would target the specific gene or protein involved in pathogenesis of above-mentioned microorganisms. In recent years, nanotechnology has gained great momentum in designing a targeted drug delivery system, wherein the targeted drug molecule is encapsulated in the nano-carrier which can be programmed for sustained drug release and has higher efficacy against the pathogens. Some of the nanoparticle platforms like liposome, dendrimers, hydrogels, metal-based nanoparticles have recently proved their efficacy at the molecular site (like as reticuloendothelial system, macrophages) where native conventional drugs could not penetrate efficiently. The major advantages of using nano-formulations in drug delivery are low toxicity, sustained release of drugs, enhanced drug uptake, etc. The chapter is primarily focused on the use of nanomedicine in pharmacological intervention for improving treatment regimen and strategies against infectious organism and is concluded by discussing the alternative strategy of monoclonal antibody therapy.

Keywords

Emerging infectious diseases (EID) · Pharmacology · Pharmacotherapeutics · Polypharmacology · Nanotherapeutics

3.1 Introduction

The world has constantly been under grave threat of a disease outbreak, sometimes leading into a devastating epidemic or more severe pandemic as witnessed several times during this century. The disease results in an outbreak when there is an unanticipated surge in number of diseased individuals in a particular geographic area. The outbreak results in epidemic if it transmits to a larger area and in pandemic when it spreads across the globe (Piret and Boivin 2021). The world has experienced several outbreaks and epidemics in different parts and more than 18 global pandemics till date attributed to the bacteria *Yersinia pestis*, *Vibrio cholerae*, and viruses SARS-CoV, MERS-CoV, strains of Influenza Virus and the most recent SARS-CoV-2 (Huremović 2019). These spread of infectious diseases often result in high mortality rate and have devastating effect on the world economy and cripple healthcare sector. Some of the factors contributing to the widespread infections are the mutations in the genome resulting in novel virus, development of drug resistance, and lack of specific treatment against a particular pathogen.

3.2 Transmission of Infectious Diseases

The widespread transmission of infectious diseases could be through direct contact (like STDs) or indirect contact (e.g., Malaria) (van Seventer and Hochberg 2017). A carrier is usually an infected asymptomatic individual, who unintentionally facilitate

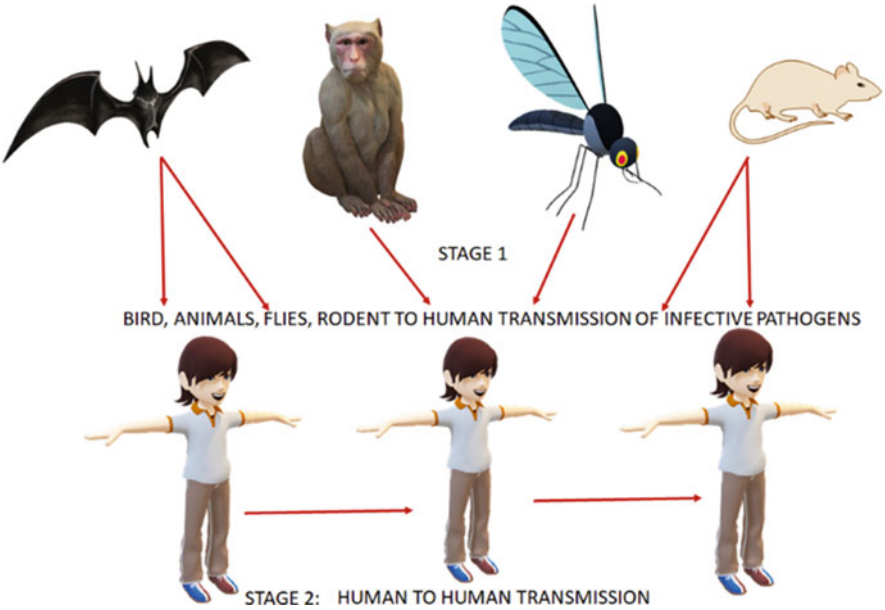

Fig. 3.1 Diagrammatic representation of transmission of infectious pathogens from lower animals to human and human to human

the spread of infection. The direct mode of transmission is mediated by direct physical contact or transfer of bodily fluids between person to person (e.g., STDs, like gonorrhea) or through the spread of infection via droplets generated during coughing, sneezing, or sometimes speaking (e.g., influenza virus, RSV) (Cortez and Weitz 2013) (Fig. 3.1). The indirect mode of transmission is orchestrated through air (e.g., SARS-CoV-2, *M. tuberculosis*), fomite (e.g., *Vibrio cholerae*, *Staphylococcus aureus*, respiratory and enteric viruses), contaminated food or water (*Escherichia coli*, *Clostridium botulinum*), animal reservoirs (Fig. 3.1) commonly known as zoonotic diseases (West Nile Virus (via bird), *Yersinia pestis* (via rodents), *Bacillus anthracis* (via sheep)), insect vectors (e.g., Chikungunya virus (mosquitoes), *Plasmodium falciparum* (mosquitoes), *Leishmania donovani* (sand flies)), etc. (van Seventer and Hochberg 2017).

3.3 Infectious Diseases

3.3.1 Bacterial Diseases

Bacteria are ubiquitously present and are mostly harmless or even beneficial (inside the human gut), however there are various species of bacteria that contribute to the burden of infectious diseases around the world (Doron and Gorbach 2008). Bacterial species can cause gastrointestinal tract discomfort (*Salmonella enteritis*, *Escherichia*

coli) (OnHealth 2021), respiratory ailments (*Streptococcus* species) (Cappelletty 1998), urinary tract infections (*Escherichia coli*, *Enterococcus faecalis*) (Flores-Mireles et al. 2015), sexually transmitted diseases (*Chlamydia trachomatis*, *Neisseria gonorrhoeae*) (OnHealth 2021), or sometimes life threatening diseases like bacterial meningitis (*Streptococcus pneumoniae*, *Neisseria meningitides*, *Listeria monocytogenes*) (Hoffman and Weber 2009).

Salmonella and *Campylobacter* colonize the intestinal mucosa of humans and proliferate, leading to the food borne illness known as salmonellosis and campylobacteriosis (Lacey 1993). The symptoms associated with these bacteria include diarrhea, fever, abdominal pain, nausea, and vomiting. *Salmonella* can rarely disseminate into the blood stream infecting other organs like liver (Gerba 2009). The severe infection of *Campylobacter* can result in bacteremia, pancreatitis, and sometimes miscarriage (Acheson and Allos 2001). The mode of transmission of *Salmonella* is primarily through fecal-oral route (Gerba 2009). However, *Campylobacter* adopts food borne mode of transmission via raw or undercooked meat, contaminated milk, or water (Acheson and Allos 2001). These bacterial infections are self-limiting, hence does not require the antibiotic treatment. However, the people with severe diarrhea might require administration of intravenous fluids.

The bacterial pathogens colonizing the respiratory tracts often lead to mild to severe cases of infections. The common upper respiratory tract infection (streptococcal pharyngitis) is caused by *Streptococcus pyogenes*, which damages the mucosal membrane of pharynx via release of toxins like streptolysins (Helal et al. 2020). The infection is characterized by high fever, pharyngeal inflammation, swollen lymph nodes, and tonsils. The mode of transmission is primarily by direct contact or by droplet mode (Helal et al. 2020). *Corynebacterium diphtheria* causes the infection of oropharynx via production of diphtheria toxins that guards the bacteria against the host immune response and kills the host cells. The infection results in formation of pseudo membrane consisting of dead host cells, pus cells, fibrins, which masks the mucous membrane of nasal cavity, pharynx, and larynx. As the disease progresses, it results in more severe manifestations with the enlargement of pseudo membrane obstructing the openings of trachea and pharynx leading to suffocation and can also result in myocarditis. The bacteria is transmitted through aerosols and droplets released during coughing by an infected person (Helal et al. 2020). *Mycobacterium tuberculosis* infection results in one of the deadliest infectious diseases known as tuberculosis (TB). The mode of transmission of the bacteria is through droplets or aerosols, which when inhaled causes chronic granulomatous disease manifestation in lungs or other body parts. TB is a chronic disease of grave concern due to increasing number of multi-drug resistant (MDR) and extensively drug resistant TB (XDR) cases due to reckless use of antibiotics (Helal et al. 2020). *E. coli* is the harmless natural microflora inhabitant of human intestine that assist in maintaining the healthy intestinal tract. However, some strains of *E. coli* like Shiga toxin producing *E. coli* can give rise to severe foodborne illness transmitted through contaminated food, raw milk, vegetables, meats, etc. The uropathogenic strain of *E. coli* sometimes colonizes the urinary tract, through stool resulting in urinary tract infections (UTI) (Forsyth et al. 2018). *Streptococcus pneumoniae* are commensals

residing in the mucosal surface of respiratory tract of the obligate human host. *S. pneumoniae* have the inherent ability to evade and manipulate host inflammatory and immune response to facilitate transmission and invasion of the host cells. They can alter their interaction with the host from commensal to pathogenic resulting in severe infection as a consequence of invasion to lungs, bloodstream, or meninges causing pneumonia, sepsis, and meningitis (Weiser et al. 2018). *Neisseria meningitides* is another leading cause of bacterial meningitis, a critical infection of meninges surrounding the brain, resulting in severe brain damage and high fatality. The mode of transmission is from person-to-person contact via respiratory droplets and throat secretions (Rouphael and Stephens 2012).

3.3.2 Fungal Infections

Fungi are primarily opportunistic pathogens that mostly cause infection in immunocompromised individuals. Fungi are mostly harmless in healthy individuals as the epithelial cells in GI tract will prevent the fungal invasion, and ciliary cells will prevent the inhalation of fungal spores and cells. However, the damaged tissue and compromised immune system act as breeding grounds for fungi making them the opportunistic pathogens (Pathakumari et al. 2020). Candidiasis is the fungal infection caused by *Candida* spp. (*C. albicans*, *C. glabrata*, *C. krusei*, *C. auris*), they are endogenous commensal pathogens that invade the GI tract or skin. Candidiasis is associated with mucocutaneous lesions, fungemia, urinary tract infections. Infection of *Candida* species is most common fungal infections in immunocompromised individual (Etienne and Caron 2007). *C. auris* specifically is a pathogen of concern as they are resistant to multiple drugs and is the most common fungal infection found in hospitals and healthcare facilitites (Chowdhary et al. 2017).

Aspergillosis is another opportunistic fungus that mainly infects the lower respiratory tract post-inhalation of aspergillus spores resulting in the invasive disease causing hemorrhagic necrosis and infarction (Kousha et al. 2011). *A. fumigatus* mostly causes the invasive pulmonary diseases (Dagenais and Keller 2009), whereas *A. flavus* is primarily associated with invasive extrapulmonary infections (López-Cortés et al. 2012). The invasive aspergillosis can also result in chronic disease especially in patients taking corticosteroids as recently observed in COVID-19 patients as post-COVID sequelae (Kakamad et al. 2021). Mucormycosis is the fungal infection caused by fungi of the genera *Rhizopus*, *Rhizomucor*, *Mucor*, etc. (Hassan and Voigt 2019). The symptoms associated with this fungal infection are necrotic lesions in nose, orbital cellulitis, proptosis, fever, purulent nasal discharge (Wali et al. 2011). Mucormycosis was commonly observed among the COVID-19 survivors as one of the post-COVID complications attributed to the rampant use of corticosteroids leading to uncontrolled diabetes (Raut and Huy 2021).

Blastomycosis is the pulmonary infection caused by fungus *Blastomyces dermatitidis* due to the inhalation of spores. Symptoms associated with the infection are pneumonia, dissemination to skin, fever, cough, fatigue. *Blastomyces* inhabit the moist soil comprising of decomposing material like wood, leaves, excreta; and is

most commonly found in North America, Canada, and sometimes in Africa and Middle East (Fang et al. 2007).

3.3.3 Viral Infections

Virus infections are the most commonly occurring infectious diseases in humans afflicted by viruses, the pathogenic micro-organism composed of DNA/RNA encapsulated by a layer of proteinaceous coat. Viruses can cause vast variety of infections like respiratory infections, skin infections, CNS infection, etc. (Edelman et al. 2000). Some of the commonly occurring viral infections are briefly explained in this section. Respiratory viruses like influenza A virus, RSV, coronavirus, rhinovirus infect the upper/lower respiratory tract resulting in sore throat, common cold, sinusitis, breathlessness, fever, bronchiolitis and can sometimes advance to causing lung pneumonia (Dasaraju and Liu 1996). These viruses have the potential to cause the world-wide outbreak causing the pandemic situation, as evidenced recently by SARS-CoV-2. Viral meningitis is the CNS infection caused mostly by enteroviruses, sometimes by arbovirus, herpes virus (HSV), etc. Viral encephalitis is another consequence of invasion of CNS by viruses like Japanese encephalitis, dengue virus, Nipah virus, HSV, West Nile virus, etc. (Lamadé et al. 2019). Apart from CNS manifestations, infection of HSV is also associated with skin problems like genital herpes, fever blisters, etc. (Saleh et al. 2021). Other viruses causing skin infection are human papillomavirus that cause warts and varicella virus that cause chicken pox, shingles (Winn and Walker 1994). Viruses like rotavirus and norovirus can result in gastroenteritis (stomach flu) characterized by nausea, vomiting, and diarrhea (Franco and Greenberg 2012). Viruses like HIV, HPV, HSV, hepatitis B can cause sexually transmitted diseases, which are communicable and life threatening diseases (Winn and Walker 1994). Viruses have the inherent ability to adapt to the new host and environment by undergoing rapid mutations, genetic re-assortment, develop resistance to antivirals, eventually resulting in emergence or re-emergence of novel virus, which may pose a severe threat to mankind. In 2015, Brazil observed a sudden surge in the cases of Zika virus infections resulting in congenital infections, Guillain-Barre syndrome (Cao-Lormeau et al. 2016). ZIKV is an arthropod borne virus that can infect the neural stem of the developing brain, resulting in diminishing of cortical layer. Other clinical manifestations include fever, conjunctivitis, joint and muscle pain, diarrhea, anorexia, retro-orbital pain, and abdominal discomfort. Some of the promising vaccine candidates include the DNA vaccine, subunit vaccine, inactivated vaccine, nucleic acid vaccines, etc., but none of them has completed the clinical trials due to the waning out of the Zika epidemic (Pattnaik et al. 2020). Ebola virus is one of the deadliest emerging virus infections that result in hemorrhagic fever in humans. The virus re-emerged in 2013 in Guinea and spread rapidly to the neighboring countries resulting in an epidemic with high lethality rate (Lever and Whitty 2016). The virus enters through the mucous membrane or abrasions in the skin and can infect all cells of the body. The high mortality rate is attributed to the multi-organ failure and shock associated with acute Ebola virus infection

(Aleksandrowicz et al. 2011). SARS-CoV is another emerging viral infection that is considered a serious threat to the mankind. The initial outbreak was observed in 2002 in Guangdong province of China where the virus resulted in headache, fever, respiratory failure but has low mortality rate (Fleck 2004). However, the virus re-emerged in 2019–2020 in Wuhan, China and resulted in a pandemic situation in 2020. The novel coronavirus also known as SARS-CoV-2 has acquired mutations in the spike protein region and enhanced its transmissibility making it more dangerous. The SARS-CoV-2 is transmitted by aerosol, droplets, person-person, fomite, and via fecal route of transmission. The symptoms associated with the infection include fever, body aches, headache, diarrhea, loss of taste and smell, sore throat, breathlessness (Laha et al. 2020).

3.3.4 Parasitic Infections

Parasites, as the name suggests, are microorganisms that reside inside the host or thrive on the host for their own benefit and are harmful for the host cell. Parasitic infections are mainly caused by protozoa, helminths, and ectoparasites. Protozoa are single cell organism with high tendency to infect immunocompromised individuals but are usually harmless (*Entamoeba coli*) (Issa 2014), however can sometimes result in fatal outcomes (*Entamoeba histolytica*, *Trypanosoma* spp.). *Entamoeba histolytica* causes amoebiasis which is transmitted through fecal–oral route in the areas of high population density and poor sanitation facilities (Cummings and Turco 2009). *Leishmania* is transmitted by sandflies causing leishmaniasis, that can affect skin, mucous membrane of nose, mouth and can also lead to fatality (Cummings and Turco 2009). *Trypanosoma* causes sleeping sickness or Chagas disease transmitted by tsetse fly infecting the blood, lymph and affecting the CNS and can lead to death. *Toxoplasma gondii* results in toxoplasmosis also known as parasitic pneumonia affecting heart, liver, brain, etc. The consumption of uncooked meat or milk that is contaminated with the parasite leads to manifestation of infection. The healthy individuals infected with the parasite mostly remain asymptomatic; however it is risky in immunocompromised patients and pregnant women. *Plasmodium* spp. (*P. falciparum*, *P. vivax*, etc.) causes malaria, the most deadly parasitic infection transmitted through the female anopheles mosquitoes. The parasitic infection causes fever, chills, headache, anemia and may advance to multi-organ failure leading to fatality. Helminths are multicellular parasites that thrive inside or outside the body and commonly include worms like roundworms, flatworms, tapeworms, etc. Roundworms cause ascariasis that is usually an asymptomatic disease condition. The worms enter the host cell through contaminated food or water and can be visible when eliminated via feces. The round worm of the family Toxocara can cause toxocariasis that can affect the brain, eye, and liver. The disease is mostly asymptomatic and transmitted through accidental swallowing of the eggs of parasite (Cummings and Turco 2009). Roundworm of the family Trichinella can lead to trichinosis, which causes intestinal discomfort, fever, and muscle ache. The disease is transmitted through consumption of uncooked meat manifested with the parasite.

The Taenia family of tapeworm enters the human body through consumption of infected meat and infects the intestine causing taeniasis. Ectoparasites live outside the human body and infest the skin causing severe pruritis. Examples of ectoparasites are mites, ticks, lice, fleas, bedbugs (Cummings and Turco 2009).

3.4 Emerging Infectious Diseases

Emerging Infectious Diseases (EID) are the newly recognized or previously unidentified infections that have caused or have potential to cause serious health crisis in a particular geographic area. They are majorly new infections caused by novel microorganisms or due to evolutionary changes in existing microorganisms, previously identified infections inhabiting new geographic region or old infections re-emerging due to newly acquired antibiotic resistance (Baylor College of Medicine 2021). Majority of EID are disseminated via zoonotic mode of transmission, wherein the pathogen incubates in the animal and is randomly transmitted to vulnerable human population via direct contact or through food, etc. EID may also be vector borne, food borne, or air borne. The outcome of infectious diseases is usually unpredictable; they may be contained by the host immune system, therapeutics, and vaccines or may also lead to global outbreaks. Similarly the emergence and transmission of novel infection is inevitable due to world travel and global interdependence, leading to a phenomenal socio-economic impact. The recent rise in the cases of EID is associated with drastic demographic and environmental changes and global trade. Other factors contributing to the spread of emerging infections are population density, poverty, vulnerability of population, destructive ecological changes, evolution of pathogens, drug resistance, decline in vaccine coverage, etc. (McArthur 2019). Some of the examples of the recent emerging infections are *Candida auris*, Avian influenza, Lone star tick, Ebola virus, Zika Virus, Sars-CoV-2, etc.

Candida auris is an emerging multi-drug resistant fungus identified in 2009 that results in invasive infections and has high mortality rate (Sears and Schwartz 2017). These microorganisms display resistance to azoles and are sensitive to echinocandin antifungals (Pristov and Ghannoum 2019). Avian influenza virus (H7N2) has low pathogenicity and unique way of transmission to humans through domestic animals. The virus may manifest through aerosol mode of transmission and pose a potential threat to nonimmune individuals. The treatment is majorly symptomatic; however neuraminidase inhibitors can be administered within 48 h of infection (Zhao et al. 2016). The lone star tick, *Amblyomma americanum*, results in the erythematous rash similar to Lyme disease (Wormser et al. 2006). The tick acts as a vector and expedites the spread of Bourbon virus, heartland virus, tularemia, and tick-associate rash among the humans (McArthur 2019). The treatment is primarily symptomatic with doxycycline as the antibiotic of choice and topical corticosteroid for local reactions (Wormser et al. 2006). Ebola virus infection causes a rare and deadly Ebola hemorrhagic fever and had led to an outbreak in West Africa in 2014–2016 (Mbonye et al. 2014). The virus uses wild animals like fruit bats as reservoir and then

spread among the human population via direct contact with bodily fluids or contaminated fomites (Weber et al. 2019). The current treatment strategy includes the FDA approved monoclonal antibodies: Inmazeb and Ebanga (Sivanandy et al. 2022). Zika virus is another emerging virus infection that is of concern after it led to an outbreak in Brazil in 2015 (Lowe et al. 2018). Zika is an arthropod borne virus transmitted in humans from the bite of *Aedes aegypti* mosquitoes. The virus is transmitted among the human population via bodily fluids and also from mother to fetus during pregnancy. Infection of Zika during pregnancy results in complications like loss of fetus, still birth or can also result in congenital abnormalities in the fetus or newborn. Currently, there is no available treatment against Zika virus (Lowe et al. 2018). The most recent addition to the emerging infectious diseases is the SARS-CoV-2 which led to a pandemic situation in 2020 and has a higher mortality rate than the previous SARS-CoV outbreak. The wide range of symptoms associated with the SARS-CoV-2 infection include fever, cough, breathlessness, fatigue, headache, loss of taste and smell, sore throat, diarrhea sometimes leading to pneumonia and heart complications under severe cases of infection (Hayes et al. 2021). For the treatment of COVID-19, the antiparasitic drug remdesivir has received the FDA approval. However, there are other treatment alternatives (like anti-SARS-CoV-2 monoclonal antibody therapy, tocilizumab, baricitinib, etc.), that have received Emergency Use Approval (EUA) (Rismanbaf 2020). Apart from the therapeutic interventions, vaccines against SARS-CoV-2 have also been developed on different platforms and are being administered in different parts of the world to contain the spread of virus infection. Some of the vaccines are mRNA vaccines (mRNA-1273), inactivated virus vaccine (Covaxin), viral vectored vaccine (Covishield, Sputnik V), etc. (Azevedo et al. 2021). Apart from the approved vaccines, there are more than 100 vaccine candidates that are in different phase of clinical trial, re-iterating the importance of vaccine to prohibit the further escalation of COVID-19 infections.

3.5 Pharamacotherapeutic Interventions

From several decades, pharmacists have been involved in extensive research in identifying the potential broad spectrum antibiotics, antiviral, antiparasitic, and antifungal medications. They have been under immense pressure in dealing with the increased cases of drug resistance that have been observed owing to rampant use of antibiotics, antivirals, or other therapeutic drugs. Another major issue often encountered is the emergence of a novel strain due to mutations in the genome of the pathogen which often leads to the events of epidemics and pandemics as observed in the cases of SARS-CoV-2 infection. During the COVID-19 pandemic, the initial strategy to identify the potential therapeutic agent was to check the efficacy of re-purposed drugs or the therapeutic agents that had previously shown efficacy. During the 2002 outbreak of SARS-CoV, the protease inhibitors Lopinavir/Ritonavir and Type I interferons exhibited encouraging results in in vitro studies (Singh et al. 2020). The re-purposed broad spectrum anti-coronaviral drug that achieved the

FDA approval is remdesivir, that has previously shown promising results against Ebola virus (Pardo et al. 2020).

Medical pharmacology provides vast classification of therapeutic drugs used for the treatment of infectious diseases such as antifungal, antibacterial, antiviral, antiparasitic, some of which are briefly described below.

3.5.1 Antibiotics

Antibiotics are classified under several classes and usually administered in form of a cocktail wherein different antibiotics of different classes are prescribed for the treatment of acute and chronic bacterial infections. They are co-administered during other microbial infections to prevent the spread of secondary bacterial infections in immunocompromised individuals.

3.5.1.1 Beta Lactam Antibiotics

These are among the front-runners and most commonly prescribed drugs against bacterial infectious diseases. The common biochemical feature of these antibiotics is the presence of highly reactive beta lactam ring (3-carbon, 1-nitrogen) (Page 2012). Beta lactam antibiotics interfere with the synthesis of peptidoglycan, a component of bacterial cell wall, resulting in lysis and loss of viability or autolysis within the cell wall. These antibiotics are highly efficacious against variety of diseases; however, the alarming resistance to these antibiotics is a serious challenge that needs immediate attention. The reasons for the increasing antibiotic resistance are speculated to be their inactivation by beta lactamases, inability to penetrate to the target site, efflux through pumping mechanisms, etc. (Heesemann 1993). This class of antibiotics includes penicillin, cephalosporin, carbapenems, monobactams, beta-lactamase inhibitors. Penicillins are prescribed for the treatment of diversity of bacterial infections like *Streptococcus pneumonia*, *Neisseria meningitides*, *Enterococcus faecalis*, *Listeria*, *Pseudomonas aeruginosa*, etc. Cephalosporin is mainly administered for the treatment of upper respiratory tract infections, urinary tract infections, streptococcal endocarditis, Lyme disease, meningitis, community-acquired pneumonia, etc. Carbapenems are a part of treatment regime for nosocomial infections, abdominal infections, urinary tract infections, meningitis. Monobactams are effective against aerobic gram-negative bacteria only and used for treatment of pneumonia and urinary tract infections (Page 2012).

Aminoglycosides (AG) are the oldest class of antibiotics produced naturally by actinomycetes bacterial species: *Streptomyces* spp. and *Micromonas* spp. effective against both gram-positive and negative microorganisms (Poulikakos and Falagas 2013). Some examples of AGs are neomycin, kanamycin, paromomycin, tobramycin, gentamicin, streptomycin, etc. (Poulikakos and Falagas 2013). Aminoglycosides are bactericidal in nature; they interfere with synthesis of bacterial proteins by binding to 16S ribosomal RNA component of 30S smaller subunit of prokaryotic ribosome (Hermann 2007). The major limitation and the reason for the

restricted use of AG molecules are the nephrotoxicity and ototoxicity associated with it; however sincere efforts to reduce toxicity have yielded a strategy of once daily administration to restrict its toxic side effects (Poulikakos and Falagas 2013). Despite the associated toxicity, they are still the first-line therapy against plague (streptomycin, gentamicin), tularemia (streptomycin, gentamicin), brucellosis (streptomycin or gentamicin with doxycycline), tuberculosis (amikacin, kanamycin), etc. (Poulikakos and Falagas 2013). Bacteria have developed different strategies to confer resistance against aminoglycosides; *Pseudomonas aeruginosa* has the ability to pump AG out of the cell through efflux pumps (Mingeot-Leclercq et al. 1999); *Mycobacterium tuberculosis* mutates the 16srRNA, the target of AGs, thereby resisting the bactericidal effect of streptomycin (Wachino and Arakawa 2012); inactivation by aminoglycoside-modifying enzymes (AMEs) (Poulikakos and Falagas 2013). The AGs gentamycin and streptomycin demonstrate synergistic effect against gram-positive bacteria resulting in endocarditis; tobramycin and amikacin have high efficacy against *P. aeruginosa* (Poole 2005). Amikacin is mainly administered to the pathogens that resist AGs through AMEs as it escapes the inactivation by AMEs. Aminoglycosides are vital part of empirical combination treatment against various infections attributed to MDR gram negative bacteria like sepsis and septic shock, respiratory tract infections, urinary tract infections, meningitis (caused by *Streptococcus agalactiae* or *Listeria* spp.) (van de Beek et al. 2012).

3.5.1.2 Fluoroquinolones

Fluoroquinolones are a class of broad spectrum antibiotics effective against aerobic gram positive and negative bacteria, primarily used for the treatment of urinary tract infection and respiratory pathogens (Cruciani and Bassetti 1994). The sensitive species of bacteria include gram-positive bacteria like *Staphylococci*, *Streptococcus pneumoniae*, *Listeria monocytogenes*, *Enterococcus faecalis*, and gram-negative bacteria like *Neisseria* spp. (*meningitides*, *gonorrhoeae*), *Haemophilus influenzae*, *Pseudomonas aeruginosa* (Bush et al. 2020). The mechanism of action of this class of antibiotics is by inhibition of DNA topoisomerase (Type II), which plays a crucial role in bacterial DNA replication and mRNA transcription process (Aldred et al. 2014). This class of antibiotics is classified into four generations on the basis of their antimicrobial activity. The first-generation antibiotics (cinoxacin, nalidixic acid) demonstrate moderate effect against gram-negative bacteria (*Enterobacteriaceae*) during uncomplicated urinary tract infections, but prescribed for systemic infections. The second-generation quinolones (norfloxacin, ofloxacin, etc.) are effective against wider range of gram-negative bacteria (*Enterobacteriaceae*) and ciprofloxacin which is the most effective quinolone against *Pseudomonas aeruginosa*. This class of quinolones is used for the treatment of urinary tract infections, gastroenteritis (severe diarrhea), nosocomial infections, and sexually transmitted diseases. The third-generation quinolones (levofloxacin, moxifloxacin, etc.) are prescribed for wide range of gram-negative and positive bacteria (*Enterobacteriaceae*, *Streptococci*, etc.), administered for the treatment of community-acquired pneumonia. The fourth-generation quinolone (trovafloxacin) is effective against wider range of gram positive and negative bacteria (*Enterobacteriaceae*, *P. aeruginosa*,

S. aureus, *Streptococci*, etc.) and anaerobic bacteria (Bush et al. 2020). The most frequently observed side effects of quinolones include nausea, CNS effects like headache, dizziness, insomnia, and gastrointestinal discomfort. Some rare serious adverse effects should also be evaluated before the intake of quinolones: damage of connective tissue, interstitial nephritis, severe neurotoxic effects (depression, hallucination, etc.), phototoxicity, and cardiovascular toxicity (Bush et al. 2020). Fluoroquinolones in conjugation with other classes of antibiotics play an essential role in the post-exposure treatment for pathogens used in biologic warfare. Examples: fluoroquinolone (ciprofloxacin), doxycycline (AG) are used for the treatment of *Bacillus anthracis*; fluoroquinolone (ciprofloxacin, norfloxacin), doxycycline (AG) are used for the treatment of *Vibrio cholerae*; aminoglycerides (streptomycin, gentamycin), fluoroquinolone (ciprofloxacin) are used for the treatment of *Yersinia pestis* (plague) (Bush et al. 2020).

3.5.2 Antifungals

Antifungals are a pharmacologically diverse group encompassing chemical and pharmacological agents and natural compounds, crucial for management of fungal infection (mycoses). Some commonly used antifungals are described in this section.

Amphotericin B deoxycholate is a member of polyene class of antifungals, administered during invasive fungal infections (Gallis et al. 1990). These drugs act by binding to the ergosterol in the fungal cell wall and form the ion channel which leads to loss of protons and cations resulting in membrane depolarization and concentration-dependent killing. Amphotericin also damages the cells by stimulating oxidative stress by formation of free radicals and increasing membrane permeability (Gallis et al. 1990). It is used for the treatment of candidiasis (*C. albicans*, *C. krusei*, *C. tropicalis*, *and C. parapsilosis*), mucormycosis (*Fusarium* species and penicilliosis), sporotrichosis, coccidioidomycosis, paracoccidioidomycosis, blastomycosis, aspergillosis, and parasitic infection (Leishmaniasis) (Noor and Preuss 2021). The most common side effects include loss of magnesium and potassium, renal toxicity, and cellular toxicity. However, the newer lipid made formulations (amphotericin B lipid complex) are often used instead of conventional amphotericin as they have an advantage of reduced cytotoxicity (Noor and Preuss 2021).

Azoles are systemic antifungal drugs that disrupt the phospholipid layer in fungal cell by inhibiting 14-alpha sterol demethylase (Papich 2019). They possess broad-spectrum antifungal properties against yeasts and molds and are classified into two classes: imidazole group and triazole group (Gavarkar et al. 2021). Ketoconazole (nizoral) is among the oldest member of imidazole group and is the systemic agent used for the treatment of blastomycosis, coccidioidomycosis, chromomycosis, histoplasmosis, and paracoccidioidomycosis (Sinawe and Casadesus 2021). The usage of ketoconazole is often associated with severe side effects like hepatotoxicity, gastrointestinal intolerability, reduction in concentration of testosterone and cortisol, etc. (Sinawe and Casadesus 2021). Owing to drastic side effects associated with the

member of imidazole group, they have been supplanted by more effective and less toxic triazole class of antifungals like fluconazole, isavuconazole, itraconazole, voriconazole, etc. (Papich 2019). Fluconazole demonstrated superior pharmacokinetics and reduced adverse effects profile as compared to ketoconazole. Fluconazole is administered orally during oropharyngeal and esophageal candidiasis and other invasive candidiasis. Due to their ability to penetrate CNS, they are also used for the treatment of cryptococcal meningitis and *Candida* spp. (*C. albicans*, *C. tropicalis*, and *C. parapsilosis*) mediated urinary tract infections (Fisher et al. 2011). Fluconazole however was less susceptible against *C. glabrata* and ineffective against *C. krusei*. The adverse effects of fluconazole include gastrointestinal discomfort and rashes; it can rarely lead to a severe consequence like hepatic necrosis (Bailey et al. 1990). Itraconazole is another azole group antifungal agent that is used for the treatment of lymphocutaneous sporotrichosis, mild to less severe cases of histoplasmosis, blastomycosis, and paracoccidioidomycosis. It is also effective against invasive aspergillosis, coccidioidomycosis, and some types of chromoblastomycosis. Despite its efficacy against wide spectrum of fungal species, itraconazole is not well tolerated in gastrointestinal tract, causes allergic rash and hallucinations. The higher doses of the antifungal drug can lead to hypertension, hypokalemia, and edema (Bailey et al. 1990). The triazole posaconazole has high efficacy against yeast and molds like *Cladophialophora* spp., Zygomycetes, and species causing mucormycosis (Bailey et al. 1990). It is also used as fungal prophylaxis against *Aspergillus* or *Candida* infections in neutropenic patients (Allen 2010). Voriconazole is a broad spectrum triazole used for the treatment of invasive *Aspergillus*, *Scedosporium apiospermum*, and *Fusarium* infections. It is also administered during esophageal candidiasis and systemic candida infections. The side effects associated with voriconazole include heptatoxicity, hallucination, dermatologic reactions, and visual disturbances (Bailey et al. 1990).

3.5.3 Antivirals

Antivirals are the drugs, chemical or natural compounds that are used to treat viral infection by targeting the crucial viral proteins or host proteins that facilitate viral pathogenesis inside the host cell. Viruses primarily rely on host cellular machinery to replicate themselves, hence the virologist faces a major challenge in designing antiviral compounds that would interfere with viral replication process without being deleterious for the host cells. Some of the efficient antivirals that are used to treat viral infections are mentioned in this section.

3.5.3.1 Antiviral Targeting the Viral Proteins

Nucleoside and nucleotide analogs are efficiently used as broad-spectrum antivirals, targeting viruses of different families by blocking viral polymerases. Polymerases play a centric role in viral replication and transcription process, making it an attractive target for antiviral development. Ribavirin is one of the most widely known broad-spectrum nucleoside analog (2′-C-methylcytidine) that has

demonstrated its antiviral potential against different families of positive sense RNA viruses like flavivirus, norovirus, and picornaviruses (Beaucourt and Vignuzzi 2014). Favipiravir (T-705) is a nucleotide analog that is effective against various positive (SARS-CoV) and negative sense RNA viruses (influenza virus). T-705 interferes with RNA dependent RNA polymerase in its active form and is approved for treatment of influenza virus in Japan. The drug was also assessed for its antiviral potential against SARS-CoV-2 virus infection (Shiraki and Daikoku 2020). The adenosine analog BCX4430 has demonstrated antiviral effect against filoviruses in in vitro study. Their mode of action is chain termination of viral polymerases of RNA viruses thereby eliciting antiviral effect against positive and negative sense RNA viruses (Warren et al. 2014). The major drawback of this class of drugs is the toxicity associated with them, which limit their clinical efficacy and are still under development or different stages of clinical trial studies. Another potential antiviral target that is widely studied is the viral proteases, the inhibitors of specific proteases are used for treatment of HIV and HCV infections. Identifying a protease inhibitor that would target multiple families of viruses with similar protease is a tedious task. The protease inhibitors (like darunavir, lopinavir, ritonavir, amprenavir, etc.) are efficiently used in combination with other drugs to avoid the development of drug resistance (Lou et al. 2014). Rupintrivir is one such example that irreversibly inhibits 3C-protease and has demonstrated antiviral efficacy against human rhino viruses, and other members of picornaviruses, coronaviruses, and norovirus (Kim et al. 2012).

3.5.3.2 Antivirals Targeting the Host Proteins

Another strategy of designing the broad-spectrum antivirals is by targeting the host proteins that play an imperative role in viral replication inside the host cells. However, several factors need to be addressed before designing antiviral targeting the host proteins: identifying the host factor that can be targeted and the impact that would have on the host cells and viruses and assessing its toxicity and adverse effects in the host cells. The downstream effect of the targeted protein also needs to be considered to assess the effect of masking the protein function. Many viruses hijack the host lipid metabolism process to facilitate its replication process (Thaker et al. 2019). Hence, broad spectrum antivirals interfere with cholesterol metabolism like statins and have demonstrated in vitro antiviral activity against viruses like HCV, HIV, RSV, dengue virus, cytomegalovirus, etc. (Takizawa and Yamasaki 2018). Irrespective of the promising in vitro results, the antiviral efficacy of statin as monotherapy in HCV-infected patients yielded insignificant antiviral effect (Vere et al. 2012). However, the antiviral potential was enhanced when used in combination of previous standard care treatments. Another lipid modulator, arbidol is approved for prophylaxis and treatment of various respiratory viral infections (including influenza) in China and Russia (Huang et al. 2017). Arbidol is an indole derivative that interferes with viral entry and membrane fusion process by binding to the lipid membrane and interacting with aromatic amino acids in the viral envelope (Boriskin et al. 2008). Apart from its efficacy against respiratory viruses, arbidol efficiently inhibits the replication of several enveloped and non-enveloped RNA and

DNA viruses like chikungunya virus, HCV, HBV, etc. (Blaising et al. 2014). Cyclophilin A (CypA), a member of cyclophilin family that participates in cellular processes like regulation of transcription, protein synthesis, immune function, etc., plays a crucial role in replication of viruses like HIV-1, HCV, influenza virus, vaccinia virus, SARS-CoV, HPV, etc. (Lou et al. 2014). During infection of HIV-1, CypA is proposed to interact with viral capsid protein and other proteins like Vpr and p6, and modulate viral infection (Solbak et al. 2011). CypA antagonists (inhibitors) like alisporivir, NIM811 and SCY635 have yielded promising antiviral effect against HIV and HCV infections (Lou et al. 2014). During viral infection, host cells stimulate the innate and adaptive immune response to fight the infection. Interferons, the primary molecules that mediate the innate immune response interact with interferon (IFN) receptors on the membrane and inhibit virus replication by stimulating IFN-stimulated genes. Owing to their crucial role in mediating antiviral response, IFN has been used for antiviral therapy against HBV and HCV infections (in combination with ribavirin) (Scott and Perry 2002).

3.5.4 Antiparasitic Drugs

Antiparasitic drugs are a group of medicines that is effective in management and treatment of parasitic infections. Some of the antiparasitic drugs, their efficacy and adverse effects are mentioned in this section. The antimalarial drug is dependent on the causative species of *Plasmodium*; chloroquine is effective in treatment of malaria caused by most of the species of *Plasmodium* (*P. malariae*, *P. vivax*, etc.) except *P. falciparum*, which has developed resistance against the drug (Chinappi et al. 2010). Chloroquine is an aminoquinoline that blocks nucleic acid synthesis by inhibiting detoxification of heme. Chloroquine is majorly well tolerated, however it can sometimes lead to gastrointestinal problems, or can rarely cause hypotension, seizures, blurring vision, etc. (Campbell and Soman-Faulkner 2021). For the treatment of *P. falciparum*, the combination treatment atovaquone-proguanil or artemether-lumefantrine may be the first-line treatment that could be used (Blanshard and Hine 2021). Atovaquone inhibits the cytochrome electron transport chain, whereas proguanil inhibits dihydrofolate reductase, both synergistically leading to loss of membrane potential of mitochondria. Atovaquone may result in side effects like nausea, headache, fever, gastrointestinal disturbances, etc. (Campbell and Soman-Faulkner 2021). Artemether binds to heme group and decomposes Endoperoxide Bridge, releasing the toxic free radicals that are deleterious for the parasite. Lumefantrine blocks the detoxification of heme leading to accumulation of toxic heme that is detrimental for the parasite. This combination treatment can result in disturbance of GI, dizziness, headaches, etc. (Campbell and Soman-Faulkner 2021). The infection of *Entamoeba histolytica* can be managed by antiamoebic therapy which is dependent on the presentation of the disease (Knight 1980). Amoeba infection in lumen is treated by luminal amebicides like iodoquinol, paromomycin sulfate, whereas extraintestinal infection (like hepatic abscesses) can be managed with metronidazole, tinidazole, and emetine (complemented by

chloroquine). Iodoquinol interferes with metabolism of amoeba by reducing the ferrous ions necessary for metabolism process, whereas paromomycin inhibits the protein synthesis process. Iodoquinol and paromomycin can sometimes cause adverse effect on GI, paromomycin can also lead to renal toxicity (Campbell and Soman-Faulkner 2021). Metronidazole and tinidazole initiate the redox reaction inside the parasite releasing the toxic intermediates that disrupts the DNA of amoeba and inhibits protein synthesis. Both these drugs have similar activity profile; however tinidazole is less toxic than metronidazole. Metronidazole and tinidazole often cause headache, dry mouth, nausea and can rarely result in vertigo, pancreatitis, neutropenia, and CNS toxicity (Campbell and Soman-Faulkner 2021). Infection of *Trypanosoma cruzi* (causative agent of Chagas disease) is managed by nifurtimox or benznidazole (CDC 2021m), whereas the infection of *T. brucei* (causative agent of sleeping sickness) can be treated with pentamidine during early stages and by eflornithine when CNS manifestations are observed (Campbell and Soman-Faulkner 2021). Nifurtimox generates the nitro anions which are oxidized to the reactive intermediates that are toxic to the parasite. It can cause side effects like disturbance in GI, seizures, rash, insomnia, etc. (Docampo and Moreno 1986). Pentamidine is an aromatic diamine that disturbs the membrane potential of the mitochondria of parasite. It is a highly toxic parasitic drug, that can cause hypotension, dyspnea, pancreatic toxicity, gastrointestinal disturbance, cardiac arrhythmia, etc., depending on the route of administration (Campbell and Soman-Faulkner 2021). Eflornithine acts as an irreversible inhibitor of ornithine decarboxylase, the essential enzyme in polyamine biosynthesis. The side effects associated with eflornithine include disturbance in GI and other reversible manifestations like leukopenia, thrombocytopenia, and seizures (Barman Balfour and McClellan 2001).

Another types of parasitic infection may be caused by nematodes, which can be successfully managed by antinematodal drugs like albendazole, diethylcarbamazine, ivermectin, etc. (Campbell and Soman-Faulkner 2021). Albendazole is the drug of choice for the treatment of infection associated with ascariasis, trichuriasis, hookworm, pinworm, however diethylcarbamazine is administered for the treatment of filariasis, tropical eosinophilia, and loiasis (Campbell and Soman-Faulkner 2021). The onchocerciasis infection is managed with ivermectin. Albendazole interferes with the microtubule synthesis in parasite, which leads to inhibition of glucose uptake, eventually leading to the death of parasite. Albendazole is mostly well tolerated but can sometimes cause disturbances in GI, headache, alopecia, increase in liver enzymes, etc. (Grayson et al. 2017). Diethylcarbamazine acts as opsonin ultimately facilitating the phagocytosis of the parasite (Campbell and Soman-Faulkner 2021). Ivermectin is an anthelmintic drug that acts by opening the chloride ion channels, leading to hyperpolarization and eventually killing the nematods (Campbell and Soman-Faulkner 2021).

3.6 Nanomedicine

In recent years, nanotechnology has emerged as an attractive area of research that has the potential to transform the detection and management aspect of various diseases like cancer, infectious diseases, etc. (Kirtane et al. 2021). The field of nanomedicine is the amalgamation of nanotechnology and science, wherein the main goal is designing a nanometer size particle that has the potential to create substantial impact on disease management (Ventola 2012). Nanomedicines potentially provide several benefits like better efficacy, targeted drug delivery, bioavailability, customization, and reduced toxicity in comparison to the conventional medicines (Bawa 2012). Several infectious diseases like HIV, tuberculosis require prolonged treatment extending up to several years; this can be accomplished by nanomedicine encapsulated in nanocarriers that can be delivered over sustained period of time (Kirtane et al. 2021). Nanocarriers have the ability to be engineered to ensure the release of drugs on exposure to certain stimulus, it can also enhance the uptake and hence efficacy of drugs against intracellular pathogens. One such example is the polyester-based delivery system (e.g., PLGA), that can be degraded in presence of esterase resulting in sustained diffusion of the drug (Makadia and Siegel 2011). Polymers that can be used in such nanocarriers include poly(lactide-co-glycolide), poly(anhydrides), poly(orthoesters), poly(cyanoacrylates), and poly(amides) (Langer 1998). Sustained release of drugs can also be accomplished by encasing the drugs (hydrophilic and hydrophobic) in liposomes (BeyondSpring 2020). Such liposomal nanocarriers (MiKasome) were developed encapsulating amikacin, which yielded positive clinical trial results during *Mycobacterium infections* but were later discontinued in 2000 (Fielding et al. 1998; Schiffelers et al. 2001).

Encapsulation of drugs in nanocarriers facilitates the targeted drug therapy with minimal impact on healthy cells as they are designed to have increased permeability at the diseased site as compared to healthy tissue (Kirtane et al. 2021). The nanocarriers can also be modified by addition of ligands that mediate the active targeting, also known as ligand mediated targeting. Nanocarriers have also been used for targeting drugs to macrophages which are the common target sites of various pathogens like *M. tuberculosis*, *Aspergillus species*, *HIV*, etc. Nanoparticles also enhance the penetration and accumulation of drugs in reticuloendothelial organs and other organs (Löbenberg et al. 1998). The antiretroviral drug azidothymidine encapsulated in poly(hexacyanoacrylate) nanoparticle improves its accumulation in organs like liver, spleen, and lungs.

3.6.1 Types of Nanoparticles Used in Nanomedicine

Wide varieties of nanoparticles are being explored in development of nanomedicines. These nanoparticles can be broadly categorized into organic and inorganic nanoparticles, some of which are described in this section and shown diagrammatically in Fig. 3.2.

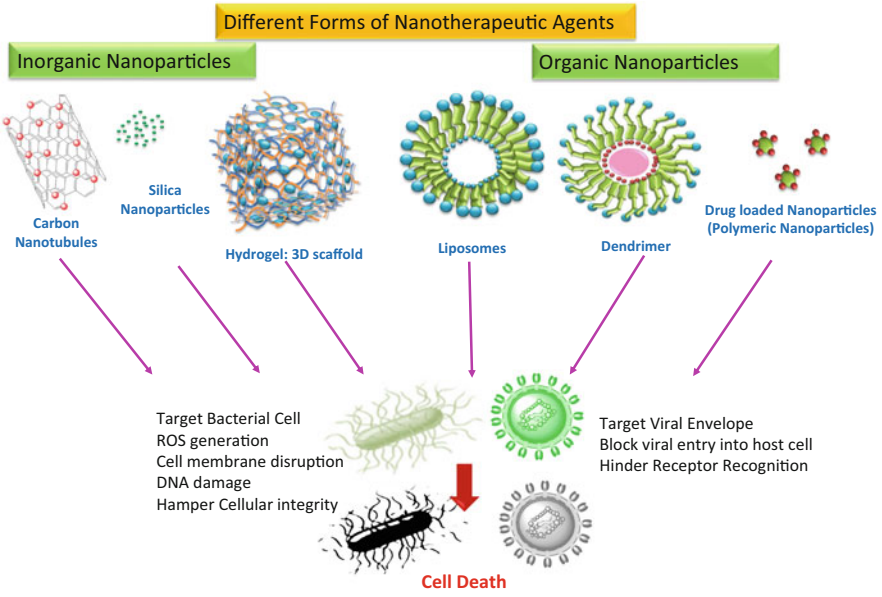

Fig. 3.2 Graphical representation of organic and inorganic nano-formulations

3.6.1.1 Organic Nanoparticles

1. **Liposomes**: Liposomes are vesicles composed of lipid bilayer (Fig. 3.2) designed to transport biomolecules (like monoclonal antibodies, antigens, etc.) which can be released at the target site by altering the pH, osmotic pressure or modifying the surface of liposomes with addition of polymers like polyethylene glycol, etc. (Ventola 2012). Liposomes are widely used in the field of nanomedicine as they can accommodate water and lipid soluble agents/drugs in its core and have excellent biodegradability, improved pharmacokinetics and biodistribution (Torchilin 2005). Example: PEGylated liposome loaded with doxorubicin delivers the higher drug concentration in the cancerous cells and decreases the effect of drug on normal cells (Wang et al. 2012).
2. **Dendrimers**: Dendrimers are synthetic polymers identified by the branched repeating units arising from the core region and exposed anionic, cationic, or neutral functional groups on the surface (Fig. 3.2). The drug can be attached to dendrimer near the core region through covalent bond or on the terminal groups (Din et al. 2017). These nanoparticles pose as excellent vehicles for drug delivery as they offer several advantages like adjustable size, high loading capacity of drugs, high penetrability and bioavailability of drugs as the drug is bound to functional groups near the surface via covalent or noncovalent bonding (Kesharwani et al. 2014). Example: The dendrimer comprising of polyanionic carbosilane yielded promising results in the delivery of antiretroviral drugs for the treatment of HIV (Vacas-Córdoba et al. 2016).

3. **Polymer Nanoparticles (PNP)**: Polymer nanoparticles are solid colloidal nanoparticle composed of biodegradable polymers like synthetic (polylactic acid (PLA), polyglycolic acid (PGA), PEG, etc.) or natural polymers (albumin, gelatin, heparin, collagen, dextran, etc.) (Wang et al. 2009). PNPs can be categorized into nanospheres (Fig. 3.2), which entrap the drug inside the polymer matrix; or nanocapsule, wherein the polymeric membrane encapsulates the drug dissolved in oil or water (Prabhu et al. 2015). The drug or therapeutic agent can also be conjugated to the surface of these nanoparticles through polymerization and can be released into the target tissue by diffusion or desorption (Prabhu et al. 2015). PNPs offer additional advantages like better stability, improved physicochemical properties, higher drug payload, and more controlled drug release (Hu et al. 2010). The release of drug at the target site can be efficiently controlled by modifying the physicochemical properties (like molecular weight, hydrophobicity, dispersity index, etc.) and degradation of the polymers (Alexis et al. 2010). Polymeric nanoparticles are often considered as promising carriers for various medications for diseases like cancer, diabetes, cardiovascular diseases, and vaccination. Example: PLGA nanoparticles are under evaluation as a delivery vehicle for the combination of anticancerous drugs like doxorubicin and paclitaxel (Wang et al. 2011).
4. **Hydrogels**: Hydrogels are the water swollen three-dimensional scaffold constituting cross-linked hydrophilic polymers which become hydrated and closely resembles a biological tissue in the aqueous medium (Fig. 3.2). The polymers undergo covalent bonding or other physical interactions, thereafter making them insoluble in water or other solvents (Basso et al. 2018). The fundamental properties that make hydrogels the favorable and safe choice for drug delivery include their biodegradable and biocompatible nature. Hydrogels can be tailor-made by chemically modifying the comprising polymers with the functional groups that respond to specific stimuli, thereby ensuring the targeted drug delivery (Chai et al. 2017). Different types of hydrogels have been designed according to their necessity such as smart gels, thermogels, stimuli-responsive gels, magnetic gels, etc. (Chai et al. 2017). The nano-emulsion based hydrogel encompassing amphotericin B, the broad-spectrum antifungal drug acted as a stable and safe vehicle for topical delivery of amphotericin for fungal infection of skin (Hussain et al. 2016). The pH-sensitive hydrogels constituting chitosan/poly-y-glutamic acid polymers, efficiently deliver amoxicillin for the treatment of *Helicobacter pylori*, protecting the drug from degradation by gastric juices and increasing its bioavailability (Chang et al. 2010).

3.6.1.2 Inorganic Nanoparticles

1. **Carbon nanotubules (CNTs)**
   Carbon nanotubules are hollow, nano sized tube-like arrangement of allotropic form of carbon, constructed by rolling up of graphene (allotropic form of carbon) sheets (Bianco 2004) (Fig. 3.2). CNTs demonstrate characteristic biological and physicochemical properties which make them one of the ideal nanocarriers for the transport of drug. Some of these properties include needle-like shape (enhances

penetration ability), high drug load, ability of surface modifications, stability, and structural flexibility (Bianco 2004; Yan et al. 2007). The drug can be encapsulated in the cavity of CNTs or attached covalently/non-covalently to the surface (Wu et al. 2009). Example: Several studies are evaluating CNTs as the delivery vehicle for anticancerous drugs like doxorubicin, paclitaxel, etc. (Din et al. 2017).

2. **Silica nanoparticles**
   The silica nanoparticles are made of mesoporous silica, having the major structural advantage owing to their honeycomb-like structure with multiple pores that can host huge amounts of drug (Slowing et al. 2008). These silica nanoparticles have high therapeutic efficacy due to their excellent biocompatibility, high loading capacity, good stability, and ability to load drugs with versatile characteristics (hydrophilic, lipophilic, etc.) (Slowing et al. 2008). Example: Silica nanoparticles have efficiently delivered anticancerous drugs like doxorubicin (Lebold et al. 2009), campthothecin (Lu et al. 2007) to the target cell as demonstrated by in vitro studies.

3.6.2 Nanoparticles in Infectious Diseases

HIV infections: Nanoparticles have been most actively studied in antiretroviral therapy against HIV infection. Injectable nanosuspension containing the two retrovirals, cabotegravir and rilpivirine are actively being pursued in clinical trials. These nanosuspensions increase the half-lives of the antivirals and are safe to use with less toxicity. Other injectable nanoparticles are also being evaluated and yielded encouraging results in non-human primates like rhesus macaque. One such formulation was developed for the prolonged delivery of highly water soluble drug dolutegravir. The drug was esterified with mystic acid, resulting in a prodrug (water insoluble) that is formulated into nanoparticles. These nanoparticles are being evaluated in rhesus macaque where they are believed to be engulfed by macrophages, resulting activation of drug and release of active moiety from macrophages. Freeling et al. are studying the efficacy of lipid nanoparticle encapsulating multiple antiretroviral drugs (lopinavir, ritonavir, and tenofovir) in primates, where the nano-formulation produced enhanced intracellular concentration of drugs in plasma and lymph nodes as compared to free drug (Freeling et al. 2015).

Malaria: The malaria causing parasite *Plasmodium* penetrates the skin and reaches the liver where it infects the hepatocytes and proliferates into merozoites. The infected hepatocytes release the merozoites into the blood stream where they invades and replicates within the RBCs (Vaughan and Kappe 2017). The researchers are developing antimalarial drugs targeting the replication of parasites in RBCs. The liposomal nanoparticle containing heparin and monoclonal antibodies covalently attached to them are being studied in vitro for selective targeting of infected RBCs (Marques et al. 2017). Another nanoparticle, NanoAbsorb, the solid emulsion pre-concentrate has been successful in

delivering antimalarial drug artemeter in vivo with increased efficacy (Joshi et al. 2008). The self-emulsifying system of long chain-triglycerides and another lipid based oral nano-emulsion containing artemether and tafenoquine, respectively, have also demonstrated promising antimalarial potential. They provide increased bioavailability and decreased toxicity in in vivo studies (Melariri et al. 2015). The multiple drug nano-formulations are also being assessed against resistant strains of parasite. For example, non-structured lipid nanoparticles encapsulating artemether and lumefantrine resulted in better clearance of the pathogen from infected mice (Parashar et al. 2016). One of the nano-formulation based malarial vaccine has demonstrated partial protection against malaria in children, however further research is needed to increase the efficacy of the proposed vaccine (Laurens 2020).

Tuberculosis (TB): Tuberculosis contributes significantly to increase the global economic burden and is one of the leading causes of deaths due to infectious diseases. The severity of the disease is attributed to the prolonged treatment of multiple drugs and the frequent development of antibiotic resistance. Hence, researchers around the globe are studying the efficacy of nanoparticles to deliver the antibiotics that would reduce the duration of treatment and decrease the chances of drug resistance crisis. The lipid nanoparticles have increased the therapeutic benefits by improving the bioavailability of rifampicin alone (Singh et al. 2015) and in combination with isoniazid and pyrazinamide (Pandey et al. 2005) in rodents, resulting in the clearance of bacilli from the lung and spleen of the animal after five doses, which would be equivalent to the 46-daily doses of free drugs (Pandey et al. 2005). Scientists have developed nebulized lipid nanoparticles and sodium alginate nanoparticles (encapsulating rifampicin, isoniazid, and pyrazinamide) for the treatment of pulmonary TB, the most common form of TB (Pandey and Khuller 2005; Zahoor et al. 2005). These nanoparticles improve the bioavailability of the drugs in the lungs and reduce hepatotoxicity. Researchers are also attempting to improve the only approved vaccine of TB, the BCG vaccine which offers limited protection. Garcia-Contreras et al. administered the guinea pigs with aerosolized BCG nano-microparticle, which offered better protection and improved resistance against infection with TB (Garcia-Contreras et al. 2008).

3.7 Conclusion

Infectious diseases are the contagious, communicable, or transmissible diseases caused by pathogenic microorganisms like bacteria, virus, fungi, or parasite. These infectious diseases sometimes result in an outbreak or epidemic and rarely into more devastating pandemic contributing significantly to the world-wide mortality rate. The infectious diseases are widely studied wherein the scientists have dissected the pathogenesis of the microorganisms inside the host cell and also unveiled the interaction of the pathogens with the host protein that facilitates the disease progression. Based on these studies, there are several chemical or natural compounds,

vaccines or alternative therapeutic interventions that are available against the disease causing pathogens. However, several pathogens like (Influenza virus, *Mycobacterium tuberculosis*, *Candida auris*, *Plasmodium falciparum*) have developed drug resistance which is a matter of grave concern. The pathogens develop drug resistance due to exorbitant use of drugs, genetic changes in the pathogen, environmental factors, etc. There are several challenges associated with the efficacy of conventional therapies like efficacy and bioavailability of drug, targeted drug therapy, absorption and sustained release of drug, drug toxicity, etc. These challenges or drawbacks can be overcome by nanomedicine, the use of nanotechnology in drug delivery; a relatively new and attractive area of research that has been the focus of interest of biologists and pharmacists. Nanocargos loaded with active drug molecules penetrate target site of action and release the active pharmacological agent in controlled/sustained release manner. They exhibit biocompatibility, protect drug from external microenvironment/degradation, maintain drug plasma concentration, and deliver multiple drugs in one shot. Some of the nano-formulations that are being analyzed for their drug delivery efficacy include organic nanoparticles like liposomes, dendrimers, hydrogels, etc.; and inorganic nanoparticles like carbon nanoparticles, silica nanoparticles, etc. Nano-formulations enclosing the drugs have yielded fruitful results against infectious diseases like HIV, Malaria, TB, etc.

Apart from exploring nanomedicine for therapeutic interventions against infectious diseases, there are other alternative modes of treatments that are being extensively researched. The use of antibody-based therapies against infectious diseases is currently experiencing renaissance fuelled by the increasing cases of drug resistance, emergence of novel microorganisms, etc. The antibody-based therapy also known as serum therapy utilizes the humanized antibodies that have high specificity and low toxicity. The serum therapy has demonstrated high efficacy against infectious diseases alone, or in conjugation with the conventional treatment (Casadevall et al. 2004). The therapeutic monoclonal antibodies are widely being evaluated for their efficacy against several diseases and many of them (alemtuzumab, aducanumab, etc.) have received FDA approval for the treatment of non-infectious diseases like cancer, neurodegenerative diseases like Alzheimer's disease, etc. (Gklinos et al. 2021). The therapeutic monoclonal antibodies have also demonstrated protection against infectious microorganisms like *Mycobacterium tuberculosis*, *Leishmania* spp., *Listeria monocytogenes*, etc. (Casadevall et al. 2004). The first monoclonal antibody (mAb) approved for clinical use against infectious diseases was palivizumab against RSV infection (Domachowske et al. 2021). Thereafter, mAb or antibody cocktail was approved for use against *Clostridium difficile*, Ebola virus, and the most recent SARS-CoV-2 (Pecetta et al. 2020). Hence antibody therapy is a promising strategy that can be further explored alone or in combination of the available treatment strategy against various infectious diseases.

References

Acheson D, Allos BM (2001) Campylobacter jejuni infections: update on emerging issues and trends. Clin Infect Dis 32(8):1201–1206. https://doi.org/10.1086/319760

Aldred KJ, Kerns RJ, Osheroff N (2014) Mechanism of quinolone action and resistance. Biochemistry 53(10):1565–1574. https://doi.org/10.1021/bi5000564

Aleksandrowicz P, Marzi A, Biedenkopf N et al (2011) Ebola virus enters host cells by macropinocytosis and clathrin-mediated endocytosis. J Infect Dis 204(Suppl 3):S957–S967. https://doi.org/10.1093/infdis/jir326

Alexis F, Pridgen EM, Langer R, Farokhzad OC (2010) Nanoparticle technologies for cancer therapy. Handb Exp Pharmacol 197(197):55–86. https://doi.org/10.1007/978-3-642-00477-3_2

Allen UD (2010) Antifungal agents for the treatment of systemic fungal infections in children. Paediatr Child Health (Oxford) 15(9):603–608. https://doi.org/10.1093/pch/15.9.603

Azevedo TCP, Freitas PV, Cunha PHPD, Moreira EAP, Rocha TJM, Barbosa FT, Sousa-Rodrigues CF, FWDS R (2021) Efficacy and landscape of Covid-19 vaccines: a review article. Rev Assoc Med Bras (1992) 67(3):474–478. https://doi.org/10.1590/1806-9282.20210073

Bailey EM, Krakovsky DJ, Rybak MJ (1990) The triazole antifungal agents: a review of itraconazole and fluconazole. Pharmacotherapy 10(2):146–153

Barman Balfour JA, McClellan K (2001) Topical eflornithine. Am J Clin Dermatol 2(3):197–201. https://doi.org/10.2165/00128071-200102030-00009

Basso J, Miranda A, Nunes S et al (2018) Hydrogel-based drug delivery nanosystems for the treatment of brain tumors. Gels 4(3):62. https://doi.org/10.3390/gels4030062

Bawa R (2012) Regulating nanomedicine – can the FDA handle it? Curr Drug Deliv 8(3):227–234. https://doi.org/10.2174/156720111795256156

Baylor College of Medicine (2021) Emerging infectious diseases. https://www.bcm.edu/departments/molecular-virology-andmicrobiology/emerging-infections-and-biodefense/emerging-infectious-diseases. Accessed 21 Jun 2021

Beaucourt S, Vignuzzi M (2014) Ribavirin: a drug active against many viruses with multiple effects on virus replication and propagation. Molecular basis of ribavirin resistance. Curr Opin Virol 8:10–15. https://doi.org/10.1016/j.coviro.2014.04.011

BeyondSpring (2020) Files for patent protection on BPI-002 for the treatment of viral infections, including COVID-19. https://drug-dev.com/beyondspring-files-for-patent-protection-on-bpi-002-for-the-treatment-of-viral-infections-including-covid-19/. Accessed 29 Aug 2020

Bianco A (2004) Carbon nanotubes for the delivery of therapeutic molecules. Expert Opin Drug Deliv 1(1):57–65. https://doi.org/10.1517/17425247.1.1.57

Blaising J, Polyak SJ, Pécheur EI (2014) Arbidol as a broad-spectrum antiviral: an update. Antivir Res 107(1):84–94. https://doi.org/10.1016/j.antiviral.2014.04.006

Blanshard A, Hine P (2021) Atovaquone-proguanil for treating uncomplicated Plasmodium falciparum malaria. Cochrane Database Syst Rev 2021(1):CD004529. https://doi.org/10.1002/14651858.CD004529.pub3

Boriskin Y, Leneva I, Pecheur E-I, Polyak S (2008) Arbidol: a broad-spectrum antiviral compound that blocks viral fusion. Curr Med Chem 15(10):997–1005. https://doi.org/10.2174/092986708784049658

Bush NG, Diez-Santos I, Abbott LR, Maxwell A (2020) Quinolones: mechanism, lethality and their contributions to antibiotic resistance. Molecules 25(23):5662. https://doi.org/10.3390/molecules25235662

Campbell S, Soman-Faulkner K (2021) Antiparasitic drugs. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/pubmed/31334971. Accessed 23 Jun 2021

Cao-Lormeau VM, Blake A, Mons S et al (2016) Guillain-Barré Syndrome outbreak associated with Zika virus infection in French Polynesia: a case-control study. Lancet 387(10027):1531–1539. https://doi.org/10.1016/S0140-6736(16)00562-6

Cappelletty D (1998) Microbiology of bacterial respiratory infections. Pediatr Infect Dis J 17(8): S55–S61. https://journals.lww.com/pidj/fulltext/1998/08001/microbiology_of_bacterial_respiratory_infections.2.aspx. Accessed 17 Jun 2021

Casadevall A, Dadachova E, Pirofski LA (2004) Passive antibody therapy for infectious diseases. Nat Rev Microbiol 2(9):695–703. https://doi.org/10.1038/nrmicro974

Chai Q, Jiao Y, Yu X (2017) Hydrogels for biomedical applications: their characteristics and the mechanisms behind them. Gels. https://doi.org/10.3390/gels3010006

Chang CH, Lin YH, Yeh CL et al (2010) Nanoparticles incorporated in pH-sensitive hydrogels as amoxicillin delivery for eradication of Helicobacter pylori. Biomacromolecules 11(1):133–142. https://doi.org/10.1021/bm900985h

Chinappi M, Via A, Marcatili P, Tramontano A (2010) On the mechanism of chloroquine resistance in Plasmodium falciparum. PLoS One 5(11). https://doi.org/10.1371/journal.pone.0014064

Chowdhary A, Sharma C, Meis JF (2017) Candida auris: a rapidly emerging cause of hospital-acquired multidrug-resistant fungal infections globally. PLoS Pathog 13(5):e1006290. https://doi.org/10.1371/journal.ppat.1006290

Cortez MH, Weitz JS (2013) Distinguishing between indirect and direct modes of transmission using epidemiological time series. Am Nat 181(2):E43–E54. https://doi.org/10.1086/668826

Cruciani M, Bassetti D (1994) The fluoroquinolones as treatment for infections caused by Gram-positive bacteria. J Antimicrob Chemother 33(3):403–417. https://doi.org/10.1093/jac/33.3.403

Cummings R, Turco S (2009) Parasitic infections. In: Essentials of Glycobiology, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1899/

Dagenais TRT, Keller NP (2009) Pathogenesis of Aspergillus fumigatus in invasive aspergillosis. Clin Microbiol Rev 22(3):447–465. https://doi.org/10.1128/CMR.00055-08

Dasaraju PV, Liu C (1996) Infections of the respiratory system. In: Baron S (ed) Medical microbiology, 4th edn. University of Texas Medical Branch at Galveston, Galveston. Chapter 93. Available from: https://www.ncbi.nlm.nih.gov/books/NBK8142/

Din FU, Aman W, Ullah I et al (2017) Effective use of nanocarriers as drug delivery systems for the treatment of selected tumors. Int J Nanomedicine 12:7291–7309. https://doi.org/10.2147/IJN.S146315

Docampo R, Moreno SN (1986) Free radical metabolism of antiparasitic agents. Fed Proc 45(10): 2471–2476. https://pubmed.ncbi.nlm.nih.gov/3017765/. Accessed 23 Jun 2021

Domachowske JB, Anderson EJ, Goldstein M (2021) The future of respiratory syncytial virus disease prevention and treatment. Infect Dis Ther 10(Suppl 1):47–60. https://doi.org/10.1007/s40121-020-00383-6

Doron S, Gorbach SL (2008) Bacterial infections: overview. In: International encyclopedia of public health. Elsevier Inc., Amsterdam, pp 273–282. https://doi.org/10.1016/B978-012373960-5.00596-7

Edelman R, Tacket CO, Wasserman SS, Bodison SA, Perry JG, Mangiafico JA (2000) Phase II safety and immunogenicity study of live chikungunya virus vaccine TSI-GSD-218. Am J Trop Med Hyg 62(6):681–685. https://doi.org/10.4269/ajtmh.2000.62.681

Etienne M, Caron F (2007) Management of fungal urinary tract infections. Press Medicale 36(12 III):1899–1906. https://doi.org/10.1016/j.lpm.2006.12.033

Fang W, Eashington L, Kumar N (2007) Imaging manifestations of Blastomycosis: a pulmonary infection with potential dissemination. Radiographics 27(3). https://doi.org/10.1148/rg.273065122

Franco MA, Greenberg HB (2012) Rotaviruses, noroviruses, and other gastrointestinal viruses. Goldman Cecil Med:2144–2147. https://doi.org/10.1016/B978-1-4377-1604-7.00388-2

Fielding RM, Lewis RO, Moon-McDermott L (1998) Altered tissue distribution and elimination of amikacin encapsulated in unilamellar, low clearance liposomes (MiKasome®). Pharm Res 15(11):1775–1781. https://doi.org/10.1023/A:1011925132473

Fisher JF, Sobel JD, Kauffman CA, Newman CA (2011) Candida urinary tract infections-treatment. Clin Infect Dis 52:S457–S466. https://doi.org/10.1093/cid/cir112

Fleck F (2004) SARS virus returns to China as scientists race to find effective vaccine. Bull World Health Organ 82(2):152–153. https://doi.org/10.1590/S0042-96862004000200016

Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ (2015) Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nat Rev Microbiol 13(5):269–284. https://doi.org/10.1038/nrmicro3432

Forsyth VS, Armbruster CE, Smith SN, Pirani A, Springman AC et al (2018) Rapid growth of uropathogenic Escherichia coli during human urinary tract infection. mBio 9(2). https://doi.org/10.1128/mBio.00186-18

Freeling JP, Koehn J, Shu C, Sun J, Ho RJY (2015) Anti-HIV drug-combination nanoparticles enhance plasma drug exposure duration as well as triple-drug combination levels in cells within lymph nodes and blood in primates. AIDS Res Hum Retrovir 31(1):107–114. https://doi.org/10.1089/aid.2014.0210

Gallis HA, Drew RH, Pickard WW (1990) Amphotericin B: 30 years of clinical experience. Rev Infect Dis 12(2):308–329. https://doi.org/10.1093/clinids/12.2.308

Garcia-Contreras L, Wong Y-L, Muttil P et al (2008) Immunization by a bacterial aerosol. Proc Natl Acad Sci U S A 105(12):4656–4660. https://doi.org/10.1073/pnas.0800043105

Grayson ML et al. (2017) Kucers' the use of antibiotics: A clinical review of antibacterial, antifungal, antiparasitic, and antiviral drugs, 7th edn. CRC Press, Boca Raton, FL, pp 3313–3329. https://doi.org/10.1201/9781315152110

Gavarkar PS, Adnaik RS, Mohite SK (2021) An overview of azole antifungals. Int J Pharm Sci Res. An-overview-of-azole-antifungals/?view=fulltext. Accessed 23 Jun 2021

Gerba CP (2009) Environmentally transmitted pathogens. Environ Microbiol:445–484. https://doi.org/10.1016/B978-0-12-370519-8.00022-5/

Gklinos P, Papadopoulou M, Stanulovic V, Mitsikostas DD, Papadopoulos D (2021) Monoclonal antibodies as neurological therapeutics. Pharmaceuticals 14(2):92. https://doi.org/10.3390/ph14020092

Hassan MA, Voigt K (2019) Pathogenicity patterns of mucormycosis: epidemiology, interaction with immune cells and virulence factors. Med Myco 57:S245–S256. https://doi.org/10.1093/mmy/myz011

Hayes LD, Ingram J, Sculthorpe NF (2021) More than 100 persistent symptoms of SARS-CoV-2 (Long COVID): a scoping review. Front Med 8:750378. https://doi.org/10.3389/fmed.2021.750378

Heesemann, J. (1993). Mechanisms of resistance to beta-lactam antibiotics Infection, 21 Suppl 1, S4-9. https://doi.org/10.1007/BF01710336

Helal ZM, Rizk DE, El-Sokkary MM, Hassan R (2020) Prevalence and characterization of streptococcus pyogenes clinical isolates from different hospitals and clinics in Mansoura. Int J Microbiol 5814945. https://doi.org/10.1155/2020/5814945

Hermann T (2007) Aminoglycoside antibiotics: old drugs and new therapeutic approaches. Cell Mol Life Sci 64(14):1841–1852. https://doi.org/10.1007/s00018-007-7034-x

Hoffman O, Weber RJ (2009) Pathophysiology and treatment of bacterial meningitis. Ther Adv Neurol Disord 2(6):1–7. https://doi.org/10.1177/1756285609337975

Hu CMJ, Aryal S, Zhang L (2010) Nanoparticle-assisted combination therapies for effective cancer treatment. Ther Deliv 1(2):323–334. https://doi.org/10.4155/tde.10.13

Huang L, Zhang L, Liu Y et al (2017) Arbidol for preventing and treating influenza in adults and children. Cochrane Database Syst Rev 2017(2):CD011489. https://doi.org/10.1002/14651858.CD011489.pub2

Huremović D (2019) Brief history of pandemics (pandemics throughout history). In: Psychiatry of pandemics. Springer International Publishing, Cham, pp 7–35. https://doi.org/10.1007/978-3-030-15346-5_2

Hussain A, Samad A, Singh SK et al (2016) Nanoemulsion gel-based topical delivery of an antifungal drug: in vitro activity and in vivo evaluation. Drug Deliv 23(2):652–667. https://doi.org/10.3109/10717544.2014.933284

Issa R (2014) Non-pathogenic protozoa. Int J Pharm Pharm Sci 6(12):30–40

Joshi M, Pathak S, Sharma S, Patravale V (2008) Solid microemulsion preconcentrate (NanOsorb) of artemether for effective treatment of malaria. Int J Pharm 362(1–2):172–178. https://doi.org/10.1016/j.ijpharm.2008.06.012

Kakamad FH, Mahmood SO, Rahim HM et al (2021) Post covid-19 invasive pulmonary Aspergillosis: a case report. Int J Surg Case Rep 82:105865. https://doi.org/10.1016/j.ijscr.2021.105865

Kesharwani P, Jain K, Jain NK (2014) Dendrimer as nanocarrier for drug delivery. Prog Polym Sci 39(2):268–307. https://doi.org/10.1016/j.progpolymsci.2013.07.005

Kim Y, Lovell S, Tiew K-C et al (2012) Broad-spectrum antivirals against 3C or 3C-like proteases of picornaviruses, noroviruses, and coronaviruses. J Virol 86(21):11754–11762. https://doi.org/10.1128/jvi.01348-12

Kirtane AR, Verma M, Karandikar P, Furin J, Langer R, Traverso G (2021) Nanotechnology approaches for global infectious diseases. Nat Nanotechnol 16(4):369–384. https://doi.org/10.1038/s41565-021-00866-8

Knight R (1980) The chemotherapy of amoebiasis. J Antimicrob Chemother 6(5):577–593. https://doi.org/10.1093/jac/6.5.577

Kousha M, Tadi R, Soubani AO (2011) Pulmonary aspergillosis: a clinical review. Eur Respir Rev 20(121):156–174. https://doi.org/10.1183/09059180.00001011

Lacey RW (1993) Food-borne bacterial infections. Parasitology 107(S1):S75–S93. https://doi.org/10.1017/S0031182000075521

Laha S, Chakraborty J, Das S, Manna SK, Biswas S, Chatterjee R (2020) Characterizations of SARS-CoV-2 mutational profile, spike protein stability and viral transmission. Infect Genet Evol 85:104445. https://doi.org/10.1016/j.meegid.2020.104445

Langer R (1998) Drug delivery and targeting. Nature 392(6679 Suppl):5–10. https://pubmed.ncbi.nlm.nih.gov/9579855/. Accessed 16 Jun 2021

Lamadé M, Craemer E, Schnitzler P (2019) Emerging and re-emerging viruses affecting the nervous system. Neurol Res Pract 1(20). https://doi.org/10.1186/s42466-019-0020-6

Laurens MB (2020) RTS, S/AS01 vaccine (Mosquirix™): an overview. Hum Vaccin Immunother 16(3):480–489. https://doi.org/10.1080/21645515.2019.1669415

Lebold T, Jung C, Michaelis J, Bräuchle C (2009) Nanostructured silica materials as drug-delivery systems for doxorubicin: single molecule and cellular studies. Nano Lett 9(8):2877–2883. https://doi.org/10.1021/nl9011112

Lever RA, Whitty CJM (2016) Ebola virus disease: emergence, outbreak and future directions. Br Med Bull 117(1):95–106. https://doi.org/10.1093/bmb/ldw005

Löbenberg R, Araujo L, Von Briesen H, Rodgers E, Kreuter J (1998) Body distribution of azidothymidine bound to hexyl-cyanoacrylate nanoparticles after i.v. injection to rats. J Control Release 50(1–3):21–30. https://doi.org/10.1016/S0168-3659(97)00105-3

López-Cortés LE, Garcia-Vidal C, Ayats J, Gudiol C, Bodro M et al (2012) Invasive aspergillosis with extrapulmonary involvement: pathogenesis, clinical characteristics and prognosis. Rev Iberoam Micol 29(3):139–143. https://doi.org/10.1016/j.riam.2011.10.001

Lou Z, Sun Y, Rao Z (2014) Current progress in antiviral strategies. Trends Pharmacol Sci 35(2):86–102. https://doi.org/10.1016/j.tips.2013.11.006

Lowe R, Barcellos C, Brasil P, Cruz OG, Honorio NA et al (2018) The Zika virus epidemic in Brazil: from discovery to future implications. Int J Environ Res Public Health 15(1):96. https://doi.org/10.3390/ijerph15010096

Lu J, Liong M, Zink JI, Tamanoi F (2007) Mesoporous silica nanoparticles as a delivery system for hydrophobic anticancer drugs. Small 3(8):1341–1346. https://doi.org/10.1002/smll.200700005

Makadia HK, Siegel SJ (2011) Poly Lactic-co-Glycolic Acid (PLGA) as biodegradable controlled drug delivery carrier. Polymers (Basel) 3(3):1377–1397. https://doi.org/10.3390/polym3031377

Marques J, Valle-Delgado JJ, Urbán P et al (2017) Adaptation of targeted nanocarriers to changing requirements in antimalarial drug delivery. Nanomed Nanotechnol Biol Med 13(2):515–525. https://doi.org/10.1016/j.nano.2016.09.010

Mbonye AK, Wamala JF, Nanyunja M, Opio A, Makumbi I et al (2014) Ebola viral hemorrhagic disease outbreak in West Africa- lessons from Uganda. Afr Health Sci 14(3):495–501. https://doi.org/10.4314/ahs.v14i3.1

McArthur DB (2019) Emerging infectious diseases. Nurs Clin North Am 54(2):297–311. https://doi.org/10.1016/j.cnur.2019.02.006

Melariri P, Kalombo L, Nkuna P et al (2015) Oral lipid-based nanoformulation of tafenoquine enhanced bioavailability and blood stage antimalarial efficacy and led to a reduction in human red blood cell loss in mice. Int J Nanomedicine 10:1493–1503. https://doi.org/10.2147/IJN.S76317

Mingeot-Leclercq MP, Glupczynski Y, Tulkens PM (1999) Aminoglycosides: activity and resistance. Antimicrob Agents Chemother 43(4):727–737. https://doi.org/10.1128/aac.43.4.727

Noor A, Preuss CV (2021) Amphotericin B – StatPearls - NCBI Bookshelf. https://www.ncbi.nlm.nih.gov/books/NBK482327/. Accessed 23 Jun 2021

OnHealth (2021) Bacterial infections 101: types, symptoms, and treatments. https://www.onhealth.com/content/1/bacterial_infections. Accessed 17 Jun 2021

Page MGP (2012) Beta-lactam antibiotics. In: Antibiotic discovery and development, vol 9781461414001. Springer, New York, pp 79–117. https://doi.org/10.1007/978-1-4614-1400-1_3

Pandey R, Khuller GK (2005) Solid lipid particle-based inhalable sustained drug delivery system against experimental tuberculosis. Tuberculosis 85(4):227–234. https://doi.org/10.1016/j.tube.2004.11.003

Pandey R, Sharma S, Khuller GK (2005) Oral solid lipid nanoparticle-based antitubercular chemotherapy. Tuberculosis (Edinb) 85:415–420. https://doi.org/10.1016/j.tube.2005.08.009

Papich MG (2019) Pharmacotherapeutics of infectious disease. In: Pharmacotherapeutics for veterinary dispensing. Wiley, Hoboken, NJ, pp 189–230. https://doi.org/10.1002/9781119404576.ch9

Parashar D, Aditya NP, Murthy RSR (2016) Development of artemether and lumefantrine co-loaded nanostructured lipid carriers: physicochemical characterization and in vivo antimalarial activity. Drug Deliv 23(1):123–129. https://doi.org/10.3109/10717544.2014.905883

Pardo J, Shukla AM, Chamarthi G, Gupte A (2020) The journey of remdesivir: from Ebola to COVID-19. Drug Context 9. https://doi.org/10.7573/DIC.2020-4-14

Pathakumari B, Liang G, Liu W (2020) Immune defence to invasive fungal infections: a comprehensive review. Biomed Pharmacother 130:110550. https://doi.org/10.1016/j.biopha.2020.110550

Pattnaik A, Sahoo BR, Pattnaik AK (2020) Current status of zika virus vaccines: successes and challenges. Vaccine 8(2):266. https://doi.org/10.3390/vaccines8020266

Pecetta S, Finco O, Seubert A (2020) Quantum leap of monoclonal antibody (mAb) discovery and development in the COVID-19 era. Semin Immunol 50:101427. https://doi.org/10.1016/j.smim.2020.101427

Pérez-Molina JA, Sojo-Dorado J, Norman F, Monge-Maillo B, Díaz-Menéndez M et al (2013) Nifurtimox therapy for Chagas disease does not cause hypersensitivity reactions in patients with such previous adverse reactions during benznidazole treatment. Acta Trop 127(2):101–104. https://doi.org/10.1016/j.actatropica.2013.04.003

Piret J, Boivin G (2021) Pandemics throughout history. Front Microbiol 11:631736. https://doi.org/10.3389/fmicb.2020.631736

Poole K (2005) Aminoglycoside resistance in Pseudomonas aeruginosa. Antimicrob Agents Chemother 49(2):479–487. https://doi.org/10.1128/AAC.49.2.479-487.2005

Poulikakos P, Falagas ME (2013) Aminoglycoside therapy in infectious diseases. Expert Opin Pharmacother 14(12):1585–1597. https://doi.org/10.1517/14656566.2013.806486

Prabhu RH, Patravale VB, Joshi MD (2015) Polymeric nanoparticles for targeted treatment in oncology: current insights. Int J Nanomedicine 10:1001–1018. https://doi.org/10.2147/IJN.S56932

Pristov KE, Ghannoum MA (2019) Resistance of Candida to azoles and echinocandins worldwide. Clin Microbiol Infect 25(7):792–798. https://doi.org/10.1016/j.cmi.2019.03.028

Raut A, Huy NT (2021) Rising incidence of mucormycosis in patients with COVID-19: another challenge for India amidst the second wave? Lancet Respir Med. https://doi.org/10.1016/S2213-2600(21)00265-4

Rismanbaf A (2020) Potential treatments for COVID-19; a narrative literature review. Arch Acad Emerg Med 8(1):e29

Rouphael NG, Stephens DS (2012) Neisseria meningitidis: biology, microbiology, and epidemiology. Methods Mol Biol (Clifton NJ) 799:1–20. https://doi.org/10.1007/978-1-61779-346-2_1

Saleh D, Yarrarapu SNS, Sharma S (2021) Herpes simplex type 1. In: StatPearls. StatPearls Publishing, Treasure Island. Available from: https://www.ncbi.nlm.nih.gov/books/NBK482197/

Schiffelers R, Storm G, Bakker-Woudenberg I (2001) Liposome-encapsulated aminoglycosides in pre-clinical and clinical studies. J Antimicrob Chemother 48(3):333–344. https://doi.org/10.1093/jac/48.3.333

Scott LJ, Perry CM (2002) Interferon-α-2b plus ribavirin: a review of its use in the management of chronic hepatitis C. Drugs 62(3):507–556. https://doi.org/10.2165/00003495-200262030-00009

Sears D, Schwartz BS (2017) Candida auris: an emerging multidrug-resistant pathogen. Int J Infect Dis 63:95–98. https://doi.org/10.1016/j.ijid.2017.08.017

Shiraki K, Daikoku T (2020) Favipiravir, an anti-influenza drug against life-threatening RNA virus infections. Pharmacol Ther 209:107512. https://doi.org/10.1016/j.pharmthera.2020.107512

Sinawe H, Casadesus D (2021) Ketoconazole-StatPearls. StatPearls Publishing, Treasure Island. Available from: https://www.ncbi.nlm.nih.gov/books/NBK559221/

Singh H, Jindal S, Singh M, Sharma G, Kaur IP (2015) Nano-formulation of rifampicin with enhanced bioavailability: development, characterization and in-vivo safety. Int J Pharm 485(1–2):138–151. https://doi.org/10.1016/j.ijpharm.2015.02.050

Singh TU, Parida S, Lingaraju MC, Kesavan M, Kumar D, Singh RK (2020) Drug repurposing approach to fight COVID-19. Pharmacol Rep 72(6):1479–1508. https://doi.org/10.1007/s43440-020-00155-6

Sivanandy P, Jun PH, Man LW, Wei NS, Mun NF et al (2022) A systematic review of Ebola virus disease outbreaks and an analysis of the efficacy and safety of newer drugs approved for the treatment of Ebola virus disease by the US Food and Drug Administration from 2016 to 2020. J Infect Public Health 15:285–292. https://doi.org/10.1016/j.jiph.2022.01.005

Slowing II, Vivero-Escoto JL, Wu CW, Lin VSY (2008) Mesoporous silica nanoparticles as controlled release drug delivery and gene transfection carriers. Adv Drug Deliv Rev 60(11):1278–1288. https://doi.org/10.1016/j.addr.2008.03.012

Solbak SM, Wray V, Horvli O et al (2011) The host-pathogen interaction of human cyclophilin A and HIV-1 Vpr requires specific N-terminal and novel C-terminal domains. BMC Struct Biol 11(1):1–16. https://doi.org/10.1186/1472-6807-11-49

Takizawa N, Yamasaki M (2018) Current landscape and future prospects of antiviral drugs derived from microbial products. J Antibiot (Tokyo) 71(1):45–52. https://doi.org/10.1038/ja.2017.115

Thaker SK, Ch'ng J, Christofk HR (2019) Viral hijacking of cellular metabolism. BMC Biol 17(1):59. https://doi.org/10.1186/s12915-019-0678-9

Torchilin VP (2005) Recent advances with liposomes as pharmaceutical carriers. Nat Rev Drug Discov 4(2):145–160. https://doi.org/10.1038/nrd1632

Vacas-Córdoba E, Maly M, De la Mata FJ, Gómez R, Pion M, Muñoz-Fernández MÁ (2016) Antiviral mechanism of polyanionic carbosilane dendrimers against HIV-I. Int J Nanomedicine 11:1281–1294. https://doi.org/10.2147/IJN.S96352

van de Beek D, Brouwer MC, Thwaites GE, Tunkel AR (2012) Advances in treatment of bacterial meningitis. Lancet 380(9854):1693–1702. https://doi.org/10.1016/S0140-6736(12)61186-6

van Seventer JM, Hochberg NS (2017) Principles of infectious diseases: transmission, diagnosis, prevention, and control. Int Encyclop Pub Health:22–39. https://doi.org/10.1016/B978-0-12-803678-5.00516-6

Vaughan AM, Kappe SHI (2017) Malaria parasite liver infection and exoerythrocytic biology. Cold Spring Harb Perspect Med 7(6). https://doi.org/10.1101/cshperspect.a025486

Ventola CL (2012) The nanomedicine revolution: part 1: emerging concepts. P T Peer Rev J Form Manag 37(9):512–525. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3462600/

Vere CC, Streba CT, Streba L, Rogoveanu I (2012) Statins in the treatment of hepatitis C. Hepat Mon 12(6):369–371. https://doi.org/10.5812/hepatmon.5998

Wachino JI, Arakawa Y (2012) Exogenously acquired 16S rRNA methyltransferases found in aminoglycoside-resistant pathogenic Gram-negative bacteria: an update. Drug Resist Updat 15(3):133–148. https://doi.org/10.1016/j.drup.2012.05.001

Wali U, Balkhair A, Al-Mujaini A (2011) Cerebro-rhino orbital mucormycosis: an update. J Infect Public Health 5:116–216. https://doi.org/10.1016/j.jiph.2012.01.003

Wang X, Wang Y, Chen ZG, Shin DM (2009) Advances of cancer therapy by nanotechnology. Cancer Res Treat 41(1):1. https://doi.org/10.4143/crt.2009.41.1.1

Wang H, Zhao Y, Wu Y et al (2011) Enhanced anti-tumor efficacy by co-delivery of doxorubicin and paclitaxel with amphiphilic methoxy PEG-PLGA copolymer nanoparticles. Biomaterials 32(32):8281–8290. https://doi.org/10.1016/j.biomaterials.2011.07.032

Wang AZ, Langer R, Farokhzad OC (2012) Nanoparticle delivery of cancer drugs. Annu Rev Med 63:185–198. https://doi.org/10.1146/annurev-med-040210-162544

Warren TK, Wells J, Panchal RG et al (2014) Protection against filovirus diseases by a novel broad-spectrum nucleoside analogue BCX4430. Nature 508(7496):402–405. https://doi.org/10.1038/nature13027

Weber DJ, Sickbert-Bennett EE, Kanamori H, Rutala WA (2019) New and emerging infectious diseases (Ebola, Middle Eastern respiratory syndrome coronavirus, carbapenem-resistant Enterobacteriaceae, Candida auris): focus on environmental survival and germicide susceptibility. Am J Infect Control 47:A29–A38. https://doi.org/10.1016/j.ajic.2019.03.004

Weiser JN, Ferreira DM, Paton JC (2018) Streptococcus pneumoniae: transmission, colonization and invasion. Nat Rev Microbiol 16(6):355–367. https://doi.org/10.1038/s41579-018-0001-8

Winn WC, Walker DH (1994) Viral infections. In: Pulmonary pathology. Springer, New York, pp 429–464. https://doi.org/10.1007/978-1-4757-3935-0_12

Wormser GP, Dattwyler RJ, Shapiro ED, Halperin JJ et al (2006) The clinical assessment, treatment and prevention of lyme disease, human granulocytic anaplasmosis, and babesiosis: clinical practice guidelines by the Infectious Diseases Society of America. Clin Infect Dis 43(9):1089–1134. https://doi.org/10.1086/508667

Wu W, Li R, Bian X et al (2009) Covalently combining carbon nanotubes with anticancer agent: preparation and antitumor activity. ACS Nano 3(9):2740–2750. https://doi.org/10.1021/nn9005686

Yan Y, Chan-Park MB, Zhang Q (2007) Advances in carbon-nanotube assembly. Small 3(1):24–42. https://doi.org/10.1002/smll.200600354

Zhao T, Qian Y-H, Chen S-H, Wang G-L et al (2016) Novel H7N2 and H5N6 Avian Influenza A viruses in sentinel chickens: a sentinel chicken surveillance study. Front Microbiol 7:1766. https://doi.org/10.3389/fmicb.2016.01766

Zahoor A, Sharma S, Khuller GK (2005) Inhalable alginate nanoparticles as antitubercular drug carriers against experimental tuberculosis. Int J Antimicrob Agents 26(4):298–303. https://doi.org/10.1016/j.ijantimicag.2005.07.012

Part II

Nanomaterials as Anti-infection Therapeutics

Advanced Nanomaterials for Infectious Diseases Therapeutics

4

Irfana Zahoor, Jaffar Farooq Mir, and M. A. Shah

Abstract

The leading cause of deaths and morbidity worldwide are infectious diseases. The affect is more common in people with weakened immune systems and children. Development of multiple drug resistance limits the use of ongoing therapies to infections. This is one of the main disadvantages of conventional antimicrobial agents and leads to frequent administration of high doses of drugs, followed by adverse after-effects. Thus, stating that there is a serious and urgent need to overcome drug resistance by developing new therapeutics. The utility of nanoparticle systems may prove beneficial to control such issues and elevate the effectiveness of drugs. There is currently substantial interest in utilizing them as antimicrobial agents towards several microbes such as bacteria, parasites, viruses, and fungi. This chapter will examine the available reports on metal nanoparticles, encapsulated nanoparticles, and nanoantibiotics as new promising medically helpful tools for treating infectious diseases and their efficacy.

Keywords

Infectious diseases · Metal-based nanoparticles · Nanoantibiotics · Therapeutics

Globally infectious diseases due to bacteria, parasites, viruses, and fungi are responsible for many deaths and can be emerging infectious diseases, i.e., new diseases or

I. Zahoor
School of Life Sciences, Jaipur National University, Jaipur, India

J. F. Mir · M. A. Shah (✉)
Laboratory for Multifunctional Nanomaterials, P.G Department of Physics, National Institute of Technology Srinagar, Srinagar, Jammu and Kashmir, India
e-mail: shah@nitsri.net

S. Hameed, S. Rehman (eds.), *Nanotechnology for Infectious Diseases*,
https://doi.org/10.1007/978-981-16-9190-4_4

re-emerging diseases referring to infections that reappear and endure from drug resistance, complicating them to control or treat (Parrish et al. 2008; Fauci and Morens 2012; Morens and Fauci 2013; Singh et al. 2020). In spite of the fact that the immune system of man is able to safeguard the body from infections, certain infections are exceptionally contagious and harmful (Aderibigbe 2017), while others are not communicable. Generally, infections are transmitted once microorganisms that cause infections enter the body of host through natural body openings, which leads to the microorganism growing at the entry site and after that multiplying within the host cells, followed by tissue damage (Aderibigbe 2017; Fatima et al. 2021). According to the definition of the WHO, a microorganism is considered to be resistant in case it does not respond to standard medications and the infection is subsequently exceptionally untreatable and its spread to be ceased (WHO 2014). Some strains that are multidrug resistant are *Mycobacterium tuberculosis*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococci* sp., *Streptococcus pneumoniae*, *Acinetobacter baumannii*, *Neisseria gonorrhoeae*, *Shigella* sp., and Nontyphoidal *Salmonella* (WHO 2014; Singh et al. 2014; Alkharsah et al. 2018). This indicates that the treatment for infections need to second line medications. These medications are not always accessible, can be the reason for various and adverse after-effects and must be given through intravenous route, which is costly (WHO 2014). However, the real issue is that the microbes have turned resistant to second line medications, for instance, methicillin-resistant *Staphylococcus aureus* and *Klebsiella pneumoniae* to vancomycin and carbapenems, respectively, and also to third generation drugs. It is assessed that nearly 20% of cases are safe to right now accessible medications for HIV and tuberculosis diseases (WHO 2014; Zazo et al. 2016). Drug resistance has hampered the infectious diseases treatment, indicating towards the immediate need to look for novel therapeutics to overcome the drug resistance (Rehman et al. 2019). Therapeutics based on antibodies metal-based nanoparticles, etc., for infectious diseases treatment are progressing (Casadevall 1996; Saylor et al. 2009). The small size of metal-based nanoparticles (10–100 nm) accounts their great interaction on surface and in the cell with biomolecules. Their surface area being large promotes permeability of cell. Moreover, metal nanoparticles have great physicochemical properties. Due to these distinctive properties, metal nanoparticles become potent therapeutics for infectious diseases treatment. For biomedical applications, metal-based nanoparticles need to meet certain conditions, e.g., being stable and non-aggregated, biocompatible, specific for target cells/tissue, non-toxic, and economical (Mody et al. 2010). They can moreover be modified by conjugating chosen antibodies, proteins, ligands, and drugs that have specified binding affinity to targeted cells, subsequently making them more capable for drug delivery to targeted cells and therapeutic efficacy (Mody et al. 2010). The amalgamation of antibodies, drugs, proteins, etc. on metal nanoparticles safeguard nanoparticles from the body's defense system (Mody et al. 2010; Aderibigbe 2017). Also, different nanoscale drug carriers are accessible for the effective administration of antibodies by ameliorating aggregation and pharmacokinetics, whereas decreasing the after-effects of antibiotics. Abstractly, nanoparticles remain within the body for much longer time than antibiotics, which

may be useful for accomplishing enduring remedial effects (Allaker and Ren 2008; Huh and Kwon 2011; Zhang et al. 2022). This chapter presents metal-based nanoparticles, encapsulated nanoparticles, and nanoantibiotics that are potent therapeutics for treating infectious diseases (i.e., bacterial, parasitic, viral, and fungal) and their efficacy.

4.1 Metal-Based Nanoparticles

Metal-based nanoparticles are specified by their high surface area and minute size (Arvizo et al. 2012; Al-Jameel et al. 2021). Their morphology and surface charge impact their cellular uptake. Moreover, their cellular interactions are enhanced on account of the presence of superficial functionalities on certain metal nanoparticles. Several kinds of metal-based nanoparticles studied by many researchers are without a known mechanism of action. The toxicity mechanism of metal nanoparticles varies among different strains. Mostly, metals show toxicity towards all types of cells, but discriminating among strains (Aderibigbe 2017; Nahvi et al. 2021). Metal-based nanoparticles studied to date that have antibacterial, antifungal, antiviral, and antiparasitic properties include silver, gold iron oxide, zinc oxide, copper oxide, gallium, titanium dioxide, and aluminum oxide nanoparticles (Alahmari et al. 2021; Almessiere et al. 2020a; Baig et al. 2020) .

4.1.1 Silver Nanoparticles

Silver as one of the commonly utilized nanomaterials has been known long-standing. In spite of the fact that the disputable genotoxic effects, cytotoxic effects, and lower selectivity of silver nanoparticles raise question on their utilization as antimicrobial agents, their physicochemical properties such as morphology, load, stabilization, and concentration amalgamate their consideration within the desirable agents of the new era (Natalia et al. 2019; Rehman et al. 2020a). Silver nanoparticles after interaction with microbes discharge silver particles, causing cellular deactivation of proteins, hinder permeability of membrane and cell death (Choi et al. 2008; Sondi and Salopek-Sondi 2004). Sondi and Salopek-Sondi (2004) proposed that exposing bacteria *Escherichia coli* to Ag_2O nanoparticles resulted in loss of replication potential of DNA and the cell cycle ceased due to DNA damage at the G2/M stage. At that point cells were influenced by oxidative stress followed by initiation of apoptosis (Sondi and Salopek-Sondi 2004). Another approach by Hernández-Sierra et al. (2008) described the antibacterial property of silver nanoparticles against *Streptococcus mutans*, suggesting that they might be utilized to treat dental caries. The bactericidal activity of silver nanoparticles was explored towards multidrug-resistant pathogens *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*. Silver nanoparticles appeared critical against *Klebsiella pneumonia* and *Staphylococcus aureus* with an inhibition zone of 18 and 17 mm at 1000 μg/mL of concentration with MIC 62.5 and 15.6 μg/mL,

respectively (Mujaddidi et al. 2021; Ravinayagam and Rehman 2020; Rehman et al. 2020b). Prabha et al. studied silver nanoparticles as potential therapeutics for tuberculosis and showed growth inhibiting effects of *Mycobacterium tuberculosis*, dependent on nanoparticle concentrations. *Mycobacterium tuberculosis* growth was inhibited 25–50 mM concentration of the nanoparticle hindered the development of *Mycobacterium tuberculosis* (Praba et al. 2013). Amalgamation of zinc oxide and silver nanoparticles was reported by Jafari et al. (2016), silver nanoparticles were showed very low cytotoxicity (in vitro) and did not repressed the growth of *M. tuberculosis*. But combining zinc oxide and silver nanoparticles at an optimized condition showed significant antibacterial activity towards *M. tuberculosis* (Jafari et al. 2016).

Silver nanoparticles were studied as potent therapeutics for the treating sexually transmitted infections like gonorrhea, syphilis, and chlamydia (Alexander 2009; Yilma et al. 2013). Chlamydia is a sexually transmitted disease, *Chlamydia trachomatis* being the responsible pathogen, that more often causes serious inflammatory responses. Silver polyvinylpyrrolidone nanoparticles were prepared with great anti-inflammatory properties. In vitro trial on a mouse model J774 macrophage of *C. trachomatis* disease showed that the nanoparticles were able to control mediators that caused inflammation due to the infection by *Chlamydia trachomatis*. The nanoparticles moreover restrained cytokines and chemokines produced by the macrophage infection (Yilma et al. 2013). The nanoparticles were exceptionally portable and stable without agglomeration. Silver nanoparticles have too been known to treat infections of urinary tract. In a research, Inbaneson et al. proposed the impact of silver nanoparticles to infections of urinary tract caused by *Enterobacter* and *Pseudomonas aeruginosa* (Inbaneson et al. 2011). The mechanism of silver nanoparticles activity was accepted to be by the nanoparticles interaction with cell membrane surface and the hindrance of the respiratory capacities of the cell, which disturbs the ATP generation and replication of the microbial DNA (Inbaneson et al. 2011). It has too been proposed that the urinary catheter incorporated with silver nanoparticles leads to decreased risk of infections of urinary tract (Pickard et al. 2012; Ritter et al. 2013).

The antifungal action of silver nanoparticles is very less considered than to its antibacterial action; though there are significant reports that prove silver nanoparticles possess fungicidal properties. Silver nanoparticles showed significant antifungal action than the standard drug natamycin studied against above 200 strains from the patients enduring serious keratitis caused by *Alternaria alternate*, *Aspergillus niger*, *Fusarium oxysporum*, *Fusarium verticillioides*, *Fusarium solani*, *Aspergillus fumigates*, *Aspergillus flavus*, *Aspergillus versicolor* (Xu et al. 2013).

Researchers have also reported the antiplasmodial action of silver nanoparticles. Murugan et al. synthesized silver nanoparticles utilizing spongeweed and *C. tomentosum* for reduction and stabilization purpose. The synthesized nanoparticles were tested to chloroquine sensitive and chloroquine resistant microbes of *Plasmodium falciparum* (IC50) were 72.45 and 76.08 μg/mL, respectively (Murugan et al. 2016).

4.1.2 Gold Nanoparticles

With the advancement of antibacterial agents, gold nanoparticles are regarded to be exceptionally valuable due to their capacity for large functionalization, easy detection, non-toxic behavior, polyvalent impacts and their photothermal property (Dizaj et al. 2014). Researchers have discussed the reasons behind the bactericidal activity of gold nanoparticles, that comprised of binding to cell membrane of bacteria, consequently alteration within the bacterial membrane potential and reduction in ATP levels, also hampering attachment of transfer RNA to the ribosome (Cui et al. 2012). Mohamed et al. fabricated gold nanoparticles that were potent towards bacteria *Corynebacterium pseudotuberculosis*, that infect the sheep. The bactericidal action of gold nanoparticles is based on the capacity of the nanoparticles to cross the cell wall barrier (Mohamed et al. 2017).

Sametband et al. elucidated gold nanoparticles as therapeutic agents against the flu infection. The viricidal action of the nanoparticles was based on inhibiting the binding of viral strain to the cell surface (Sametband et al. 2011). Dutta et al. prepared gold nanoparticles utilizing bark and leaf of *Syzygium jambos* (Dutta et al. 2017). These gold nanoparticles displayed significant antiplasmodial activity towards the chloroquine sensitive pathogen and chloroquine resistant pathogen of *P. falciparum* (Dutta et al. 2017). Evaluation of anthelmintic activity of gold nanoparticles synthesized by reducing gold chloride with mycelium-free culture filtrate of fungus. The fabricated gold nanoparticles ceased the parasitic physiological action, followed by numbness and death. Changes within the parasitic protein activity after treating with gold nanoparticles were critical and showed the potential of gold nanoparticles (Kar et al. 2014).

4.1.3 Iron-Oxide Based Nanoparticles

Iron and iron-oxide nanoparticles have attained much scrutiny in recent period (Mir et al. 2020a, b). Their mechanism of action have also been studied by some researchers. The bactericidal action of iron-oxide nanoparticles is due to oxidative stress by ROS, damaging bacterial protein and DNA (Rafi et al. 2015; Balasamy et al. 2019). Fabrication of iron-oxide nanoparticles with the peel extract of *Punica granatum* showed strong bactericidal activity against *Pseudomonas aeruginosa* (Irshad et al. 2017). Ahmad et al. synthesized hexagonal shaped iron-oxide nanoparticles through co-precipitation approach and after that amended them with leaf extract of *Ocimum sanctum*. The amended approach of the nanoparticles with the extract of *Ocimum sanctum* showed significant bactericidal activity towards *Staphylococcus aureus* (Ahmad et al. 2017). Iron-oxide nanoparticles prepared using *Withania coagulans* resulted in strong antibacterial action against *P. aeruginosa* and *S. aureus* when compared with iron-oxide nanorods by chemical approach, revealing that green synthesized iron-oxide nanorods are much effective than chemically synthesized nanorods (Qasim et al. 2020). Figure 4.1 shows iron-oxide nanorods synthesized through chemical approach. Iron-oxide nanoparticles

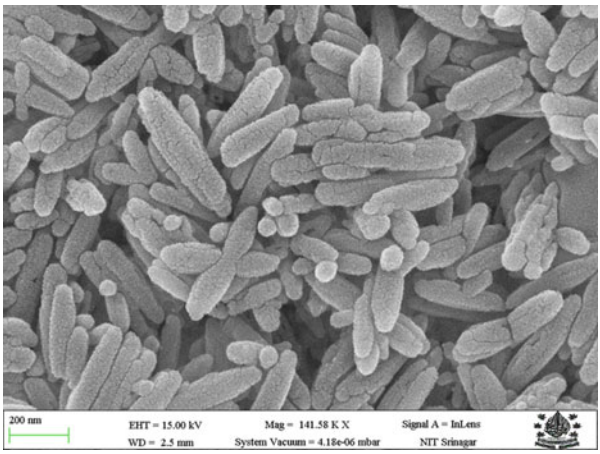

Fig. 4.1 SEM image of iron oxide nanoparticles

showed concentration dependent antimicrobial activity when synthesized using *Euphorbia hirta* towards *Arthogrophis cuboida*, *Aspergillus niger*, and *Aspergillus fumigatus* (Ahmad et al. 2021).

Abo-zeid et al. studied the virucidal action of iron-oxide nanoparticles against HCV and SARS-CoV-2 by molecular docking. Models showed that the association of Fe_2O_3 and Fe_3O_4 was efficient with HCV glycoproteins E1 and E2 and SARS-CoV2-S1-RBD. It was proposed that Fe_3O_4 was much stable in combining with S1-RBD and for HCV E1 and E2 much stable combination was made with Fe_2O_3. It is anticipated that these interactions are related to changes in conformations in structural proteins of virus, followed by virus inactivation (Abo-Zeid et al. 2020).

Exploitation of iron nanoparticles for DNAzyme delivery was studied by Ryoo et al., for treating hepatitis C. Damage was induced to gene NS3 of the hepatitis C virus. The HCV NS3 gene encodes for protease and helicase, necessary for viral replication. After the nanoparticles were administered in the animal models, accumulation of nanoparticles was observed within the macrophages and hepatocytes inside the liver, thus revealing their ability to treat hepatitis C (Ryoo et al. 2012).

4.1.4 Zinc Oxide Nanoparticles

Zinc oxide nanoparticles have been studied against different microbes and showed potent antibacterial activity. Figure 4.2 shows SEM images of (a) zinc and (b) zinc oxide nanorods. Reddy et al. fabricated zinc oxide nanoparticles with great antibacterial action towards *K. pneumonia* that causes respiratory infections. These synthesized nanoparticles destroyed cell wall of bacteria and restrained the internalization of the intrusion by non-phagocytic cells (Reddy et al. 2014).

Rago et al. examined the antimicrobial activity of nanorods and microrods of zinc oxide against gram-positive strain. Both nanomaterials showed noteworthy antibacterial activity, but that of nanorods were effective and higher. ROS

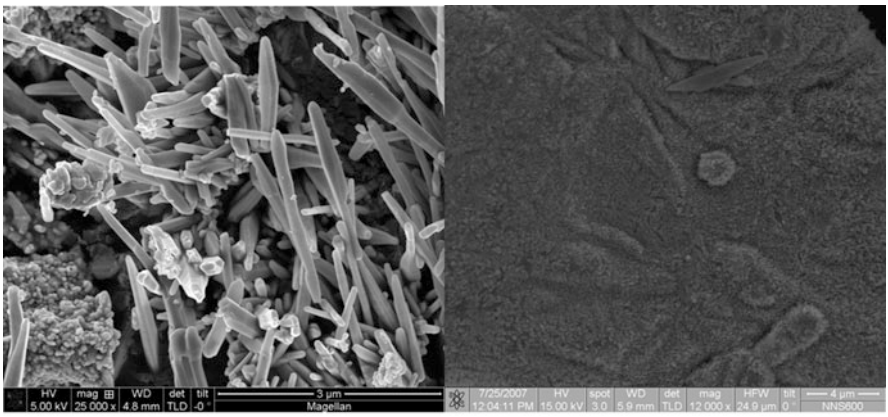

Fig. 4.2 SEM images of (**a**) zinc and (**b**) zinc oxide nanoparticles

production was not observed, but *B. subtilis* cells were encountered with high damage than to *S. aureus*, proposing that the cell morphology influences the bactericidal action of nanomaterials (Rago et al. 2014; Almessiere et al. 2020b). Zinc oxide nanoparticles were fabricated using extract of *Petroselinum crispum* and resulted in significant antibacterial activity. The green synthesized nanoparticles showed enhanced activity than nanoparticles fabricated by chemical approach (Stan et al. 2015). Pseudo stem extract of banana (*Musa balbisiana* Colla), an agro-biowaste has been utilized for reduction purpose for the green fabrication of zinc oxide nanoparticles and its application towards antibiofilm and bactericidal activity. Zinc oxide nanoparticles were effective against pathogens including *B. subtilis*, *S. aureus*, *E. coli*, and *P. aeruginosa*. Significant antibiofilm activity was also revealed in case of *P. aeruginosa*. The authors concluded that due to strong activities of zinc oxide nanoparticles such as antibiofilm and antibacterial they can be used as water disinfectants, drug industry, and food conservation (Akhtar et al. 2020; Basumatari et al. 2021).

It has been reported that green synthesis of zinc oxide nanoparticles has an improved antibacterial impact against selected bacterial pathogens compared to nanoparticles fabricated using a chemical approach (Gunalan et al. 2012; Venkataraju et al. 2014). The variation in antibacterial activity is assigned to the size of nanoparticles. The mechanism of action is assigned to bacterial cell damage, zinc (II) ions release, and the ROS production (Gunalan et al. 2012; Venkataraju et al. 2014). Zinc oxide nanoparticles possess potent antifungal properties towards the filamentous pathogens, *A. alternata* and *F. verticillioides*. The growth inhibition and reproduction of fungi were significantly elevated with the zinc oxide nanomaterials. Zinc ions can be discharged from the surface of zinc oxide nanoparticles after interaction with cell wall of fungi and accumulated within the cytosol. This hinders metabolism of cell, disassembly of ribosome, denaturation of

protein, disruption of electron chain, eventually leading to cell death (Akpomie et al. 2021).

Surwade et al. reported the alterations in bactericidal activity after charge alterations on outer membrane of *Haemophilus influenzae* using wild strain (Rd) and H446 and H543 (mutant lines). The report demonstrates that zinc oxide nanoparticles communicate with bacterial outer membrane and has an important role to play in mechanism of action. The results reveal that with a better negative charge on the external layer, the nanoparticle toxicity towards the microbes is negligible. In other case, presence of functional groups with better positive charge on membrane made the bacteria sensitive to nanoparticle toxicity. But it may vary with other pathogens tested (Surwade et al. 2020).

Zinc oxide has been assessed as a strong leishmanicidal agent (Delavari et al. 2014; Nadhman et al. 2016). Delavari et al. proposed dose-dependent leishmanicidal activity of zinc oxide nanoparticles with 37.8 μg/mL IC50 value after 1 day incubation. The nanoparticles were cytotoxic to promastigotes by initiating apoptosis. After 72 h zinc nanoparticles at 120 μg/mL induced 93.76% apoptosis (Delavari et al. 2014).

4.1.5 Copper Oxide Nanoparticles

Copper nanoparticles are nowadays gaining attention owed to their antimicrobial properties, as well as their cost effectiveness. As with other metals, copper accumulation can be toxic, but the body homeostasis is controlled with the help of copper transporters. But these nanoparticles have issues of agglomeration and quick oxidation, thereby need a stabilizer. The stabilizer should be used in low concentration otherwise it can alter the morphology nanoparticles (Usman et al. 2013). The SEM images of copper nanoparticles are shown in Fig. 4.3.

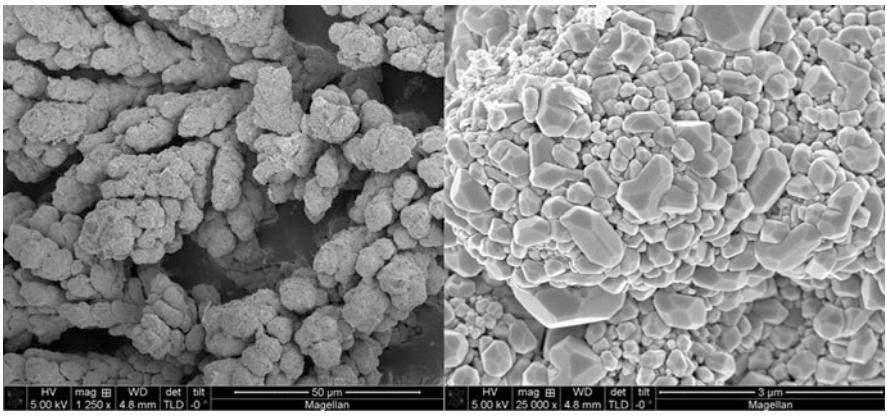

Fig. 4.3 SEM images of copper nanoparticles

Pandey et al. successfully fabricated nanorods and multi-armed nanoparticles of copper oxide, by electrochemical and wet approaches. The excellent bactericidal activity of nanoparticles was due to their shape. The multi-armed nanoparticles interacted with the bacterial cell, effectively penetrated into cell and lead to cell death. The cells were killed by destruction of cell membranes. The nanoparticles were effective against cells and endospores of *Bacillus anthracis*. CuO nanorods destroyed 92.17% of 4.5×10^4 CFU/mL cells at a dose of 1 mg/mL within 1 h. While multi-armed nanoparticles were more potent by destroying 99.92% of 7×10^5 CFU/mL cells at a dose of 0.5 mg/mL within 30 min and 99.6% of 1.25×10^4 CFU/mL cells at a dose of 2 mg/mL within 5 min (Pandey et al. 2014). It is reported that the bactericidal action of copper oxides is due to production of ROS, oxidation of proteins, lipid peroxidation, degradation of DNA in bacterial cells (Chatterjee et al. 2014).

In vitro screening of copper oxide nanoparticles prepared from *Achillea millefolium* were studied against *Albicans*, *M. canis*, *A. flavus*, and *G. glabrata*. The miconazole and amphotericin B were used as standard drugs. The copper oxide nanoparticles were found effective against all pathogens and could be due to the entry of nanoparticles to cell membranes inhibiting division of cells (Rabiee et al. 2020). In a novel study, copper oxide nanoparticles were examined on HSV-1 infection. Cytotoxicity of nanoparticles on vero cells was analyzed through the MTT assay. TCID50 and quantitative RT-PCR were used for determining antiherpetic potential. In arrange to assess the inhibitory influence of copper oxide nanoparticles on the viral antigens expression, an roundabout immunofluorescence measure was carried out to examine inhibitory effects of copper oxide nanoparticles on gene expression of viral antigens. Standard drug cyclovir was used in all tests. It was concluded that copper nanoparticles showed significant viricidal activity against HSV-1 (Tavakoli and Hashemzadeh 2020).

Other metallic nanoparticles made of aluminum, titanium, and gallium have too been studied. The reports revealed their potential in enhancing therapeutic efficacy due to their antimicrobial, antiviral, and antiparasitic (Narayanasamy et al. 2015; Nadeem et al. 2018; Hernández-Díaz et al. 2020; Fawzy et al. 2021; Qureshi et al. 2021).

4.2 Encapsulated Nanoparticles

4.2.1 Polymer/Dendrimer Encapsulation

The polymer/dendrimer-based nanoparticulated systems ameliorate therapeutic efficacy. These nanoparticles encapsulate the active materials with higher stability and show biocompatibility with cells and tissues when synthesized from biopolymers which are biodegradable and biocompatible. Importantly, polymer/dendrimer nanoparticles can be devised to efficiently deliver the medication to a target site, thereby increasing therapeutic outcome and bringing down the side effects (Lu et al. 2011; Liu et al. 2020).

In a research, chitosan nanoparticles were used for coating therapeutic proteins to study the cytokine interactions towards chronic infection, production of cytokine and elicitation of long-standing memory cell development. Chitosan nanoparticles (∼200 nm) were synthesized using ionic gelation method and bind with *Mycobacterium tuberculosis* proteins (CFP-10 and CFP-21). The CFP10 and CFP-21 proteins coated with nanoparticles resulted in increased bioavailability and insignificant cytotoxicity when compared with pristine proteins (Verma et al. 2011a). Tri polyphosphate was used as a cross-linker in ionotropic gelation method to prepare chitosan nanoparticles (∼250–300 nm). The chitosan nanoparticles entrapped 257.936 μg/mL concentration of CFP10 and 296.659 μg/mL concentration of CFP21. Minimal cytotoxic effect was shown by proteins coated with nanoparticles compared to 60% cytotoxic effects by free proteins (CFP10 and CFP21). In-vivo studies were carried out for the antigenic response of *Mycobacterium tuberculosis.* CFPs. IFN-γ were secreted in higher levels by splenocytes, i.e., ∼300 pg/mL and ∼400 pg/mL by CFP10 and CFP21 loaded nanoparticles, respectively, while free CFP10 showed ∼100 pg/mL and free CFP21 showed ∼80 pg/mL. IL-4 were secreted in low levels by splenocytes, indicating TH1 bias in immune response (T-cell response) similar to natural infections (Verma et al. 2011b).

Chitosan nanoparticles effectively transport immunogenic antigens to the APCs (antigen-presenting cells), which afterwards raise body's natural responses safely in case of infectious diseases. Hajam et al., synthesized chitosan nanoparticles which were coated over messenger RNA molecules and further surface coated with influenza proteins (H9N2 HA2 and M2e). The delivery of messenger RNA molecules into antigen-presenting cells by chitosan nanoparticles was effective and infiltrated the mucosal barrier efficiently to get to the immune induction sites. To evaluate the ability of chitosan nanoparticles transporting H9N2 HA2 and M2e to stimulate safe immunity, the chickens were vaccinated intranasally with empty chitosan nanoparticles, chitosan nanoparticles transporting influenza proteins in both messenger RNA and protein formats and chitosan nanoparticles transporting antigens only in protein format. The study concluded that HA2 and M2e antigens induced a comprehensive defense against avian influenza viruses and incorporating mRNAs in vaccine preparations is an efficient approach to stimulate better body's defense system (Hajam et al. 2020).

Dendrimers are useful nanomaterials, for investigating delivery through oral, topical, and intravenous administration routes (Mignani et al. 2013). A rapid-response, single dose, completely synthetic and adjuvant-free dendrimer nanoparticle vaccine stage was evolved where antigens are encoded by mRNA replicon encapsulation. This was the first system to be able to produce defensive immunity towards a wide range of deadly microbial challenges, including *Toxoplasma gondii*, H1N1 flu and Ebola infection. This immunization can be made with different antigen replicons, and fully able to evoke both antibody and CD8+ T-cell response (Chahal et al. 2016).

4.2.2 Metal Encapsulation

Metal nanoparticles superficial functionalization being a common technique for enhancing biocompatible nature may change from ionic ligands such as phosphates or citrate (Thanh and Green 2010). Polymers are amazing physical obstructions coating the core nanoparticles as they do not allow their direct contact with the surrounding. Particle size is increased by encapsulation (Thanh and Green 2010) which plays an imperative part required for systemic circulation, although the size of particle is controlled for biodistribution at specific site. Starch, chitosan, dextran, pullulan, and also PEG can be used for enhancing solubility of water. Metal nanoparticles can also be stabilized by dendrimers as they are well suited for encapsulation of metals within three dimensional voids present in their structure, in fact through electrostatic attractions silver and gold are easily encapsulated by dendrimers (Esumi et al. 2003; He et al. 1999). Encapsulation of metals mainly takes place via complexation reactions, hydrogen bonding, electrostatic and van der Waals interactions, and hence subsequently avoiding agglomeration (Crooks et al. 2001).

The evaluation report for potential treatment of wound infection by a pH-responsive hydrogel integrated silver nanoparticles showed hydrogel was able to quickly switch from a ceased state in a minute acidic environment (normal wound) to a hydrated state in a basic environment (infected wound). This pH-dependent extension activated an altogether more prominent rate of silver nanoparticle discharge at basic pH (7.4 and 10) in comparison to that in acidic pH (4). The bactericidal activity of the hydrogel integrated silver nanoparticles was confirmed against different bacterial strains. Vitally, the silver nanoparticles loaded hydrogel recommended greater degree of biocompatibility by not showing any toxicity to mammalian cells. The results displayed above affirm the synthesis of a novel silver nanoparticle integrated pH-responsive hydrogel that is competent of on-demand discharge of silver nanoparticles in the event of presence of pathogenic microbes in a wound. This approach may give a more proficient way forward for the clinical administration of chronically infected wounds (Haidari et al. 2021).

A study was conducted with lung surfactant model to assess the impact of the transposal of the BP100 peptide coated on a gold nanoparticle which is further encapsulated with polymers including polyethylene glycol-block-polystyrene, polyethylene glycol, and polystyrene. The impacts of nanocarriers on the lung surfactant mode can be evaluated and other than that it was suggested that putting microbicidal peptides coated on PEG capped gold nanoparticles for treatment of lung infections is feasible (Souza et al. 2020).

The act of the maintenance ability of Ag^0 and Cu^0 nanoparticles on the bactericidal activity of cellulose-based polyol dendrimer and montmorillonite was comparatively explored. An uncommon approach including thermal analysis, X-ray photoelectron spectroscopy, and surface charge measurements permitted relating the host–matrix features to the distinctive antibacterial actions of Ag^0 and Cu^0 nanoparticles against the bacterial strains *Escherichia coli* DH5α and *Bacillus subtilis* 168. High dispersion was favored by interactions of optimum metal matrix of both particles and grains of material, thus making surface contact better with

respect to culture medium. This was clarified by hydrophilic character and reasonable compromise between the metal detention by the host matrix and discharge in the impregnating media. Lewis acid–base interactions show up to happen between solid surface, MNP, and fluid media competitively. These discoveries are of awesome significance, giving a more profound understanding of the antibacterial action of metal-loaded nanomaterials. Such discoveries open promising possibilities for clay based drugs and vegetal fibers to treat gastro-intestinal and dermatological diseases (Noori et al. 2021).

4.3 Nanoantibiotics

Nanoantibiotics is among the most promising therapeutical applications of nanotechnology that utilize physicochemical fusion of minute particles with antibiotics (Soares et al. 2018; Mamun et al. 2021) or artificially fabricate pristine antibiotic particles with the size of $\leq$100 nm at least in one dimension. This nanoscale reengineering approach including antibiotics has renewed the existing drug arsenals and made them effective against a various pathogens (Mamun et al. 2021). The functionalization of antibiotics on designed nanoparticles is pivotal as the type of surface charge (neutral, +ive, -ive or zwitterionic) and their densities control the killing of bacteria. Functionalization of antibiotics on negatively surface charged particles have been proposed to increase microbicidal potential of nanoconjugates (Miller et al. 2015; Qureshi et al. 2020).

Till date, these nanoscale antibiotics reengineered systems were examined as delivery vehicle either of a single antibiotic or conjugation of various antibiotics. These nanoengineered systems include (1) polymeric nanoparticles, (2) chitosan nanoparticles, (3) lipid polymer crossover nanomaterials, (4) mesoporous nanomaterials, (5) metal nanoparticles, (6) metal-oxide nanoparticles, (7) carbon-based nanomaterials (e.g., carbon nanotubes, graphenes), (8) membrane-bound nanomaterials (e.g., dendrimers, liposomes, micelles), and other nanomaterials such as nanosheets, nanocomposites, nanomesh, strong lipid nanoparticles, etc. Among them, polymeric, membrane-bound liposomes or micelles and mesoporous nanomaterials are the most examined delivery systems (Pichavant et al. 2016; Yeh et al. 2020).

Katva et al. (2018) determined the effects of conjugating silver nanoparticles with chloramphenicol and gentamicin against *Enterococcus faecalis*, the results showed enhanced bactericidal action when silver nanoparticles were utilized with one of the antibiotics. Moreover, hemolytic activity of silver nanoparticles revealed their non-toxic behavior towards erythrocytes.

Silver nanoparticles have moreover been utilized in conjugation with chosen antibiotics to treat bacterial infections that results in increased therapeutic efficacy. Deng et al. inspected the synergistic impacts of mixing silver nanoparticles with different antibiotics (aminoglycosides, β-lactams, polypeptides, and quinolones) against the *Salmonella typhimurium*, a drug-resistant bacterium (Deng et al. 2016). Three classes of antibiotics in conjugation with silver nanoparticles appeared

restraint against growth of the *Salmonella*. The synergistic impacts of the three classes of antibiotics were assigned to complexes that were formed between the silver nanoparticles and antibiotics, which resulted in the discharge of a higher concentration of silver ions on the cell wall of bacteria and subsequently hindered growth of bacteria (Deng et al. 2016), whereas β-lactam antibiotics in conjugation with silver nanoparticles showed no synergistic impacts since they were incapable to make such complexes.

Saha et al. conjugated gold nanoparticles with streptomycin, ampicillin, and kanamycin (Saha et al. 2007). The conjugation showed effective potential towards *Micrococcus luteus*, *E. coli* DH5, and *Staphylococcus aureus*. The conjugation of gold nanoparticles with kanamycin or streptomycin resulted in strong bactericidal activity when compared to gold nanoparticles conjugated with ampicillin. It was observed that conjugation of nanoparticles with antibiotics enhanced bactericidal action than to pristine antibiotics, thereby suggesting increases in interaction of gold formulations with bacterial cell (Saha et al. 2007).

Chitosan was used as a cross-linker while preparing dextran and chondroitin sulfate nanoparticles loaded with chloramphenicol. The loaded nanoparticles were evaluated by in vitro and ex vivo methods against *Salmonella typhi*. The chitosan as a cross-linker offered a strong linkage, resulting in prolonged drug release. In macrophages the intracellular chloramphenicol delivery was fourfold higher and resulted in significant antimicrobial activity towards Salmonella Paratyphi A (Kiruthika et al. 2015). The property of chitosan as a cross-linker can be used for making new approaches which were discomfort due to regular dosing that can be avoided during therapies.

4.4 Conclusions and Future Trend

Infectious diseases categorized as microbial, viral, and parasitic and their medication is hindered by drug resistance. Mostly, the drugs that are prescribed for these infectious diseases are non-selective and also endure drug toxicity. Nanomaterials (metal-based nanoparticles, encapsulated nanoparticles, and nanoantibiotics) have been reported to overcome such hindrances. It has been proposed that nanomaterials display satisfactory cell interactions with biomolecules on the cell surface and inside the cell leading to development of therapeutics for the treating of infectious diseases. Preclinical evaluations for treatment of infectious diseases have been reported most. The antimicrobial activity of nanomaterials is due to their potential to generate reactive oxygen species damaging the cell wall of bacteria and their capability of binding with RNA or DNA, thereby disturbing replicating process of pathogens and transmembrane electron transport. The microbicidal activities of the nanomaterials depend on factors such as preparation method, morphology. Reports on parasitic infection are very less, suggesting that there is pressing need to fabricate nanomaterials that are cost effective with better therapeutic efficacy. Pharmacokinetics and toxicity of metal compounds need attention. Nanomaterials have the

potential to improve effectiveness of drugs, thus promising therapeutics for the treatment of infectious diseases in future.

References

Abo-Zeid Y, Ismail NS, McLean GR et al (2020) A molecular docking study repurposes FDA approved iron oxide nanoparticles to treat and control COVID-19 infection. Eur J Pharm Sci 153:105465

Aderibigbe BA (2017) Metal-based nanoparticles for the treatment of infectious diseases. Molecules 22:1370

Ahmad T, Phul R, Khatoon N et al (2017) Antibacterial efficacy of *Ocimum sanctum* leaf extract-treated iron oxide nanoparticles. N J Chem 41:2055–2061

Ahmad W, Kumar Jaiswal K, Amjad M (2021) *Euphorbia herita* leaf extract as a reducing agent in a facile green synthesis of iron oxide nanoparticles and antimicrobial activity evaluation. Inorg Nano-Metal Chem 51:1147–1154

Akhtar S, Rehman S, Asiri SM, Khan FA, Baig U, Hakeem AS, Gondal MA (2020) Evaluation of bioactivities of zinc oxide, cadmium sulfide and cadmium sulfide loaded zinc oxide nanostructured materials prepared by nanosecond pulsed laser. Mater Sci Eng C 116:111156

Akpomie KG, Ghosh S, Gryzenhout M et al (2021) One-pot synthesis of zinc oxide nanoparticles via chemical precipitation for bromophenol blue adsorption and the antifungal activity against filamentous fungi. Sci Rep 11:8305

Alahmari F, Rehman S, Almessiere M, Khan FA, Slimani Y, Baykal A (2021) Synthesis of Ni0.5Co0.5-xCdxFe1.78Nd0.02O4 ($x \leq 0.25$) nanofibers by using electrospinning technique induce anti-cancer and anti-bacterial activities. J Biomol Struct Dyn 39(9):3186–3193

Alexander JW (2009) History of the medical use of a silver. Surg Infect 10:289–292

Al-Jameel SS, Rehman S, Almessiere MA, Khan FA, Slimani Y, Al-Saleh NS, Manikandan A, Al-Suhaimi EA, Baykal A (2021) Anti-microbial and anti-cancer activities of Mn0.5Zn0.5DyxFe2-xO4 ($x \leq 0.1$) nanoparticles. Artif Cells Nanomed Biotechnol 49(1): 493–499

Alkharsah KR, Rehman S, Alkhamis F, Alnimr A, Diab A, Al-Ali AK (2018) Comparative and molecular analysis of MRSA isolates from infection sites and carrier colonization sites. Ann Clin Microbiol Antimicrob 17(1):1–1

Allaker RP, Ren G (2008) Potential impact of nanotechnology on the control of infectious disease. Trans R Soc Trop Med Hyg 102:1–2

Almessiere MA, Slimani Y, Rehman S, Khan FA, Polat EG, Sadaqat A, Shirsath SE, Baykal A (2020a) Synthesis of Dy-Y co-substituted manganese-zinc spinel nanoferrites induced anti-bacterial and anti-cancer activities: comparison between sonochemical and sol-gel auto-combustion methods. Mater Sci Eng C 116:111186

Almessiere MA, Slimani Y, Rehman S, Khan FA, Güngüneş ÇD, Güner S, Shirsath SE, Baykal A (2020b) Magnetic properties, anticancer and antibacterial effectiveness of sonochemically produced Ce3+/Dy3+ co-activated Mn-Zn nanospinel ferrites. Arab J Chem 13(10):7403–7417

Arvizo RR, Bhattacharyya S, Kudgus RA et al (2012) Intrinsic therapeutic applications of noble metal nanoparticles: past, present and future. Chem Soc Rev 41:2943–2970

Baig U, Gondal MA, Rehman S, Akhtar S (2020) Facile synthesis, characterization of nano-tungsten trioxide decorated with silver nanoparticles and their antibacterial activity against water-borne gram-negative pathogens. Appl Nanosci 10(3):851–860

Balasamy RJ, Ravinayagam V, Alomari M, Ansari MA, Almofty SA, Rehman S, Dafalla H, Marimuthu PR, Akhtar S, Al Hamad M (2019) Cisplatin delivery, anticancer and antibacterial properties of Fe/SBA-16/ZIF-8 nanocomposite. RSC Adv 9(72):42395–42408

Basumatari M, Devi RR, Gupta MK et al (2021) Musa balbisiana Colla pseudostem biowaste mediated zinc oxide nanoparticles: their antibiofilm and antibacterial potentiality. Curr Res Green Sustain Chem 4:100048
Casadevall A (1996) Antibody-based therapies for emerging infectious diseases. Emerg Infect Dis 2:200
Chahal JS, Khan OF, Cooper CL et al (2016) Dendrimer-RNA nanoparticles generate protective immunity against lethal Ebola, H1N1 influenza, and *Toxoplasma gondii* challenges with a single dose. Proc Natl Acad Sci 113:E4133–E4142
Chatterjee AK, Chakraborty R, Basu T (2014) Mechanism of antibacterial activity of copper nanoparticles. Nanotechnology 25:135101
Choi O, Deng KK, Kim NJ, Ross L et al (2008) The inhibitory effects of silver nanoparticles, silver ions, and silver chloride colloids on microbial growth. Water Res 42:3066–3074
Crooks RM, Zhao M, Sun L et al (2001) Dendrimer-encapsulated metal nanoparticles: synthesis, characterization, and applications to catalysis. Acc Chem Res 34:181–190
Cui Y, Zhao Y, Tian Y et al (2012) The molecular mechanism of action of bactericidal gold nanoparticles on *Escherichia coli*. Biomaterials 33:2327–2333
Delavari M, Dalimi A, Ghaffarifar F et al (2014) In vitro study on cytotoxic effects of ZnO nanoparticles on promastigote and amastigote forms of *Leishmania major* (MRHO/IR/75/ER). Iran J Parasitol 9:6–13
Deng H, McShan D, Zhang Y et al (2016) Mechanistic study of the synergistic antibacterial activity of combined silver nanoparticles and common antibiotics. Environ Sci Technol 50:8840–8848
Dizaj SM, Lotfipour F, Barzegar-Jalali M et al (2014) Antimicrobial activity of the metals and metal oxide nanoparticles. Mater Sci Eng C Mater Biol Appl 44:278–284
Dutta PP, Bordoloi M, Gogoi K et al (2017) Antimalarial silver and gold nanoparticles: green synthesis, characterization and in vitro study. Biomed Pharmacother 91:567–580
Esumi K, Akiyama S, Yoshimura T (2003) Multilayer formation using oppositely charged gold- and silver-dendrimer nanocomposites. Langmuir 19:76779–77681
Fatima Z, Kumari P, Rehman S, Hameed S (2021) Virulence traits of Candida spp.: an overview. In: Recent trends in mycological research. Springer, New York, pp 439–455
Fauci AS, Morens DM (2012) The perpetual challenge of infectious diseases. N Engl J Med 366: 454–461
Fawzy M, Khairy GM, Hesham A et al (2021) Nanoparticles as a novel and promising antiviral platform in veterinary medicine. Arch Virol 166:2673–2682
Gunalan S, Sivaraj R, Rajendran V (2012) Green synthesized ZnO nanoparticles against bacterial and fungal pathogens. Prog Nat Sci Mater Int 22:693–700
Haidari H, Kopecki Z, Sutton AT et al (2021) pH-responsive "smart" hydrogel for controlled delivery of silver nanoparticles to infected wounds. Antibiotics 10:49
Hajam IA, Senevirathne A, Hewawaduge C et al (2020) Intranasally administered protein coated chitosan nanoparticles encapsulating influenza H9N2 HA2 and M2e mRNA molecules elicit protective immunity against avian influenza viruses in chickens. Vet Res 51:37
He JA, Valluzzi R, Yang K et al (1999) Electrostatic multilayer deposition of a gold-dendrimer nanocomposite. Chem Mater 11:3268–3274
Hernández-Díaz JA, Garza-García JJ, Zamudio-Ojeda A et al (2020) Plant-mediated synthesis of nanoparticles and their antimicrobial activity against phytopathogens. J Sci Food Agric 101: 1270–1287
Hernández-Sierra JF, Ruiz F, Pena DC et al (2008) The antimicrobial sensitivity of *Streptococcus mutans* to nanoparticles of silver, zinc oxide, and gold. Nanomedicine 4:237–240
Huh AJ, Kwon YJ (2011) "Nanoantibiotics": a new paradigm for treating infectious diseases using nanomaterials in the antibiotics resistant era. J Control Release 156:128–145
Inbaneson SJ, Ravikumar S, Manikandan N (2011) Antibacterial potential of silver nanoparticles against isolated urinary tract infectious bacterial pathogens. Appl Nanosci 1:231–236
Irshad R, Tahir K, Li B et al (2017) Antibacterial activity of biochemically capped iron oxide nanoparticles: a view towards green chemistry. J Photochem Photobiol B Biol 170:241–246

Jafari AR, Mosavi T, Mosavari N et al (2016) Mixed metal oxide nanoparticles inhibit growth of *Mycobacterium tuberculosis* into THP-1 cells. Int J Mycobacteriol 5:S181–S183

Kar PK, Murmu S, Saha S et al (2014) Anthelmintic efficacy of gold nanoparticles derived from a phytopathogenic fungus, *Nigrospora oryzae*. PLoS One 9:e84693

Katva S, Das S, Moti HS et al (2018) Antibacterial synergy of silver nanoparticles with gentamicin and chloramphenicol against *Enterococcus faecalis*. Pharmacogn Mag 13:828–833

Kiruthika V, Maya S, Maneesha KS et al (2015) Comparative efficacy of chloramphenicol loaded chondroitin sulfate and dextran sulfate nanoparticles to treat intracellular *Salmonella* infections. Colloids Surf B Biointerfaces 15:1–10

Liu X, Wang Z, Feng X et al (2020) Platensimycin-encapsulated poly(lactic-co-glycolic acid) and poly(amidoamine) dendrimers nanoparticles with enhanced anti-Staphylococcal activity in vivo. Bioconjug Chem 31:1425–1437

Lu X-Y, Wu D-C et al (2011) Polymer nanoparticles. In: Villaverde A (ed) Progress in molecular biology and translational science, Nanoparticles in translational science and medicine, vol 104. Elsevier, Amsterdam, pp 299–323

Mamun MM, Sorinolu AJ, Munir M et al (2021) Nanoantibiotics: functions and properties at the nanoscale to combat antibiotic resistance. Front Chem 9:348

Mignani S, El Kazzouli S, Bousmina M et al (2013) Expand classical drug administration ways by emerging routes using dendrimer drug delivery systems: a concise overview. Adv Drug Deliv Rev 35:1316–1330

Miller KP, Wang L, Benicewicz BC et al (2015) Inorganic nanoparticles engineered to attack bacteria. Chem Soc Rev 44:7787–7807

Mir JF, Rubab S, Shah MA (2020a) Photo-electrochemical ability of iron oxide nanoflowers fabricated via electrochemical anodization. Chem Phys Lett 16:137088

Mir JF, Rubab S, Shah MA (2020b) Hematite (α-Fe_2O_3) nanosheets with enhanced photo-electrochemical ability fabricated via single step anodization. Chem Phys Lett 16:137584

Mody VV, Siwale R, Singh A et al (2010) Introduction to metallic nanoparticles. J Pharm Bioallied Sci 2:282–289

Mohamed MM, Fouad SA, Elshoky HA et al (2017) Antibacterial effect of gold nanoparticles against *Corynebacterium pseudotuberculosis*. Int J Vet Sci Med 5:23–29

Morens DM, Fauci AS (2013) Emerging infectious diseases: threats to human health and global stability. PLoS Pathog 9:e1003467

Mujaddidi N, Nisa S, Al Ayoubi S et al (2021) Pharmacological properties of biogenically synthesized silver nanoparticles using endophyte Bacillus cereus extract of *Berberis lyceum* against oxidative stress and pathogenic multidrug-resistant bacteria. Saudi J Biol Sci 28(11): 6432–6440

Murugan K, Panneerselvam C, Subramaniam J et al (2016) Eco-friendly drugs from the marine environment: spongeweed-synthesized silver nanoparticles are highly effective on *Plasmodium falciparum* and its vector *Anopheles stephensi*, with little non-target effects on predatory copepods. Environ Sci Pollut Res 23:16671–16685

Nadeem M, Tungmunnithum D, Hano C et al (2018) The current trends in the green syntheses of titanium oxide nanoparticles and their applications. Green Chem Lett Rev 11:492–502

Nadhman A, Khan MI, Nazir S et al (2016) Annihilation of *Leishmania* by daylight responsive ZnO nanoparticles: a temporal relationship of reactive oxygen species-induced lipid and protein oxidation. Int J Nanomedicine 11:2451–2461

Nahvi I, Belkahla S, Asiri SM, Rehman S (2021) Overview and prospectus of algal biogenesis of nanoparticles. In: Microbial nanotechnology: green synthesis and applications. Springer, Singapore, pp 121–134

Narayanasamy P, Switzer BL, Britigan BE (2015) Prolonged-acting, multi-targeting gallium nanoparticles potently inhibit growth of both HIV and *Mycobacteria* in co-infected human macrophages. Sci Rep 5:8824

Natalia AV, Ravetti S, Jose MB et al (2019) Metallic nanoparticles as a strategy for the treatment of infectious diseases. In: Holban A, Grumezescu AM (eds) Materials for biomedical engineering:

bioactive materials for antimicrobial, anticancer and gene therapy. Elsevier, Amsterdam. https://doi.org/10.1016/B978-0-12-818435-6.00014-1

Noori F, Neree AT, Megoura M et al (2021) Insights into the metal retention role in the antibacterial behavior of montmorillonite and cellulose tissue-supported copper and silver nanoparticles. RSC Adv 11:24156–24171

Pandey P, Packiyaraj MS, Nigam H et al (2014) Antimicrobial properties of CuO nanorods and multi-armed nanoparticles against *B. anthracis* vegetative cells and endospores. Beilstein J Nanotechnol 5:789–800

Parrish CR, Holmes EC, Morens DM et al (2008) Cross-species virus transmission and the emergence of new epidemic diseases. Microbiol Mol Biol Rev 72:457–470

Pichavant L, Carrié H, Nguyen MN et al (2016) Vancomycin functionalized nanoparticles for bactericidal biomaterial surfaces. Biomacromolecules 17:1339–1346

Pickard R, Lam T, MacLennan G et al (2012) Antimicrobial catheters for reduction of symptomatic urinary tract infection in adults requiring short-term catheterisation in hospital: a multicentre randomised controlled trial. Lancet 380:1927–1935

Praba VL, Kathirvel M, Vallayyachari K et al (2013) Bactericidal effect of silver nanoparticles against *Mycobacterium tuberculosis*. J Bionanosci 3:282–287

Qasim S, Zafar A, Saif MS et al (2020) Green synthesis of iron oxide nanorods using *Withania coagulans* extract improved photocatalytic degradation and antimicrobial activity. J Photochem Photobiol B Biol 204:111784

Qureshi F, Nawaz M, Rehman S, Almofty SA, Shahzad S, Nissapatorn V, Taha M (2020) Synthesis and characterization of cadmium-bismuth microspheres for the catalytic and photocatalytic degradation of organic pollutants, with antibacterial, antioxidant and cytotoxicity assay. J Photochem Photobiol B Biol 202:111723

Qureshi A, Blaisi NI, Abbas AA, Khan NA, Rehman S (2021) Prospectus and development of microbes mediated synthesis of nanoparticles. In: Microbial nanotechnology: green synthesis and applications. Springer, Singapore, pp 1–15

Rabiee N, Bagherzadeh M, Kiani et al (2020) Biosynthesis of copper oxide nanoparticles with potential biomedical applications. Int J Nanomedicine 15:3983–3999

Rafi MM, Ahmed KSZ, Nazeer KP et al (2015) Synthesis, characterization and magnetic properties of hematite (α-Fe2O3) nanoparticles on polysaccharide templates and their antibacterial activity. Appl Nanosci 5:515–520

Rago I, Chandraiahgari CR, Bracciale MP et al (2014) Zinc oxide microrods and nanorods: different antibacterial activity and their mode of action against Gram-positive bacteria. RSC Adv 4: 56031–56040

Ravinayagam V, Rehman S (2020) Zeolitic imidazolate framework-8 (ZIF-8) doped TiZSM-5 and mesoporous carbon for antibacterial characterization. Saudi J Biol Sci 27(7):1726–1736

Reddy LS, Nisha MM, Joice M et al (2014) Antimicrobial activity of zinc oxide (ZnO) nanoparticle against *Klebsiella pneumoniae*. Pharm Biol 52:1388–1397

Rehman S, Al Salem Z, Al Jindan R, Hameed S (2019) Microbial natural products: exploiting microbes against drug-resistant bugs. In: Pathogenicity and drug resistance of human pathogens. Springer, Singapore, pp 393–404

Rehman S, Farooq R, Jermy R, Mousa Asiri S, Ravinayagam V, Al Jindan R, Alsalem Z, Shah MA, Reshi Z, Sabit H, Alam Khan F (2020a) A wild fomes fomentarius for biomediation of one pot synthesis of titanium oxide and silver nanoparticles for antibacterial and anticancer application. Biomol Ther 10(4):622

Rehman S, Jermy R, Asiri SM, Shah MA, Farooq R, Ravinayagam V, Ansari MA, Alsalem Z, Al Jindan R, Reshi Z, Khan FA (2020b) Using Fomitopsis pinicola for bioinspired synthesis of titanium dioxide and silver nanoparticles, targeting biomedical applications. RSC Adv 10(53): 32137–32147

Ritter J, Thomas L, Lederer J et al (2013) Effectiveness of a silver-alloy and hydrogel coated urinary catheter on symptomatic catheter-associated urinary tract infections. Am J Infect Control 41: S143–S144

Ryoo SR, Jang H, Kim KS et al (2012) Functional delivery of DNAzyme with iron oxide nanoparticles for hepatitis C virus gene knockdown. Biomaterials 33:2754–2761
Saha B, Bhattacharya J, Mukherjee A et al (2007) In vitro structural and functional evaluation of gold nanoparticles conjugated antibiotics. Nanoscale Res Lett 2:614–622
Sametband M, Shukla S, Meningher T et al (2011) Effective multi-strain inhibition of influenza virus by anionic gold nanoparticles. Med Chem Commun 2:421–423
Saylor C, Dadachova E, Casadevall A (2009) Monoclonal antibody-based therapies for microbial diseases. Vaccine 27:G38–G46
Singh R, Smitha MS, Singh SP (2014) The role of nanotechnology in combating multi-drug resistant bacteria. J Nanosci Nanotechnol 14:4745–4756
Singh S, Rehman S, Fatima Z, Hameed S (2020) Protein kinases as potential anticandidal drug targets. Front Biosci (Landmark Ed) 23:1412–1432
Soares S, Sousa J, Pais A et al (2018) Nanomedicine: principles, properties, and regulatory issues. Front Chem 6:360
Sondi I, Salopek-Sondi B (2004) Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. J Colloid Interface Sci 275(1):177–182
Souza FR, Fornasier F, Carvalho AS et al (2020) Polymer-coated gold nanoparticles and polymeric nanoparticles as nanocarrier of the BP100 antimicrobial peptide through a lung surfactant model. J Mol Liq 314:113661
Stan M, Popa A, Toloman et al (2015) Enhanced antibacterial activity of zinc oxide nanoparticles synthesized using *Petroselinum crispum* extracts. In: Proceedings of the AIP conference, Cluj-Napoca, Romania, pp 23–25
Surwade P, Luxton T, Clar J et al (2020) Impact of the changes in bacterial outer membrane structure on the anti-bacterial activity of zinc oxide nanoparticles. J Nanopart Res 22:1–8
Tavakoli A, Hashemzadeh MS (2020) Inhibition of herpes simplex virus type 1 by copper oxide nanoparticles. J Virol Methods 275:113688
Thanh NT, Green LA (2010) Functionalisation of nanoparticles for biomedical applications. Nano Today 5:213–230
Usman MS, Zowalaty MEEI, Shameli K et al (2013) Synthesis, characterization, and antimicrobial properties of copper nanoparticles. Int J Nanomedicine 8:4467–4479
Venkataraju JL, Sharath R, Chandraprabha MN et al (2014) Synthesis, characterization and evaluation of antimicrobial activity of zinc oxide nanoparticles. J Biochem Technol 3:151–154
Verma AK, Pandey RP, Chanchal A et al (2011a) Immuno-potentiating role of encapsulated proteins of infectious diseases in biopolymeric nanoparticles as a potential delivery system. J Biomed Nanotechnol 7:63–64
Verma AK, Pandey RP, Chanchal A et al (2011b) Encapsulation of antigenic secretory proteins of *Mycobacterium tuberculosis* in biopolymeric nanoparticles for possible aerosol delivery system. J Bionanosci 5:88–95
World Health Organization (WHO) (2014) Anti microbial resistance, Global Report on Surveillance. Summary. WHO Press, Geneva
Xu Y, Gao C, Li X et al (2013) In vitro antifungal activity of silver nanoparticles against ocular pathogenic filamentous fungi. J Ocular Pharmacol Therapeut 29:270–274
Yeh YC, Huang TH, Yang SC et al (2020) Nanobased drug delivery or targeting to eradicate bacteria for infection mitigation: a review of recent advances. Front Chem 8:286
Yilma AN, Singh SR, Dixit S et al (2013) Anti-inflammatory effects of silver-polyvinyl pyrrolidone (Ag-PVP) nanoparticles in mouse macrophages infected with live *Chlamydia trachomatis*. Int J Nanomedicine 8:2421–2432
Zazo H, Colino CI, Lanao JM (2016) Current applications of nanoparticles in infectious diseases. J Control Release 224:86–102
Zhang JF, Rehman S, Alghamdi T, Sheikh FA, Hassan MS, Amna T (2022) Bionanotechnology approaches to combat biofilms and drug resistance. In: Innovative approaches for nanobiotechnology in healthcare systems. IGI Global, Pennsylvania, pp 230–248

Metal-Based Nanoparticles for Infectious Diseases and Therapeutics

5

Ebin K. Baby, Catherine Reji, and Nidhin M

Abstract

Infectious diseases that are easily transmitted by microorganisms like bacteria, protozoa, fungus, etc. are a menace to humans. The greatest threat to human race is to mitigate the impact of these diseases. People with less immunity and children are prone to these diseases. Even healthy people get infected due to its easy transmission. Microorganisms causing these diseases are becoming more resistant to the drugs that are available in the market. So, there is a need to find new therapeutic which is facile, sensitive, and selective, is an important challenge for the medical field and this is where nanotechnology is having a greater chance. Nanoparticles especially metal-based nanoparticles have the ability to act against infectious and non-infectious diseases, this is because of their unique properties like small size, high surface area, etc. They do not have a specific binding site on the bacterial cell, which lead to the failure of bacterial resistant towards the nanoparticle mechanism. There are many nanoparticles which are efficient against particular diseases. In this review we are discussing about the advanced nanomaterials as therapeutics for infectious diseases. We have also discussed about antiviral activities which gives us a ray of hope for the solution of the SARS-COV-2.

Keywords

Nanomaterials · Infectious diseases · Metal-based nanoparticles · Peptidoglycan layer · Antimicrobial activity · Reactive oxygen species · Capping agents · Doping

E. K. Baby
Department of Chemistry, Institute of Chemical Technology, Mumbai, India

C. Reji · Nidhin M (✉)
Department of Chemistry, CHRIST (Deemed to be University), Bengaluru, India
e-mail: nidhin.m@christuniversity.in

S. Hameed, S. Rehman (eds.), *Nanotechnology for Infectious Diseases*,
https://doi.org/10.1007/978-981-16-9190-4_5

5.1 Introduction

Nanotechnology is the study about matters having size ranging from 1 to 100 nm (McNeil 2005). Nanoparticles like gold, silver, platinum, and copper have attained interest because of their applications in both fundamental and technological way. Material properties change when they are converted into nanoparticles. This is due to its high surface area per weight than conventional materials. They have unique catalytic, electronic, and optical properties which differ from one nanoparticle to other. They are mainly synthesized by physical, chemical, and biological methods. First two methods involve the use of strong and weak reducing agents, with the help of gamma rays, etc. Biological methods are economical and eco-friendly. It is achieved through biological agents like bacteria, fungi, plants, etc. Temperature, pressure, time, and pH play a crucial role in developing the shape and morphology, so these things need to be taken into account in order to prepare specific characteristic product (Duran and Rai 2011).

Nanoparticles can be broadly divided into categories depending on their morphology, size, and chemical properties. Some of the well-known classes are carbon-based nanoparticles, metal-based nanoparticles, ceramics nanoparticles, semiconductor nanoparticles, polymeric nanoparticles, lipid-based nanoparticles. It is the physicochemical properties such as mechanical strength, optically activity, chemical reactivity, etc. making them useful for various applications. Nanoparticles showing magnetic properties can be used in various disciplines like biomedicine, magnetic fluids, data storage, magnetic resonance imaging (MRI), etc. This property arises due to unequal electronic distributions which depends on the synthetic methods. Bulk materials of these nanoparticles do not show similar mechanical properties as that of nanoparticles. Metal nanoparticles have higher thermal conductivities as compared to fluids in solid form (Saeed et al. 2019). In order to get more ideas about the properties of the nanoparticles synthesized one must use specific characterization techniques. There are many techniques to characterize the properties of nanoparticles such as UV-Vis spectroscopy, FTIR spectroscopy, SEM, TEM, etc. The nanoparticles can form different dimensions, shapes, and sizes. A nanoparticle can be zero dimensional like nano dots where its parameters are fixed at a single point, one dimensional like graphene only has one parameter, two dimensional like carbon nanotubes whose length and breadth are the parameters or three dimensional where it has all the parameters. It can have various shapes like spherical, cylindrical, tubular, etc. and different sizes. The nanoparticle surface can show surface variations and they can either be crystalline or amorphous (Ealias and Sravankumar 2017). Due to this reason, their reactivity varies. These particles have great applications in the field of drug delivery, cosmetics, etc. They also have an important role against infectious diseases.

We know that infectious diseases are threat to our lives due to their easy transmission. It is caused by various microorganisms like viruses, bacteria, protozoa, etc. Certain microorganisms are normally harmless or even helpful, but under certain conditions, some may cause diseases. They can spread from person to person, through contaminated food, insects, animals, and even through water. Many

infectious diseases can be cured by the implication of vaccines, but there are some diseases for which no vaccines have yet been identified and no therapeutics has been implemented. Nanoparticles especially metal-based nanoparticles can interact with the human body. This is due to their unique properties like small size, high surface area. So, they have a crucial role in medicinal field, especially against infectious diseases. Before going into the role of metal-based nanoparticles against infectious diseases, let us discuss how microorganisms like bacteria resist to antibiotics and what is the role of metal-based nanoparticles here.

5.2 Bacteria and Antibacterial Drug

Bacteria is classified into two types based on their cell structure (Fig. 5.1), gram-positive and gram-negative. Gram-positive have a thick peptidoglycan layer on their cell wall, but in gram-negative bacteria, along with the peptidoglycan layer, they have an additional outer membrane consisting of lipopolysaccharide.

They can adhere with the host cell by which they cause diseases. There are many molecular strategies bacteria adapt to adhere to the host cell. One of them is with the help of a hair-like structure found in their outer membrane which is known as *pili*. Another method is by forming a biofilm which protects them from the host environment which also provides them resistance to the drugs (Slavin et al. 2017). The common methods bacteria adapt to resist drugs are shown in Fig. 5.2.

The antimicrobial activity of metal-based nanoparticles is widely studied for the last 20 years. Silver and copper nanoparticles have been used as antimicrobial agents thousands of years ago. Nanoparticles are non-specific, which means they do not bind to any specific area of the bacteria, which makes the resistance of bacteria towards nanoparticles mechanisms difficult. They act as antimicrobial agents by the release of metal ions, generation of Reactive Oxygen Species (ROS), binding with DNA or RNA of bacteria thereby preventing their replication, disruption of the bacterial membrane, etc. (Ding et al. 2018). Before going into the details of the mechanism of metal-based nanoparticles against infectious diseases, let us discuss what are metal-based nanoparticles, their synthesis, characterization, properties, and classifications.

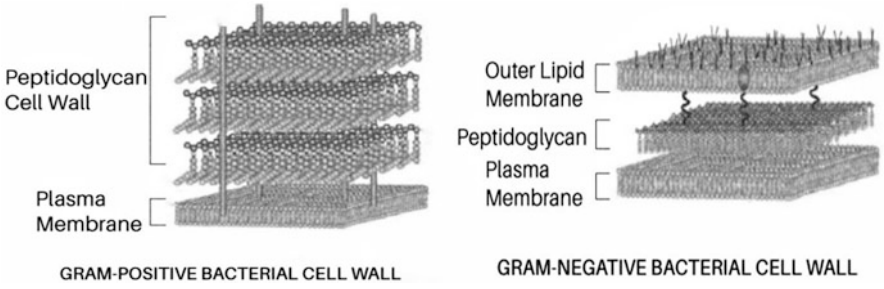

Fig. 5.1 Bacterial cell wall (Ismail Aiad et al. 2016)

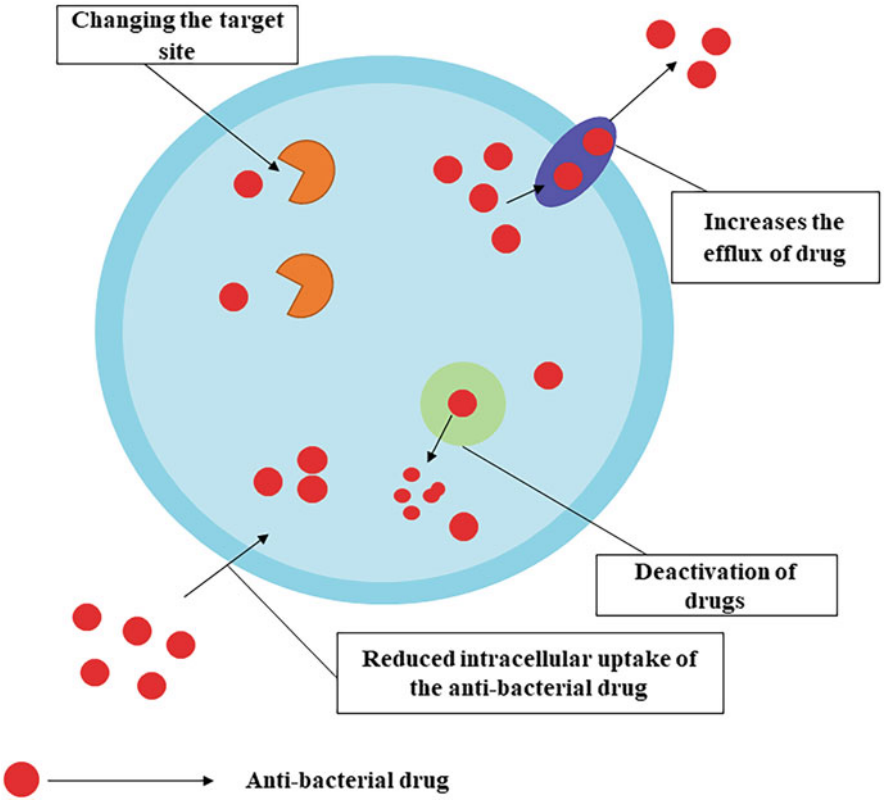

Fig. 5.2 Common mechanism of bacteria against drugs

5.3 Metal-Based Nanoparticles

The main concern of nanotechnology is the creation of nanoparticles with specific applications. Metal-based nanoparticles specifically noble metal-based nanoparticles are having one such application, that is, it can be used in medical field. It shows high biocompatibility, stability, and the possibility of production in large scale in eco-friendly methods. They are made of pure metals like gold, silver, titanium, etc., or oxides of metals like CuO, NiO, etc., or nanoparticles involving metal as one of its constituents. They control the release of different drugs and they can be frozen to powder formulation. It can be prepared using different methods. One among them is the chemical reduction of the noble metal precursor using a proper reducing agent, which can also act as a stabilizer thereby preventing agglomeration of particles. By changing the reaction conditions one can synthesize nanoparticles with various shapes and sizes. Most recent methods follow the principles of green

chemistry. It is used because it reduces the usage of harmful substances. These can also be prepared using other synthetic methods like sonochemical, microwave assisted and electrochemical methods (Klebowski et al. 2018).

5.3.1 Synthesis, Characterization, and Properties of Metal-Based Nanoparticles

Nanoparticles can be synthesized mainly through two different approaches, they are top-down and bottom-top approach. In the former, bulk materials are made into small size by any mechanical methods. Using this approach, it is difficult to get the expected size. Later these particles are stabilized to the required size. In the latter, we start with atomic and molecular size particles, which is then made to the required size. This approach is preferred over the other one because this begins with small and simpler molecules. These two approaches can be acquired mainly through physical, chemical, and biological methods. Summary of synthesis of nanoparticles is shown in Fig. 5.3.

Physical methods are used in top-down and bottom-up approaches (Kolahalam et al. 2019). Some examples of physical methods are listed in Fig. 5.3. One of it is arc discharge method. In this method, the material is vaporized in an arc discharge which is in between electrodes. Then it undergoes condensation, nucleation and then leads to the growth of nanoparticles (Virji 2014). Ball milling is also an example of a

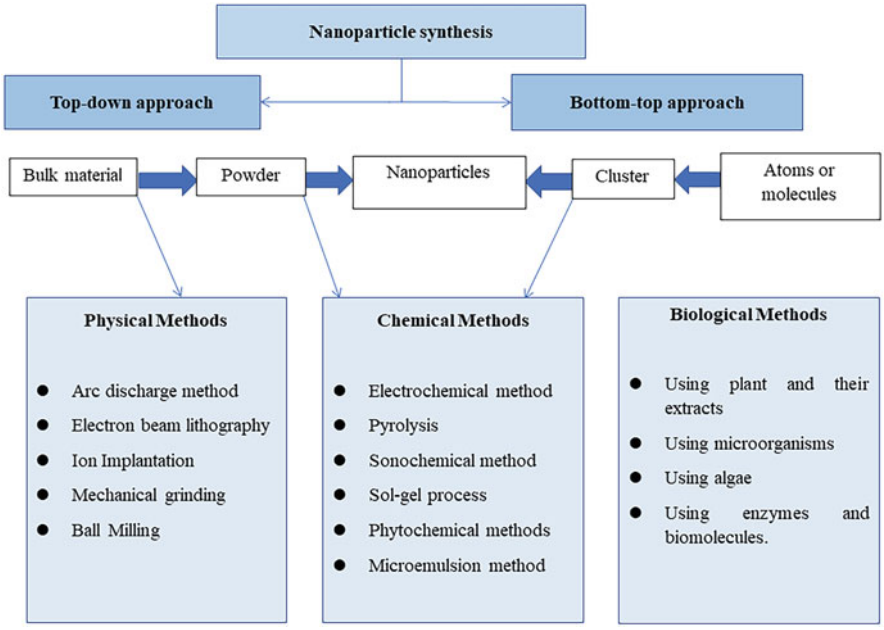

Fig. 5.3 Methods of synthesis of nanoparticles

physical method. It is a method in which the particle is grinded into fine powder (Gou et al. 2012). Chemical methods are mainly used in bottom-up approaches. These bottom up approaches can be used to provide materials with pure and controlled particle sizes. Sol-gel method is also an example of bottom up approach. In this method the precursor is allowed to undergo hydrolysis, polycondensation, and growth, of the nanoparticles. This method follows the basis of inorganic polymerization reactions. In addition to physical and chemical methods, biological methods are also there, where the use of microorganisms like fungi, bacteria, plant extracts, or template of DNA are used to create nanostructures. Biological methods are cost-effective and environment friendly (Kolahalam et al. 2019).

It is important to characterize the newly synthesized nanoparticles. Morphological studies of the synthesized materials can be carried out using the techniques like Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), Atomic Force Microscopy (AFM), and Dynamic Light Scattering (DLS). Surface studies of the materials can be carried out using X-ray diffraction and BET techniques. The Physiochemical properties of the materials can be studied using UV-vis spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), and Energy dispersive X-ray spectroscopy while optical properties are studied using microscopy, Raman spectroscopy, Surface Plasmon Resonance (SPR), and Photon correlation spectroscopy. Biological properties are found out using In vitro cell viability, In vivo and microbial colony viability studies etc. Electrical properties are studied using Zeta potential measurements (Dixit and Kumar 2017).

Metal nanoparticles have properties like optical polarizability, antimicrobial activity, electrical conductivity, biocompatibility, etc. In order to get benefit of nanoparticle, it is necessary that the nanoparticles should be stable, non-expensive, non-toxic, and selective. The stability of metal-based nanoparticles can be increased by protecting or passivating them with ligand surfactant shells which avoid coalescence at higher densities. It helps in handling and thereby studying the metal-based nanoparticles (Johnstone and Wilcoxon 2012).

5.3.2 Classification of Metal-Based Nanoparticles

Metal-based nanoparticles are mainly classified into three nanoparticles: metal nanoparticles, metal oxide nanoparticles, and metal sulfide nanoparticles. The first classification is one of the common categories of nanoparticles, some examples are gold, silver, platinum nanoparticles, etc. Metal oxide nanoparticles are also not uncommon, it includes nickel oxide, cobalt oxide, copper oxide nanoparticles, etc. Nanoparticles formed as sulfide comes under the category of metal sulfide. Figure 5.4 shows classification of metal-based nanoparticles.

They have significant role against infectious diseases, because of their small size and large surface area which helps them to enter into the bacterial cell. So, they can interact with the biomolecules and can destroy the cell. The treatment of infectious diseases is becoming difficult because of the drug resistance of microorganisms. So, it is necessary to develop new therapeutics against diseases. Metal-based

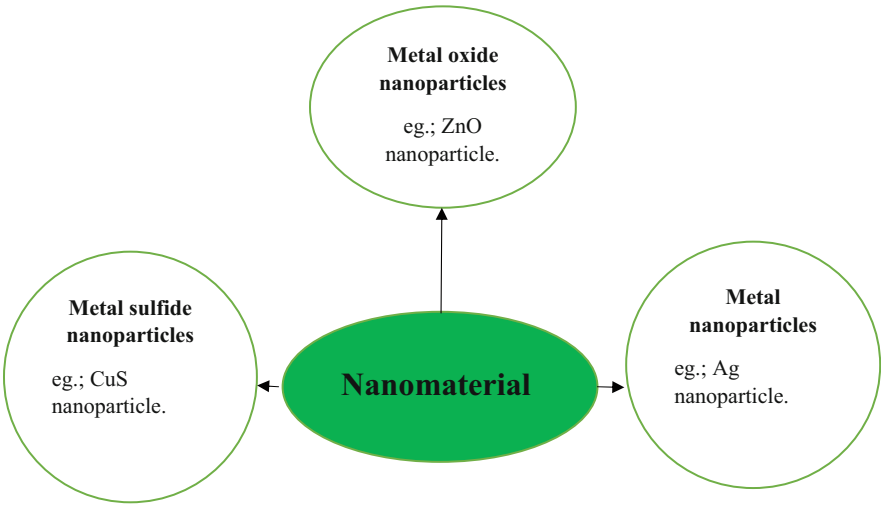

Fig. 5.4 Different types of metal-based nanoparticles

nanoparticles have been developed for infectious diseases as therapeutic agents (Aderibigbe 2017). For example, noble metals can interact with the biological system with non-toxicity and thereby can act as an antimicrobial agent (Yaqoob et al. 2020). Not only metals, some metal oxide nanoparticles show antimicrobial activities, for example, ZnO, FeO, MnO_2, CuO, Al_2O_3, and many more. Metal sulfide nanoparticles also have an important role against infectious diseases. Size, shape, charge, doping, etc. can bring changes in the activity of metal-based nanoparticles against infectious diseases. Doped (adding impurities to enhance the properties) metal/metal oxide nanoparticles show excellent inhibition towards infectious diseases, for example, doped copper/TiO_2 nanoparticles with carbon-based allotropes showed antimicrobial activities (Ebrahim-Saraie et al. 2018). Now let us discuss the important features and the role of each class of metal-based nanoparticles in infectious diseases.

5.3.3 Metal Nanoparticles Against Infectious Diseases

Metal nanoparticles are made of pure metals which have wide range of applications such as anticancer drug, drug delivery, antimicrobial agent, diagnostic assays, etc. It can be synthesized through different methods as we discussed earlier. One approach is done by Muhammad Asif Asghar et al., prepared Fe, Cu, Ag nanoparticles from green and black tea leaves extracts and characterized by FTIR spectroscopy and SEM (Asghara et al. 2018). The newly synthesized nanoparticle shows zone on inhibition (circle in which bacteria colonies not able to grow, because of the antimicrobial action of nanoparticle) against methicillin-resistance *Staphylococcus aureus* and against vancomycin-resistance *Staphylococcus aureus* (Asghara et al.

Table 5.1 Metal nanoparticles and their role against infectious diseases

No.	Nanoparticles	Role of nanoparticles against infectious diseases	References
1	Copper	• Antibacterial agent • Effective against hepatitis by inhibiting the entry and hindering viral replication	Yaqoob et al. (2020), Hang et al. (2015)
2	Silver	• Antimicrobial agent • Effective against HIV • Effective against influenza virus • Effective against malaria by inhibition of *Plasmodium falciparum* • Antileishmanial activity • Anthelmintic activity • Effective against herpes by inhibition of virus replication, viral entry, and prevention of infection	Samberg et al. (2012), Keat et al. (2015), Feng et al. (2000), Elechiguerra et al. (2005), Xiang et al. (2013), Mishra et al. (2013), Ahmad et al. (2015), Rashid et al. (2016), Hu et al. (2014)
3	Silver nanoparticles capped with starch	• Antimicrobial agent	Wang et al. (2019)
4	Gold	• Antibacterial activity against *Corynebacterium pseudotuberculosis* (animals), *Escherichia coli* • Inhibition of HIV viral entry • Effective against herpes by inhibiting viral attachment and penetration to cells • Effective against influenza A • Effective against malaria	Mohamed et al. (2017), Kesarkar et al. (2017), Baram-Pinto et al. (2010), Feng et al. (2013), Dutta et al. (2017)
5	Silicon nano substrate linked with Ag/Cu	• Antibacterial agent	Fellahi et al. (2013)
6	Tin	• Effective for herpes	Trigilio et al. (2012)
7	Gallium	• Effective against HIV and tuberculosis by suppression of its co-infection • Antibacterial properties against *Pseudomonas aeruginosa*	Choi et al. (2017), Kurtjak et al. (2016)

2018). Recently, different types of metal and its derivative nanoparticles were found to have antimicrobial properties, for example, nanosized metals are found to have bactericidal effect (kills the bacteria) (Mohammadi et al. 2011). Metals usually contain a positive charge and they will bind to the negative surface of bacteria which may help in bactericidal effect (Yaqoob et al. 2020; Bera et al. 2014). Copper nanoparticle (size <2 nm) synthesized by using L-ascorbic acid is an efficient

antibacterial agent against gram-positive and gram-negative bacteria (Xiong et al. 2011) (Table 5.1).

5.3.4 Metal Oxide Nanoparticles Against Infectious Diseases

Metal oxide nanoparticles contain metal cation and an oxide anion. It can be synthesized by different methods as discussed above. For example, metal oxide nanoparticles of Zn, Fe, Cu were synthesized by Ameer Azam et al. by dissolving metal nitrates of Zn, Cu, and Fe in citric acid and distilled water with a molar ratio of 1:1. Then the solution is stirred with a magnetic stirrer at 100 °C, until the formation of gel and it is allowed to burn at 200 °C. This light fluffy mass obtained is annealed at 400 °C for 1 h which resulted in the formation of metal oxide nanoparticles (sol-gel combustion method) (Azam et al. 2012).

A large variety of metal oxide nanoparticles show activity against infectious as well as non-infectious diseases and has many biomedical applications. Metal oxides like ZnO, MgO, TiO_2, MnO_2, FeO, Ag_2O, CaO, Al_2O_3, Bi_2O_3, Fe_2O_3 show antimicrobial activities (Yaqoob et al. 2020) (Table 5.2).

5.3.5 Metal Sulfide Nanoparticles Against Infectious Diseases

Metal sulfide nanoparticles are found to have a variety of biomedical applications (Fig. 5.5) because of different properties like high fluorescence, fine optical band gap, structural stability, etc. (Subramaniyan et al. 2018).

Metal sulfide nanoparticles can be synthesized in different ways. For example, Akl M. Awwad et al. prepared metal sulfide nanoparticles by dissolving 1 g silver nitrate in 100 ml of rosemary aqueous extract and stirring using a magnet at room temperature for 10 min. To this, sodium sulfide solution is added dropwise with continuous stirring. The yellow color of the extract changes to a suspended gray black color. This indicates the formation of silver sulfide nanoparticles. It can be characterized using UV-Vis spectroscopy, SEM, X-ray, and XRD analysis (Awwad et al. 2020). They have a significant role in antimicrobial activities. Metal sulfide nanoparticles and their role against infectious diseases are given in Table 5.3.

5.4 General Mechanism of Metal-Based Nanoparticles Against Infectious Diseases

Metal nanoparticles are found to have antimicrobial activities. Their properties like good thermal resistance, sustainability, and stability make them suitable to act as an antimicrobial agent. Figure 5.6 shows general mechanism of metal-based nanoparticles as antimicrobial agent.

Now let us discuss the mode of action of nanoparticle. There are mainly three mechanism which describes the action of nanoparticles. They are oxidative stress,

Table 5.2 Some metal oxide nanoparticles and their role against infectious diseases

No.	Nanoparticle	Role of nanoparticles against infectious diseases	References
1	Ag_2O	• Antimicrobial agent (against *Escherichia coli*)	Salas et al. (2019)
2	TiO_2	• Antibacterial against *Escherichia coli*, *Streptococcus mutans* • Antifungal agent • Enhanced cytotoxic effects on promastigotes of *Leishmania major*	Yaqoob et al. (2020), Besinis et al. (2014), Planchon et al. (2013), Jebali and Kazemi (2013)
3	ZnO	• Antibacterial against *Klebsiella pneumoniae*, *Campylobacter jejuni*, *Escherichia coli* (bactericidal efficiency increases with decrease in size of the nanoparticles) • Effective for herpes by prevention of viral entry and infection • Enhanced cytotoxic effects on promastigotes of *Leishmania major* • Anthelmintic activity	Azam et al. (2012), Jebali and Kazemi (2013), Reddy et al. (2014), Xie et al. (2011), Liu et al. (2009), Antoine et al. (2016), Ruhollah et al. (2017)
4	Iron oxide	• Antibacterial activity • Effective against hepatitis by inducing knockdown • Antiplasmodial activity against *Plasmodium falciparum* • Anthelmintic activity	Liu et al. (2009), Prabhu et al. (2015), Ryood et al. (2012), Samuel and Sundaram (2013)
5	CuO	• Antibacterial activity towards *Escherichia coli* and *Enterococcus faecalis* • Antiviral activity	Hang et al. (2015), Ahamed et al. (2014)
6	Magnesium oxide	• Antiplasmodial activity against *Plasmodium falciparum* • Enhanced cytotoxic effects on promastigotes of *Leishmania major*	Jebali and Kazemi (2013), Samuel and Sundaram (2013)
7	Cerium oxide	• Antiplasmodial activity against *Plasmodium falciparum*	Samuel and Sundaram (2013)
8	Aluminum oxide	• Antiplasmodial activity against *Plasmodium falciparum*	Samuel and Sundaram (2013)
9	Zirconium oxide	• Antiplasmodial activity against *Plasmodium falciparum*	Samuel and Sundaram (2013)

nonoxidative and dissolved metal ions. Metal nanoparticles release metal ions in presence of aqueous medium which can interact with the cell membrane of the bacteria (Yaqoob et al. 2020). Metal ions being positive charge can bind with the

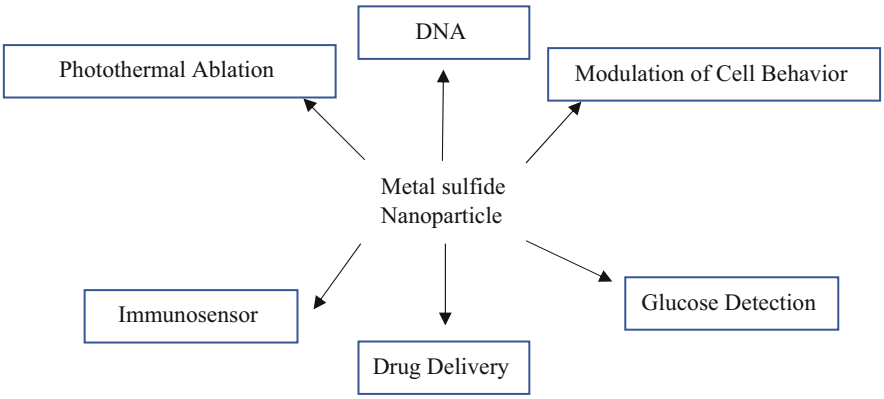

Fig. 5.5 The various applications of metal sulfide nanoparticles

Table 5.3 The role of metal sulfide nanoparticles in infectious diseases

No.	Nanoparticle	Role of nanoparticle against infectious diseases	References
1	Ag_2S	• Antimicrobial activities	Ayodhya and Veerabhadram (2016)
2	Au/CuS core/Shell nanoparticles	• Antimicrobial activities	Addae et al. (2014)
3	Fe_3S_4/Ag composite particles	• Antimicrobial activities	Quanguo et al. (2014)
4	ZnS	• Antifungal activities • Antimicrobial activities	Suyana et al. (2014), Malarkodi et al. (2014)

surface of bacteria which is negative for both gram-positive and gram-negative bacteria and can act as an antibacterial agent. Metal nanoparticles can efficiently act as an antibacterial agent in gram-negative bacteria because the nanoparticles can easily enter into the cell of bacteria through the outer membrane. For example, silver nanoparticles can interact with the bacteria and it releases silver ions which deactivate the cellular enzymes, hinders the cell permeability thereby causing cell death (Aderibigbe 2017). When it comes to gram-positive bacteria, nanoparticles cannot easily enter into the cell because of the presence of thick peptidoglycan membrane (Slavin et al. 2017). Another approach of metallic nanoparticles against microbes is by Reactive Oxygen Species (ROS)–induced oxidative stress. Nano metallic ions stimulate molecular oxygen and form ROS, which disturbs the microbial activities (Aderibigbe 2017).

The hydroxyl radical, superoxide radical, hydrogen peroxide, and single oxygen are related to ROS which disturbs the cellular activity. There is an equilibrium between generation and removal of ROS in the cells. But if there is an increase in the amount of ROS in the cell it causes the fluctuation in the membrane penetrability and will damage the cell membrane of microbes. ROS can easily penetrate into the

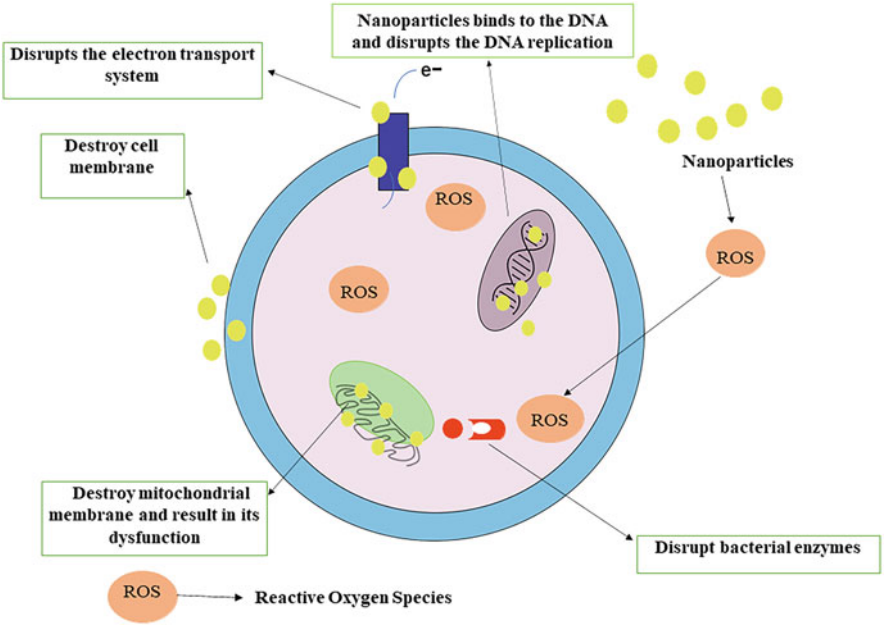

Fig. 5.6 Suggested mechanism of metal-based nanoparticles in antibacterial activity

cell membrane and can kill the bacteria. Figure 5.7 demonstrates how ROS disturbs the cell conditions and kills the cell. ROS are reactive molecule formed because of the receptivity of O_2.

Metal nanoparticles have not only antibacterial properties but also have antiviral activities against HIV, herpes, hepatitis, influenza, etc. They generally prevent the growth and replication of viruses. The viral growth and replication of HIV can be reduced by metal-based nanoparticles (Aderibigbe 2017). Silver, gallium, and gold nanoparticles are found to have antiviral activity (Fig. 5.8).

5.5 Factors Affecting the Antimicrobial Activities of Nanoparticles

The activities of nanoparticles as antimicrobial agent are influenced by many factors like size, shape, charge, doping, capping, etc. on the nanoparticles. The size of metal-based nanoparticles ranges from 1 to 100 nm which helps them to bind with the cell and cell surfaces. It also increases the cell permeability. These factors favor the nanoparticles to act as a therapeutic agent (Aderibigbe 2017). Small nanoparticles are more toxic than large nanoparticles, due to the increased production of Reactive Oxygen Species. It will damage and inactivate essential biomolecules, including DNA (Karakoti et al. 2006). Nanoparticles are supposed to take part in sub-cellular reactions due to their size comparable to that of biological molecules (Thill et al.

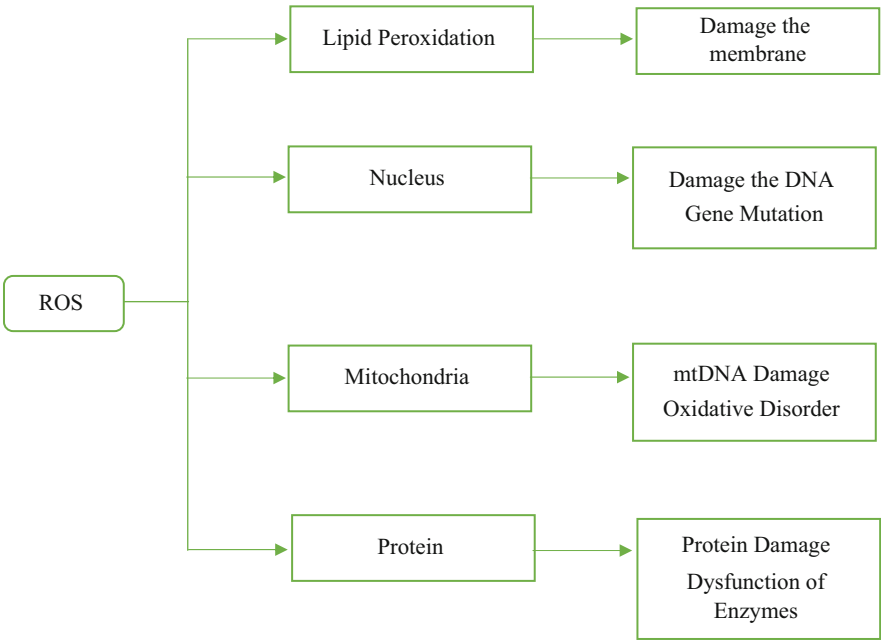

Fig. 5.7 Effects of ROS on biomolecules

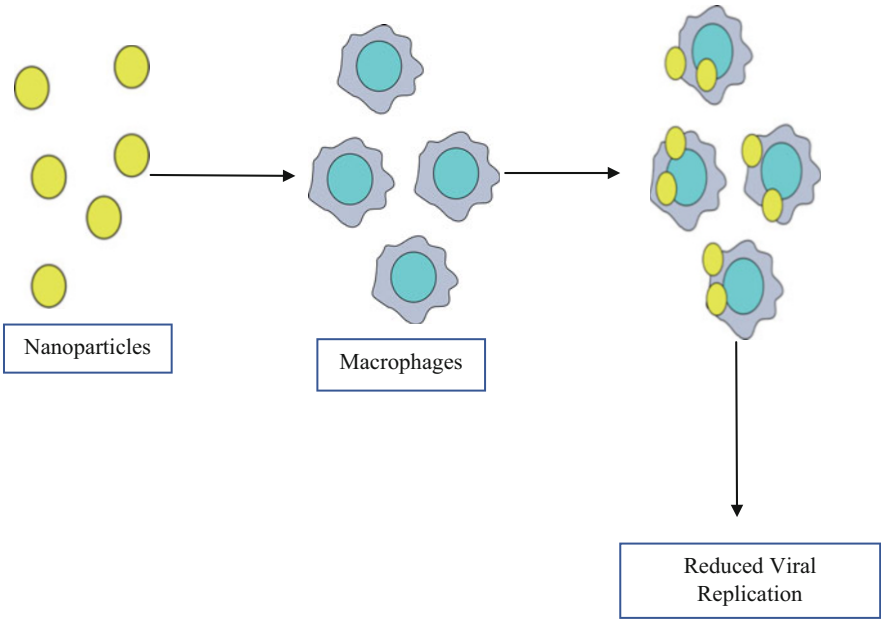

Fig. 5.8 Action of nanoparticles on macrophages

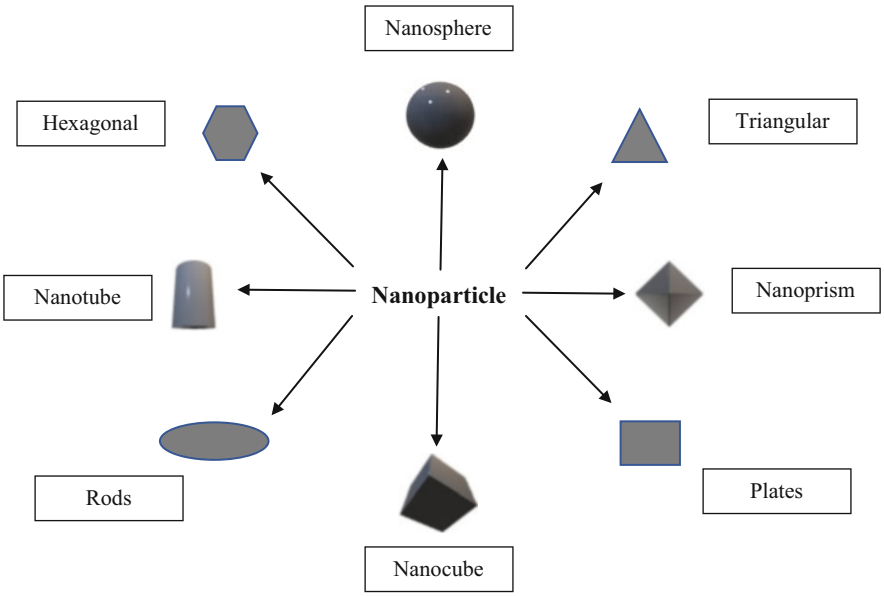

Fig. 5.9 Different shapes of metal-based nanoparticles

2006). So, nanoparticles have the ability to inhibit the growth of bacteria and thereby can act as an antimicrobial agent. There is an important role for the shape of nanoparticles in their antimicrobial activities. Common shape observed in nanoparticle is spherical, plates, rods, etc. (Fig. 5.9).

The presence of corners, edges, or defects (increased abrasiveness) increases the activity of nanoparticles because defects increase its surface area and there by increasing the binding capabilities (Slavin et al. 2017).

Table 5.4 shows the antimicrobial activities of nanoparticles with change in size and shape.

Charge has a very significant role in determining antimicrobial activities of nanoparticles. Positively charged nanoparticles, like amino functionalized polystyrene particles have the ability to change the function of the electron transport chain in bacteria (Ivask et al. 2014). Study on an *E. coli* single gene deletion library identified that bacteria with mutations on ubiquinone biosynthesis related genes were more sensitive when exposed to the positively charged nanoparticles (Ivask et al. 2012). Ubiquinone is an essential constituent which helps in aerobic respiration of bacteria, present in the electron transport chain. When bacteria are exposed with these nanoparticles, produce Reactive Oxygen Species which induces oxidative stress and thereby damages the essential parts of the cell ultimately leading to the death of the cell. It was found that a positive charge on nanoparticles enhanced the toxicity. This is due to the attraction of the negative charge of the bacterial cell wall to the opposite charge making them more effective in antimicrobial action (Slavin et al.

Table 5.4 The antimicrobial activities with varying size and shape

No.	Nanoparticle	Size (nm)	shape	Strain	Reference
1	Ag	9	Spherical	*Escherichia coli*	Ivask et al. (2014)
		19			
		43			
		18			
		23			
		9.5	Spherical	*Streptococcus mutans*	Pérez-Díaz et al. (2015)
		10		Gram-positive strains and *Bacillus*	El Badawy et al. (2011)
2	Ag/CeO_2		Rod	*Escherichia coli* ATCC 8099	Wang et al. (2014)
3	Al_2O_3	11	Spherical	*Escherichia coli* MG 1655	Simon-Deckers et al. (2009)
4	Au	8.4	Spherical	*Acinetobacter baumannii*, *Escherichia coli* J96, *Escherichia coli* O157:H7, MRSA, *Pseudomonas aeruginosa*, PDRAB, *Staphylococcus aureus*	Railean-Plugaru et al. (2016)
5	CeO_2	6	Square	*Hay bacillus* ATCC 6333 *Escherichia coli* ATCC 700926	Pelletier et al. (2010)
		15	Circular, ovoid	*Hay bacillus* ATCC 6333 *Escherichia coli* ATCC 700926	
		22	Ovoid, rectangular, triangular	*Hay bacillus* ATCC 6333 *Escherichia coli* ATCC 700926	
6	MgO	4	Square, polyhedral	*Escherichia coli* C3000, *Bacillus megaterium* ATCC 14581	Stoimenov et al. (2002)
7	TiO_2	12	Spherical	*Escherichia coli* MG 1655	Simon-Deckers et al. (2009)
		17			
		21			
		25			
8	ZnO	12	Spherical	*Escherichia coli*	Padmavathy and Vijayaraghavan (2008)

2017). For example, MgO nanoparticles can destroy the cell wall of *B. subtilis* cells (Stoimenov et al. 2002). A capping agent is generally a surfactant or polymer which is added to coat the nanoparticles and it acts as a stabilizing agent (Fig. 5.10). Synthesized nanoparticles have high surface energy which may lead to agglomerate and uncontrolled growth. Capping agents generally prevent this and stabilizes the nanoparticles. For example, when comparing Ag nanoparticles with Ag

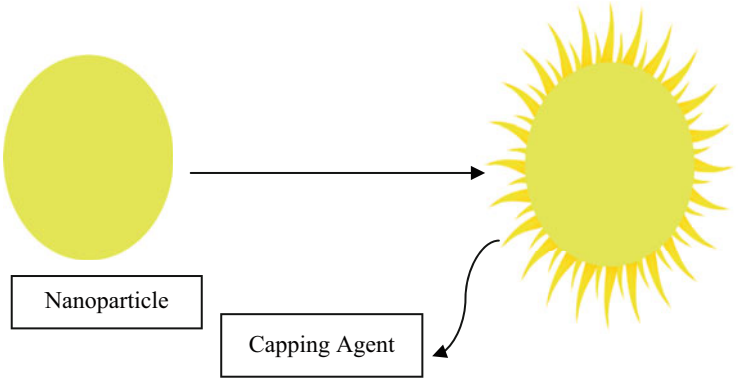

Fig. 5.10 Capping of nanoparticle

nanoparticles stabilized with citrate, chitosan, capped are more effective as antibacterial agent than silver nanoparticles without capping agents. It may be due to the accelerated generation of Ag^+ ions (Yaqoob et al. 2020). Treatment of nanoparticles with halogen also enhances the antimicrobial activities. It may be due to the abrasiveness, high surface area, and oxidizing power of the halogen (Stoimenov et al. 2002).

Doping of the nanoparticles (metal/metal oxide) using a suitable dopant can amplify its properties. The doped metal/metal oxide nanoparticles show high efficiency as antimicrobial agent. Table 5.5 shows some of the doped metal/metal oxide-based nanoparticles and their role against infectious diseases.

5.6 Conclusion

This chapter helps to understand the role of metal-based nanoparticles against infectious diseases. As we know the treatment of infectious diseases is becoming difficult because of the drug resistance of microorganisms. So, it is necessary to develop new therapeutics against diseases. Metal-based nanoparticles have been developed for infectious diseases as therapeutic agents (Aderibigbe 2017). These nanoparticles not only act against infectious diseases but also against non-infectious diseases. For example, gold, silver, nanoparticles can target cancer cells and also can act as antimicrobial agents while CdS nanoparticles can act as an anticancer drug, which is more effective than normal drugs, because they are found to be extremely active on cancerous cells (Shivashankarappa and Sanjay 2020). We also went through the antiviral activities of metal-based nanoparticles, which gives a hope to find solution for the pandemic of SARS-COV-2 (Severe Acute Respiratory Syndrome Coronavirus-Viral antigen) (Fouad 2021). The scope of nanoparticles in the medical field is not limited and yet lots to be discovered. The literature reveals that nanoparticles can show good antibacterial activity, also they have an important role

Table 5.5 The role of doped metal/metal oxide nanoparticles against infectious diseases

No.	Nanoparticles	Dopant	Role of nanoparticles against infectious diseases	References
1	LDPE/Fe-ZnO NPs	Fe	• Antibacterial activity against *Escherichia coli*	Lam et al. (2021)
2	Co-ZnO	Co	• Antimicrobial activity	Oves et al. (2015)
3	Mn-polycrystalline ZnO	Mn	• Antimicrobial activity	Rekha et al. (2010)
4	Zn-Ag	Zn	• Antimicrobial activity (*Escherichia coli* and *Vibrio cholerae*)	Salem et al. (2015)
5	Ta-ZnO	Ta	• Antibacterial application	Guo et al. (2015)
6	Ag-Zirconium Titanium phosphate	Ag	• Antibacterial applications (*Escherichia coli*)	Biswal et al. (2011)

in improving the efficiency of medicine thereby improving health conditions. The usage of antibiotics has extensively increased the resistance of bacteria, which makes the situation worse. Nanoparticles are an effective solution against bacteria in cases where antibiotics are not effective, but there is a need for more studies. Hence, we cannot confirm nanoparticles as an alternative for antibiotics. Before applying this in human beings, proper studies must be done to find out the dosage, response, and after-effects. Firstly, an extensive test must be done to study its toxicity and advantages. Then pre-clinical test must be done in appropriate infected animals to check their toxicity and benefits. Different metal nanoparticles are having different activities. These are evaluated from the animal models. These evaluations help us to study more about the short-term and long-term toxicity. The rate at which these nanoparticles are absorbed can also give us significant information about their toxicology safety profile. Therefore, it is always safe to use them after addressing these issues.

Nanoparticles are having the ability to activate multiple pathways which makes their elucidation difficult but due to this reason they become effective. So, they are non-specific, which means that they do not bind to any specific area of the bacteria, which makes the resistance of bacteria towards nanoparticles difficult and till now a perfect mechanism has not been suggested for the activity of nanoparticles. The literature shows only hypothetical mechanism, hence there is a need for further studies. Therefore, the use of metal-based nanoparticles as an antimicrobial agent will become significant in the coming decades. Metal nanoparticles can tackle the problems faced by the present medical field against infectious diseases effectively. Currently, we are halfway, further studies on the properties and effectiveness of nanoparticles can certainly give us desired products in the future.

Acknowledgement The authors thank Department of Chemistry (CHRIST) Deemed to be University, Bangalore for the successful completion of this work.

References

Addae E, Dong X, McCoy E, Yang C, Chen W, Yang L (2014) Investigation of antimicrobial activity of photothermal therapeutic gold/copper sulfide core/shell nanoparticles to bacterial spores and cells. J Biol Eng 8:11

Aderibigbe BA (2017) Metal-based nanoparticles for the treatment of infectious diseases. Molecules 22(8):1–2

Ahamed M, Alhadlaq HA, Majeed Khan MA, Karuppiah P, Al-Dhabi NA (2014) Synthesis, characterization, and antimicrobial activity of copper oxide nanoparticles. J Nanomater 2014: 1–4

Ahmad A, Syed F, Shah A, Khan Z, Tahir K, Khana AU, Yuan Q (2015) Silver and gold nanoparticles from Sargentodoxa cuneata: synthesis, characterization and antileishmanial activity. RSC Adv 5(90):73793–73806

Antoine TE, Hadigal SR, Yakoub AM, Mishra YK, Bhattacharya P, Haddad C, Valyi-Nagy T, Adelung R, Prabhakar BS, Shukla D (2016) Intravaginal zinc oxide tetrapod nanoparticles as novel immunoprotective agents against genital herpes. J Immunol 196(11):4566–4575

Asghara MA, Zahir E, Shahid S, Khan MN, Asghar MA, Iqbal J, Walker G (2018) Iron, copper and silver nanoparticles: green synthesis using green and black tea leaves extracts and evaluation of antibacterial, antifungal and aflatoxin B1 adsorption activity. LWT 90:98–106

Awwad AM, Jordan A, Salem NM, Aqarbeh MM, Abdulaziz FM (2020) Green synthesis, characterization of silver sulfide nanoparticles and antibacterial activity evaluation. Int Sci Organiz 6(1):42–48

Ayodhya D, Veerabhadram G (2016) Green synthesis, characterization, photocatalytic, fluorescence and antimicrobial activities of Cochlospermum gossypium capped Ag2S nanoparticles. J Photochem Photobiol B Biol 157:57–69

Azam A, Ahmed AS, Oves M, Khan MS, Habib SS, Memic A (2012) Antimicrobial activity of metal oxide nanoparticles against Gram-positive and Gram-negative bacteria: a comparative study. Int J Nanomedicine 2012:6003–6008

Baram-Pinto D, Shukla S, Gedanken A, Sarid R (2010) Inhibition of HSV-1 attachment, entry, and cell-to-cell spread by functionalized multivalent gold nanoparticles. Small 6:1044–1050

Bera RK, Mandal SM, Raj CR (2014) Antimicrobial activity of fluorescent Ag nanoparticles. Lett Appl Microbiol 58:520–526

Besinis A, De Peralta T, Handy RD (2014) The antibacterial effects of silver, titanium dioxide and silica dioxide nanoparticles compared to the dental disinfectant chlorhexidine on Streptococcus mutans using a suite of bioassays. Nanotoxicology 8(1):1–6

Biswal N, Martha S, Subudhi U, Parida K (2011) Incorporation of silver ions into zirconium titanium phosphate: a novel approach toward antibacterial activity. Ind Eng Chem Res 50(16): 9479–9486

Choi S-r, Britigan BE, Narayanasamy P (2017) Ga(III) nanoparticles inhibit growth of both Mycobacterium tuberculosos and HIV and release of interleukin-6 (IL-6) and IL-8 in coinfected macrophages. Antimicrob Agents Chemother 61:e02505–e02516

Ding X, Yang YY, Xu Q-H, Yuan P (2018) Metal nanoparticles for diagnosis and therapy of bacterial infection. Adv Healthc Mater 7(1701392):1–17

Dixit CK, Kumar A (2017) 3-Methods for characterization of nanoparticles. In: Advances in nanomedicine for the delivery of therapeutic nucleic acids. Woodhead Publishing, Sawston, UK, pp 43–58

Duran N, Rai M (2011) Metal nanoparticles in microbiology. Springer, New York

Dutta PP, Bordoloi M, Gogoi K, Roy S, Narzary B, Bhattacharyya DR, Mohapatra PK, Mazumder B (2017) Antimalarial silver and gold nanoparticles: green synthesis, characterization and in vitro study. Biomed Pharmacother 91:567–580

Ealias AM, Sravankumar MP (2017) A review on the classification, characterization, synthesis of nanoparticles and their applications. Mater Sci Eng 263:032019

Ebrahim-Saraie HS, Heidari H, Rezaei V, Mortazavi SMJ, Motamedifar M (2018) Promising antibacterial effect of copper oxide nanoparticles. Pharm Sci 24:213–218

El Badawy AM, Silva RG, Morris B, Scheckel KG, Suidan MT, Tolaymat TM (2011) Surface charge-dependent toxicity of silver nanoparticles. Environ Sci Technol 45:283–287

Elechiguerra JL, Burt JL, Morones JR, Camacho-Bragado A, Gao X, Lara HH, Yacaman MJ (2005) Interaction of silver nanoparticles with HIV-1. J Nanobiotechnol 3:6

Fellahi O, Sarma RK, Das MR, Saikia R, Marcon L, Coffinier Y, Hadjersi T, Maamache M, Boukherroub R (2013) The antimicrobial effect of silicon nanowires decorated with silver and copper nanoparticles. Nanotechnology 24:1–6

Feng QL, Wu J, Chen GQ, Cui FZ, Kim TN, Kim JO (2000) A mechanistic study of the antibacterial effect of silver ions Escherichia coli and Staphylococcus aureus. J Biomed Mater Res 52(4):662–668

Feng F, Sakoda Y, Ohyanagi T, Nagahori N, Shibuya H, Okamastu M, Miura N, Kida H, Nishimura S-I (2013) Novel thiosialosides tethered to metal nanoparticles as potent influenza A virus haemagglutinin blockers. Antivir Chem Chemother 23(2):59–65

Fouad GI (2021) A proposed insight into the anti-viral potential of metallic nanoparticles against novel coronavirus disease-19 (COVID-19). Bull Natl Res Cent 45(36):1–22

Gou J, Zhuge J, Liang F (2012) Processing of polymer nanocomposites. In: Manufacturing techniques for polymer matrix composites (PMCs). Woodhead Publishing, Sawston, UK, pp 95–119

Guo B-L, Han P, Guo L-C, Cao Y-Q, Li A-D, Kong J-Z, Zhai H-F, Di W (2015) The antibacterial activity of Ta-doped ZnO nanoparticles. Nanoscale Res Lett 10(1):1047

Hang X, Peng H, Song H, Qi Z, Miao X, Xu W (2015) Antiviral activity of cuprous oxide nanoparticles against hepatitis C Virus in vitro. J Virol Methods 222:150–157

Hu RL, Li SR, Kong FJ, Hou RJ, Guan XL, Guo F (2014) Inhibition effect of silver nanoparticles on herpes simplex virus 2. Genet Mol Res 13(3):7022–7028

Ismail Aiad M, Riya A, Tawfik SM, Abousehly MA (2016) Synthesis, surface properties and biological activity of N,N,N-tris(hydroxymethyl)-2-oxo-2-(2-(2-(alkanoyloxy)ethoxy)ethoxy) ethanaminium chloride surfactants. Egypt J Pet 25:306

Ivask A, Suarez E, Patel T, Boren D, Ji Z, Holden P, Telesca D, Damoiseaux R, Bradley KA, Godwin H (2012) Genome-wide bacterial toxicity screening uncovers the mechanisms of toxicity of a cationic polystyrene nanomaterial. Environ Sci Technol 46:2398–2405

Ivask A, ElBadawy A, Kaweeteerawat C, Boren D, Fischer H, Ji Z, Chang CH, Liu R, Tolaymat T, Telesca D, Zink JI, Cohen Y, Holden PA, Godwin HA (2014) Toxicity mechanisms in Escherichia coli vary for silver nanoparticles and differ from ionic silver. ACS Nano 8:374–386

Jebali A, Kazemi B (2013) Nano-based antileishmanial agents: a toxicological study on nanoparticles for future treatment of cutaneous leishmaniasis. Toxicol In Vitro 27(6): 1896–1904

Johnstone R, Wilcoxon J (2012) In: Palmer RE (ed) Metal nanoparticles and nanoalloys. Academic Press, Elsevier, Amsterdam

Karakoti AS, Hench LL, Seal S (2006) The potential toxicity of nanomaterials. JOM 58:77–82

Keat CL, Aziz A, Eid AM, Elmarzugi NA (2015) Biosynthesis of nanoparticles and silver nanoparticles. Bioresour Bioprocess 2:47–58

Kesarkar RN, Oza G, Pandey S, Dahake R, Mukherjee S, Chowdhary A, Sharon M (2017) Gold nanoparticles: effective as both entry inhibitors and virus neutralizing agents against HIV. J Microbiol Biotechnol Res 2:276–283

Klebowski B, Depciuch J, Parlińska-Wojtan M, Baran J (2018) Applications of noble metal-based nanoparticles in medicine. Int J Mol Sci 19(12):4031

Kolahalam LA, Viswanath IK, Diwakar BS, Govindh B, Reddy V, Murthy Y (2019) Review on nanomaterials: synthesis and applications. Mater Today Proc 18:2182–2190

Kurtjak M, Vukomanović M, Kramer L, Suvorov D (2016) Biocompatible nano-gallium/hydroxyapatite nanocomposite with antimicrobial activity. J Mater Sci Mater Med 27:170

Lam S-M, Sin J-C, Zeng H, Lin H, Li H, Chai Y-Y, Choong M-K, Mohamed AR (2021) Green synthesis of Fe-ZnO nanoparticles with improved sunlight photocatalytic performance for polyethylene film deterioration and bacterial inactivation. Mater Sci Semicond Process 123: 105574

Liu Y, He L, Mustapha A, Li H, Hu ZQ, Lin M (2009) Antibacterial activities of zinc oxide nanoparticles against Escherichia coli O157:H7. J Appl Microbiol 107:1193–1201

Malarkodi C, Rajeshkumar S, Paulkumar K, Vanaja M, Gnanajobitha G, Annadurai G (2014) Biosynthesis and antimicrobial activity of semiconductor nanoparticles against oral pathogens. Bioinorg Chem Appl 2014:1–10

McNeil SE (2005) Nanotechnology for the biologist. J Leukoc Biol 78(3):585–594

Mishra A, Kaushik NK, Sardar M, Sahal D (2013) Evaluation of antiplasmodial activity of green synthesized silver nanoparticles. Colloids Surf B Biointerfaces 111:713–718

Mohamed MM, Fouad SA, Elshoky HA, Mohammed GM, Salaheldin TA (2017) Antibacterial effects of gold nanoparticles against Corynebacterium pseudotuberculosis. Int J Vet Sci Med 5(1):23–29

Mohammadi G, Nokhodchi A, Barzegar-Jalali M, Lotfipour F, Adibkia K, Ehyaei N, Valizadeh H (2011) Physicochemical and anti-bacterial performance characterization of clarithromycin nanoparticles as colloidal drug delivery system. Colloids Surf B Biointerfaces 88(1):39–44

Oves M, Arshad M, Khan MS, Ahmed AS, Azam A, Ismail IMI (2015) Anti-microbial activity of cobalt doped zinc oxide nanoparticles: targeting water borne bacteria. J Saudi Chem Soc 19(5): 581–588

Padmavathy N, Vijayaraghavan R (2008) Enhanced bioactivity of ZnO nanoparticles—an antimicrobial study. Sci Technol Adv Mater 9:035004–035010

Pelletier DA, Suresh AK, Holton GA, McKeown CK, Wang W, Gu B, Mortensen NP, Allison DP, Joy DC, Allison MR, Brown SD, Phelps TJ, Doktycz MJ (2010) Effects of engineered cerium oxide nanoparticles on bacterial growth and viability. Appl Environ Microbiol 76:7981–7989

Pérez-Díaz MA, Boegli L, James G, Velasquillo C, Sánchez-Sánchez R, Martínez-Martínez R-E, Martínez-Castañón GA, Martinez-Gutierrez F (2015) Silver nanoparticles with antimicrobial activities against Streptococcus mutans and their cytotoxic effect. Mater Sci Eng 55:360–366

Planchon M, Ferrari R, Guyot F, Gélabert A, Menguy N, Chanéac C, Thill A, Benedetti MF, Spalla O (2013) Interaction between Escherichia coli and TiO_2 nanoparticles in natural and artificial waters. Colloids Surf B Biointerfaces 102:158–164

Prabhu YT, Venkateswara Rao K, Siva Kumari B, Kumar VSS, Pavani T (2015) Synthesis of Fe_3O_4 nanoparticles and its antibacterial application. Int Nano Lett 5:85–92

Quanguo H, Chunyan H, Jun L (2014) Preparation, characterization and antibacterial activity of magnetic Greigite and Fe_3S_4/Ag nanoparticles. Nanosci Nanotechnol Lett 6(1):10–17

Railean-Plugaru V, Pomastowski P, Rafinska K, Wypij M, Kupczyk W, Dahm H, Jackowski M, Buszewski B (2016) Antimicrobial properties of biosynthesized silver nanoparticles studied by flow cytometry and related techniques. Electrophoresis 37:752–761

Rashid MMO, Ferdous J, Banik S, Islam MR, Mazbah Uddin AHM, Robel FN (2016) Anthelmintic activity of silver-extract nanoparticles synthesized from the combination of silver nanoparticles and M. charantia fruit extract. BMC Complement Altern Med 16:242

Reddy LS, Nisha MM, Joice M, Shilpa PN (2014) Antimicrobial activity of zinc oxide (ZnO) nanoparticle against Klebsiella pneumoniae. Pharm Biol 58:1388–1397

Rekha K, Nirmala M, Nair MG, Anukaliani A (2010) Structural, optical, photocatalytic and antibacterial activity of zinc oxide and manganese doped zinc oxide nanoparticles. Phys B Condens Matter 405(15):3180–3185

Ruhollah D, Majdedin G, Ali N, Mahdi T, Hashemzadeh, Sadegh M (2017) Anthelmintic effects of zinc oxide and iron oxide nanoparticles against Toxocara vitulorum. Int Nano Lett 7:157–164

Ryood S-R, Jang H, Kim K-S, Lee B, Kim KB, Kim Y-K, Yeo W-S, Lee Y, Kim D-E, Min D-H (2012) Functional delivery of DNAzyme with iron oxide nanoparticles for hepatitis C virus gene knockdown. Biomaterials 33(9):2754–2761

Saeed K, Khan I, Khan I (2019) Nanoparticles: properties, applications and toxicities. Arab J Chem 12(7):908–931

Salas P, Odzak N, Echegoyen Y, Kägi R, Sancho MC, Navarro E (2019) The role of size and protein shells in the toxicity to algal photosynthesis by ionic silver delivered from silver nanoparticles. Sci Total Environ 692:233–239

Salem W, Leitner DR, Zingl FG, Schratter G, Prassl R, Goessler W, Reidl J, Schild S (2015) Antibacterial activity of silver and zinc nanoparticles against Vibrio cholerae and enterotoxic Escherichia coli. Int J Med Microbiol 305(1):85–95

Samberg ME, Loboa EG, Oldenburg SJ, Monteiro-Riviere NA (2012) Silver nanoparticles do not influence stem cell differentiation but cause minimal toxicity. Nanomedicine 7:1197–1209

Samuel JI, Sundaram R (2013) In vitro antiplasmodial activity of PDDS-coated metal oxide nanoparticles against Plasmodium falciparum. Appl Nanosci 3:197–201

Shivashankarappa A, Sanjay KR (2020) E. coli-based synthesis of cadmium sulfide nanoparticles, characterization, antimicrobial and cytotoxicity studies. Braz J Microbiol 51:939–948

Simon-Deckers A, Loo S, Mayne-L'hermite M, Herlin-Boime N, Menguy N, Reynaud C, Gouget B, Carrière M (2009) Size-, composition- and shape-dependent toxicological impact of metal oxide nanoparticles and carbon nanotubes toward bacteria. Environ Sci Technol 43: 8423–8423

Slavin YN, Asnis J, Häfeli UO, Bach H (2017) Metal nanoparticles: understanding the mechanisms behind antibacterial activity. J Nanobiotechnol 15(65):1–17

Stoimenov PK, Klinger RL, Marchin GL, Klabunde KJ (2002) Metal oxide nanoparticles as bactericidal agents. Langmuir 18:6679–6686

Subramaniyan SB, Megarajan S, Vijayakumar S, Mariappan M, Anbazhagan V (2018) Evaluation of the toxicities of silver and silver sulfide nanoparticles against Gram-positive and Gram-negative bacteria. IET Nanobiotechnol 13(3):326–330

Suyana P, Nishanth Kumar S, Dileep Kumar BS, Nair BN, Pillai SC, Peer Mohamed A, Warrier KGK, Hareesh US (2014) Antifungal properties of nanosized ZnS particles synthesised by sonochemical precipitation. RSC Adv 4(17):8439–8445

Thill A, Zeyons O, Spalla O, Chauvat F, Rose J, Auffan M, Flank AM (2006) Cytotoxicity of CeO_2 nanoparticles for Escherichia coli. Physico-chemical insight of the cytotoxicity mechanism. Environ Sci Technol 40:6151–6156

Trigilio J, Antoine TE, Paulowicz I, Mishra YK, Adelung R, Shukla D (2012) Tin oxide nanowires suppress herpes simplex virus-1 entry and cell-to-cell membrane fusion. PLoS One 7(10): e48147

Virji AMA (2014) A review of engineered nanomaterial manufacturing processes and associated exposures. In: Comprehensive materials processing, vol 8. Elsevier, Oxford, England, pp 103–125

Wang L, He H, Yunbo Y, Sun L, Liu S, Zhang C, He L (2014) Morphology-dependent bactericidal activities of Ag/CeO_2 catalysts against Escherichia coli. J Inorg Biochem 135:45–53

Wang S, Li J, Zhou Z, Zhou S, Hu Z (2019) Micro-/nano-scales direct cell behavior on biomaterial surfaces. Molecules 24:75–86

Xiang D, Zheng Y, Duan W, Li X, Yin J, Shigdar S, O'Connor ML, Marappan M, Zhao X, Miao Y, Xiang B, Zheng C (2013) Inhibition of A/Human/Hubei/3/2005 (H3N2) Influenza virus infection by silver nanoparticles in vitro and in vivo. Int J Nanomedicine 8(1):4103–4114

Xie Y, He Y, Irwin PL, Jin T, Shi X (2011) Antibacterial activity and mechanism of action of zinc oxide nanoparticles against Campylobacter jejuni. Appl Environ Microbiol 77:2325–2331

Xiong J, Wang Y, Xuea Q, Wu X (2011) Synthesis of highly stable dispersions of nanosized copper particles using L-ascorbic acid. Green Chem 13:900–904

Yaqoob AA, Ahmad H, Parveen T, Ahmad A, Oves M, Ismail IMI, Qari HA, Umar K, Ibrahim MNM (2020) Recent advances in metal decorated nanomaterials and their various biological applications: a review. Front Chem 8:1–23

The Future Therapy of Nanomedicine Against Respiratory Viral Infections

6

Heba S. Abbas, Hossam Saleh, Esraa M. M. Mohammad, Hala A. Abdelgaid, Amira S. H. Mohamed, Ebthal F. M. Elzayat, Salma E. S. Ismail, Noha M. Gamil, and Amany Y. El-Sayed

Abstract

Nowadays, respiratory viruses are the most common cause of diseases in humans, with a substantial effect on the morbidity and mortality around the world. In addition, the emergence of SARS-CoV-2 in recent years threats the public health. Till now, there is no effective therapy for COVID-19, and many techniques are being tried. The current anti-respiratory viral drugs destroy not only the respiratory viruses, but also the host's metabolic processes.

H. S. Abbas (✉)
Microbiology Department, National Organization for Drug Control and Research (NODCAR), Egyptian Drug Authority, Giza, Egypt

H. Saleh
National Institute of Oceanography & Fisheries, NIOF, Cairo, Egypt

E. M. M. Mohammad
Faculty of Science, Tanta University, Cairo, Egypt

H. A. Abdelgaid
Biochemistry Division, Faculty of Science, Cairo University, Cairo, Egypt

A. S. H. Mohamed
Faculty of Science, Suez University, Suez, Egypt

E. F. M. Elzayat
Faculty of Science, Menofia University, Menofia, Egypt

S. E. S. Ismail
Faculty of Pharmacy, Ain Shams University, Cairo, Egypt

N. M. Gamil
Faculty of Pharmaceutical Sciences and Drug Manufacturing, Misr University for Science and Technology, Cairo, Egypt

A. Y. El-Sayed
Biochemistry Department, Faculty of Science, Ain Shams University, Cairo, Egypt

S. Hameed, S. Rehman (eds.), *Nanotechnology for Infectious Diseases*,
https://doi.org/10.1007/978-981-16-9190-4_6

Nanotechnology has transformed the world by providing advanced solutions to a wide range of challenges in healthcare nowadays. Current advancements in the strategy and manufacturing of nanomedicines have provided a variety of benefits over traditional techniques of respiratory viral infection therapy. Upcoming research can be aimed on functionalized nanomaterials in order to enable site-specific, concomitant delivery of several medicines in order to treat a wide range of diseases. Designing nanomaterials and the issue of long-term toxicity should be prioritized. However, with rapid advances nanomaterials, there is hope that the overall treatment of respiratory viral infections can be more efficient. This review displays viruses' classification according to the genomic materials, respiratory viruses' threat, the antiviral efficiency of nanodrugs, and nanomaterials against respiratory viruses, and the possible antiviral mechanism of nanomaterials.

Keywords

Respiratory viruses · SARS-CoV-2 · Antiviral drugs · Toxicity · Nanomaterials · Lactoferrin nanoparticles · Silica nanocarriers · Antiviral mechanism

6.1 Introduction

Globally, respiratory diseases are the most public diseases. These diseases are generally restricted to the upper air routes and are self-limiting. In some case, the infection can proceed to he lower respiratory tract, such as bronchitis and pneumonia. Youngsters and the old people are particularly vulnerable, particularly in underdeveloped nations. Respiratory viruses have a considerably larger role in the infection of the respiratory tract and bronchitis in children, but bacteria are the leading cause of pneumonia, particularly in adults and the clinical symptoms are very overlapping, and there is a growing evidence of bacterial infections followed by the viral diseases (Van Doorn and Yu 2020). There are various respiratory viruses that regularly circulate across all ages and are identified as suited for the effective transmission of individuals to individuals. Furthermore, in recent years, the risks posed to public health are SARS Coronavirus (SARS-CoV), COVID-19 (SARS-CoV-2), and H5N1 avian influenza. However, H5N1 avian influenza virus has caused a few outbreaks of human illnesses. Despite the fact that respiratory viruses cause a large number of illnesses, there is now just a few preventative or therapeutic interventions available (Boncristiani et al. 2009). The most significant task persisting in the progress of active antiviral agents is the viruses' replication in the host cell. The host immunity in this state is weakened. Furthermore, due to the intricacies of viruses, cure is mostly accompanied with symptoms, and complete treatment of viruses might be impossible. Treatments are regarded as a red sign by the researcher, and innovative tools have been investigated in order to conquer the restrictions of current therapies (Zhu et al. 2015).

Because of the nanotechnology efficacy in treating the viral diseases, it has appeared as one of the most hopeful breakthroughs, overcoming the restrictions of

conventional antiviral medications. It not only allowed us to conquer difficulties with drug solubility, bioavailability, bio-distribution, and toxicity, but it also offered medicines distinctive characteristics, which improved their potency and selectivity toward viral cells over host cells (Milovanovic et al. 2017a, b). The use of nanotechnology to combat SARS-CoV-2 may involve processes that affect the viruses' entrance into the host cell, where blocking of the proteins of viruses' surface may render the virus inactive. Also, specific targeting nanoparticles to certain viral protein may stop the internalization of the virus (Kerry et al. 2019). More research is needed to understand the interaction between nanoparticles and SARS-CoV-2 in order to plan coherent targeted curatives (Mainardes and Diedrich 2020). The current review compiles the threat of respiratory viral infections, the toxicity of conventional antiviral medicine, and the recent advancements of nanomedine that opens up innovative ways for advance research for the management of respiratory viral diseases.

6.2 Viruses Classification According to the Genetic Materials

Genetic materials of viruses are the main key for survivability and replication for viruses. They store the genetic database and information necessary for virus evolution and revolution (Brister et al. 2014). Viruses can be classified into different types according to the type of genetic material into two types: DNA virus and RNA virus. The DNA virus may be either double strand DNA (DSDNA) as iridoviridae and herpesviridae, or single strand DNA (ss-DNA) as anellovirus and parvoviridae (Wolf et al. 2018). RNA virus also have two types, double strand RNA (ds-RNA) as birnaviridae and reoviridae, and single strand RNA (ss-RNA) in which there are two sub-categories of it: positive sense RNA (+RAN) as coronaviridae, and negative sense RNA (−RNA) as orthomyxoviridae (Fig. 6.1). Additionally, according to the presence and albescence of envelope virus, they are categorized into: enveloped and non-enveloped virus. Hepadna viruses are an example of enveloped DNA, and corona virus, hepatitis D, and retroviruses are enveloped RNA viruses. However, examples of non-enveloped viruses are adenoviruses (DNA viruses) and hepatitis A and E viruses (RNA viruses).

Virus replication and mechanism of action in host cell vary according to type of genetic material, and number of strands (ss or ds) for each genetic material. In the double stranded DNA (ds-DNA) viruses, viruses invade the cell nucleus firstly, and then it begins to replicate using the polymerase enzyme inside the host cell to replicate itself as in herpesviridae family. The ss-DNA viruses have a circular genome, the replication process occurs inside the nucleus with a mechanism called rolling cycle, the single stranded genome is converted into ds-DNA intermediates then transcription to mRNA is occurred as in parvoviridae family (Wolf et al. 2018; Kaufman et al. 2015; Malathi and Renuka 2019; Modrow et al. 2013). However, the double stranded (ds-RNA) virus enters the host cell and uses the core capsid to replicate in the cell cytoplasm using the host cell polymerase enzyme for replication. The ds-RNA then splits and one strand acts as a template for mRNA generation as in

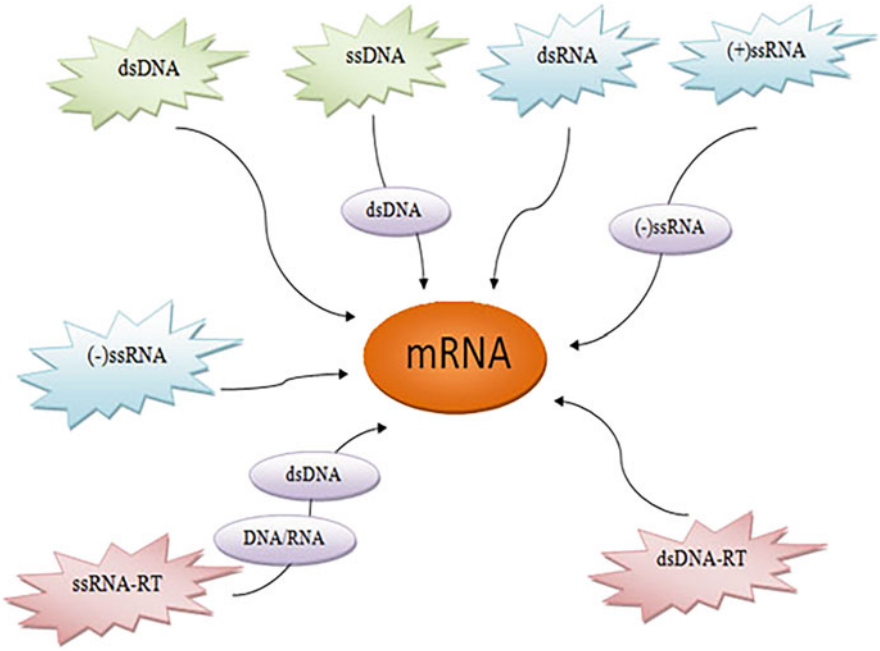

Fig. 6.1 Classification of Viruses according to their genetic materials and their replication mechanism. (Conducted by Hosam Saleh author)

rheoviridae family (Malathi and Renuka 2019; Modrow et al. 2013). Additionally, the replication processes in the ssRNA (+RNA) viruses occur in the cytoplasm where poly proteins are translated from the genomic RNA and polymerase enzyme synthesizing the complementary strand (negative polarity) for the genomic RNA strand (positive sense RNA template). The new produced complementary strand acts as a template for production of new viral genome and sub-genomic mRNA as in corona virus. Another type of RNA virus is the positive sense ssRNA reverse transcriptase virus. This type of virus contains two copies of (ssRNA), which use the reverse transcriptase enzyme to be converted to ds-DNA. The newly produced transcribed DNA is then transported to the host cell genome and start transcription and translation to mRNA using integrase enzyme as retrovirus. Moreover, there is a ds-DNA reverse transcriptase virus, which contains partial class ds-DNA genome, and produces the ssRNA intermediate (act as mRNA). The produced mRNA can be reverse transcribed to ds-DNA using reverse transcriptase enzyme to reproduction (Fig. 6.1) (Matamoros et al. 2011).

6.3 The Threat of Respiratory Viral Infections

Globally, viral diseases provide a substantial threat to both public health and global economy. Current infectious diseases, such as influenza, coronavirus, ebola, and dengue that disseminated directly or indirectly from individual to another, have emphasized the urgent need for new antiviral therapeutics (Lozano et al. 2012).

Respiratory viruses are the utmost common cause of infection in humans, having a considerable influence on morbidity and death globally, particularly in children. Severe respiratory infections are responsible for around 20% of all pediatric fatalities globally, particularly among poor people in tropical climates, where the percentage of cases of severe respiratory infections per fatality can be considerably higher than in temperate countries. Several respiratory viruses infected human of all ages, and

Table 6.1 Several human respiratory viruses, their families, and their general symptoms

Respiratory viruses	Family	Symptoms	References
Human respiratory syncytial virus	Paramyxoviridae	Mild symptoms as cough, rhinorrhea, sore throat, and fever. Severe signs as bronchiolitis and pneumonia	Shafagati and Williams (2018)
Human parainfluenza virus	Paramyxoviridae	Otitis media, pharyngitis, conjunctivitis, croup, tracheobronchitis, and pneumonia. Unusual respiratory signs	Branche and Falsey (2016)
Human rhinovirus	Picornaviridae	Respiratory pain, apnea, rhinorrhea, and hypothermia; all babies need respiratory aid	Jacobs et al. (2013)
Human metapneumovirus	Paramyxoviridae	Mild upper respiratory tract infections signs to severe pneumonia	Walsh et al. (2008), Haas et al. (2013)
Severe acute respiratory syndrome (SARS)	Coronaviridae	Flu-like signs as productive cough, rhinorrhea, sore throat, fever, and troubles of breath and pneumonia	WHO (2003)
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)	Coronaviridae	Fever, dry cough, troubles in breathing, diarrhea, conjunctivitis, headache, loss of taste or smell, rash on skin, and chest pain	National Center for Immunization and Respiratory Diseases (NCIRD), Division of Viral Diseases (2021)
Human bocavirus	Parvoviridae	Hypoxia, respiratory distress, wheezing, cough, and fever	Körner et al. (2011)

well-adapted to cause transmission from person to other (Table 6.1) (Boncristiani et al. 2009).

The mucosa of human respiratory system is the greatest noteworthy portion and the initial locate of the entry of several viruses infection. These infectious pathogens primarily invade the upper respiratory tract and then influence the lower respiratory tract, leading to morality. The diseases of lower respiratory tract clarify a principal cause of the spread human disease and mortality with ~3 million deaths annually worldwide (WHO 2016).

The respiratory viruses often come into the host via airborne spreads, and then disseminate through direct adherent or droplets/aerosols, efficiently propagate in the respiratory tract, and generally origin of clinical indications, specifically fever, dyspnea, cough, bronchiolitis, and/or pneumonia as in Table 6.1 (Kutter et al. 2018). For instance, the pandemic corona viruses, which are a large family of RNA viruses, pass on a disease to the upper respiratory and gastrointestinal tract of vertebrates. The key symptoms in human comprise cough, fever, and, in more critical cases, troubles in breathing have been described with potential death from SARS-CoV, MERS-CoV, and SARS-CoV-2 (Gurunathan et al. 2020a, b).

6.3.1 The SARS-CoV-2 Threat

Coronaviruses belong to the subfamily *Coronavirinae* (order: *Nidovirales*, family: *Coronaviridae*), which are enclosed rounded viruses with the ssRNA genome (Dhama et al. 2014; Schoeman and Fielding 2019). The beta-coronavirus, which is responsible for COVID-19, was discovered for the first time in Wuhan, in the end of 2019. SARS-CoV-2 is a zoonotic virus, or animal origin virus, that be adherent to coronaviridae viruses family, has challenged the world due to its vigorously spreading, about 85 million confirmed cases and 2 million death up till now have been confirmed with COVID-19 with (in 218 countries) (WHO 2022). This virus is thought to be originated in bats hosts then jump from other animals to infect human then spread between infected people via droplets of sneezing and coughing either in air or surfaces (Das et al. 2020; Ahmed et al. 2020a, b; Wu et al. 2020a, b). Unfortunately countries cannot lockdown completely due to the economic issues thus early detection of infected individuals besides monitoring and treatment is required as quick as possible. Infected hosts display various clinical types, extending from asymptomatic to serious symptoms in their reproductive organs, circulatory, respiratory, and digestive systems (Gurunathan et al. 2020a, b). Thus, the viral infection can fight with by controlling the chain of transmission especially till now no available approved drug specific for this virus and all available vaccines are still under trials.

SARS-CoV-2 is about 50–500 nm ssRNA virus composed of four proteins N, S, M, and E proteins which refer, respectively, to nucleocapsid that hold the viral RNA, spike, membrane for attachment of virus with host cells, while M for membrane and E for envelope proteins both of them lie exterior to complete the viral structure (Yan et al. 2020). The S protein can attach to the angiotensin-converting

enzyme 2 receptors (ACE2Rs) at the membrane surface of the host cell that help the virus to enter the host (e.g., human) cells via attachment to this cell receptor. ACE2Rs are focused at the lung, intestine, and kidney of human body proposing that corona virus can actually target these organs (Mehranfar and Izadyar 2020). Gene sequence analysis of SARS-CoV-2 suggested a great similarity to the RNA sequence of SARS and MERS which are two coronaviruses emerged in 2003 and 2012, respectively, and were epidemic which are reason for the deadly acute respiratory diseases in humans and flu-like diseases (Badgujar et al. 2020). Moreover, Zhou et al. (2020) reported the genome sequence of the novel SARS-CoV-2 is similar to the genome of bat coronavirus by 96.2%. However, the comparison between SARS-CoV and SARS-CoV-2 in human to human transmission is in the interest of the last one, that can spread much faster, which has already WHO declared it as a global pandemic on 11 March 2020 (Wan et al. 2020). However, this homology can be useful in two points; the first is that antiviral drugs used for these viruses can be used temporarily for COVID-19 infection (Zhou et al. 2020). Drugs such as chloroquine, acyclovir, hydroxychloroquine, ganciclovir, remdesivir, ganciclovir, ribavirin, lopinavir, and ritonavir are applied for COVID-19 treatment, but no drug is permitted by the FDA for the COVID-19 treatment, these drugs display better interactions with the active site of SARS-CoV-2 because of greater electrostatic and dispersion interfaces (Badgujar et al. 2020; Wang 2020). The second important point of homology is the available steps of vaccine designing for SARS/MERS in literature data that can decrease the steps of designing rapid vaccines for COVID19 that can take 2–3 years (Lu et al. 2020). Recent studies have revealed that novel SARS-CoV-2 and SARS-CoV infect host cells by using the same receptor (angiotensin-converting enzyme 2, ACE2), and the adhering of SARS-CoV-2 to the surface receptors of host cells is facilitated by the S proteins (Wu et al. 2020a, b; Wan et al. 2020; Hofmann et al. 2020). Moreover, it was detected that cells that possess ACE2, and not possess the enzymes aminopeptidase N and human dipeptidyl peptidase-4, were more liable for SARS-CoV-2 infection (Wrapp et al. 2020).

6.3.2 Toxicity of Conventional Antiviral Drugs

Chemical antiviral drugs such as Emtricitabine, Lamivudine, Aciclovir, Nevirapine, and others are currently in use. Most of those chemical drugs, on the other hand, may cause side effects or dose-limiting toxicity (Guo et al. 2019). One of the most difficult aspects of treating viral infections is getting enough drugs to reach pathogens inside their intracellular compartments (Li and Armstead 2011). Furthermore, antiviral drug may have short half-life, necessitating repeated and outsized doses to produce a therapeutic effectiveness, resulting in high costs, poor patient compliance, and serious side effects. Moreover, drug resistance can arise when patients do not adhere to their treatment protocols perfectly (Goossens 2009) or when infections are exposed to suboptimal drug dosages for a prolonged period of time (Sandegren and Andersson 2009).

Amantadine drugs prevent replication by inhibiting the action of the M2 protein. Amantadine is only effect on influenza A, not influenza B, since influenza B lacks an M2 protein and instead uses a replacement protein known as NB, which is unaffected by amantadine (Betakova et al. 1996). However, amantadine was related with central nervous system (CNS) toxic effects such as irritability, anxiety, insomnia, agitation, concentration disorder, ataxia, lisping, depression, and hallucinations (Keyser et al. 2000). Furthermore, lower extremity oedema and involuntary myoclonic jerks were also reported after treatment with amantadine (Yarnall and Burn 2012).

Similarly, oseltamivir is an oseltamivir carboxylate medication (Ro 64-0802; GS4071), which is a powerful and specific neuraminidase inhibitor of the glycoprotein that is crucial to influenza A and B viruses' propagation (McClellan and Perry 2001). However, severe toxic effects of oseltamivir, such as hepatitis, an increase in liver enzymes, and allergic reactions that lead to anaphylaxis, are less common. It also has the potential to cause Stevens-Johnson syndrome (Simón-Talero et al. 2012). In recent years, toxic epidermal necrolysis, cardiac arrhythmia, convulsions, elevated diabetes, and hemorrhagic colitis have all been recorded (Chen and Lai 2013). Neurological effects of oseltamivir included abnormal behaviors and hallucinations (Guisado-Macías et al. 2012). Its safety is not clear whether in pregnant women or in pediatrics (Kiso et al. 2004). Unfortunately, as previously mentioned, current antiviral medications also damage not only the viral infection but also the host's metabolic processes. There are plenty of other challenges to overcome in the development of effective antiviral therapies (Sumbria et al. 2021). Current advances in nanotechnology can help to resolve these hurdles, opening up new possibilities for the development of innovative broad-spectrum nanotherapeutic platforms to fight viral infections which presents a very promising approach (Fang et al. 2018). Antiviral drugs may be incorporated into nanoparticles to improve bioavailability, thus decreasing systemic toxicity, improving effectiveness, and keeping the therapeutic window for longer time (Stephen et al. 2020; Sharmin et al. 2021).

Any nanomaterials have an intrinsic toxicity that helps them to destroy viruses directly (Zhou et al. 2021). As nanoparticles invade the human body, they can pass through numerous cell barriers to influence the most sensitive organs, such as the lungs, liver, and kidney, causing mitochondrial impairment, DNA mutations, and ultimately cell apoptosis or death (Gulati et al. 2018).

6.4 Nanodrugs and Their Efficacy in Killing Viruses

A particle's antibacterial and antiviral activity is totally related with chemicals that kill bacteria and viruses or decrease their rate of growth without being very hazardous to neighboring tissues. The most recently found antibacterial agents are natural substances that have been chemically changed (von Nussbaum et al. 2006). Nanotechnology has emerged as one of the most promising developments, overcoming the shortcomings of conventional antiviral medicines, because of its efficiency to deal with viral diseases. Not only did it allow us to solve snags associated with the

solubility, bioavailability, bio-distribution, and drugs toxicity, but it also gave drugs distinctive properties, which consequently improved their effectiveness and selectivity in the direction of viral cells against the host cells. Nanoformulations can act as antiviral agents through different mechanisms as well. One of the most influential properties of nanoparticles is having immunochemically inert surfaces that minimize their enzymatic degradation and uptake by phagocytes of the reticuloendothelial system and give them high in vivo retention in turn. Also, nanoparticles have improved deposition to the diseased sites and high efficacy, and this is attributed to the enhanced permeability phenomenon that causes vasculatures to be compromised. Several nanoparticles have been proposed over the years as carriers for antiviral agents (Milovanovic et al. 2017a, b). Nanoparticles have been known with their ability to interfere with the cycle of viral infections in an efficient manner. Since the contact of viruses with the host cells is mediated by multivalent interactions and given that nanostructure has multivalent character that allows for their attachment to several ligands, nanostructures are capable of interfering with viral attachment and blocking viral entry into host cells (Łoczechin et al. 2019).

Nanoparticles (NPs) in the range of 1–100 nm size were applied as a tool for drug delivery, identification, and cure of various infectious diseases (Aderibigbe 2017; Maduray and Parboosing 2020; Prasad et al. 2018). Multiple nanomaterials and nanocarriers can act as viral activity inhibitor, they utilized in many new pharmaceutical applications due to its high accuracy in delivering of drugs to the target sites devoid of the healthy cells, detecting the viral infections in early stages, delivering nanotherapeutic molecules or nano-vaccines to certain specific organs or cells. Several nanomedicines are undergoing investigation for the treatment of viral infections. For instances, silver nanoparticles (AgNPs), gold nanoparticles (AuNPs), organic nanoparticles, graphene oxides, zinc oxide, liposomes, quantum dots nanoparticles (Lei et al. 2008; Gurunathan et al. 2020b; Rafiei et al. 2016; Michalet et al. 2005). These nanoparticles may display a brilliant perspective in the future of virus therapy especially in the pandemic Coronaviruses (CoVs). There are many studies providing a deep view about these particles and the mechanism of their action as antiviral in the cell. This short review represents some of these studies about part of these nanoparticles and how they work in the cell.

6.4.1 Nanomedicine Weapon Against SARS-CoV-2 Threat

Nanomedicine has an influence on all sectors of medicine and is regarded as a significant tool for innovative diagnostics, imaging therapy, nanomedicine treatments, vaccinations, and the development of biological materials for regeneration human cells, and organs (Fluhmann et al. 2018). Polymeric nanoparticles, liposomal and protein nanoparticles have been utilized in nanodrugs, particularly for drug delivery. A basis for their usage in various medical purposes is the amount of interactions between nanoparticles and biologically active molecules (Patra et al. 2018). Nanodrugs have been produced for years, and numerous are now being tested in experimental trials for treating several diseases such as infectious, cancer,

cardiovascular, and any inflammation. However, only a handful has been authorized for the human practice (Kupferschmidt and Cohen 2020). Furthermore, nanoparticles can enhance particular medication targeting and regulated the rate of drug release, consequently influencing therapy effectiveness and safety. Also, metallic nanoparticles have also been used in nanodrugs, owing to their antimicrobial properties (Kupferschmidt and Cohen 2020).

The use of nanoparticles to battle SARS-CoV-2 might entail processes that affect the virus's entrance into the cell of the host until it is inactivated. Because blocking viral surface proteins may inactivate the virus, tailored nanoparticles specific to virus produced proteins may limit the internalization of the virus (Kerry et al. 2019). Metallic nanoparticles have been demonstrated to impede viral adhesion to the surface of the cell, hence inhibiting internalization of the virus and reducing the replication of virus. Titanium, silver, gold, and zinc nanoparticles have already demonstrated their antiviral activity against HIV, influenza virus, respiratory syncytial virus, and others (Kupferschmidt and Cohen 2020). The nanoparticles bind to the viral envelope or protein, affecting the adherence with the host cell, according to the mode of action. The treatment's efficacy is related to the dimensions, shape, and the charge of nanoparticles' surface; however, precautions must be taken concerning the nanoparticles' concentration to bypass their cytotoxicity (Singh et al. 2017).

Organic nanoparticles have been utilized to administer antiviral drugs such as zidovudine, dapivirine, acyclovir, and others with the goal of increasing the bioavailability of drug, promoting effective drug delivery, and promoting targeted antiviral action. The fundamental restriction of antiviral drugs is the absence of particular targeting, which causes the cytotoxic effect on the host cell. This issue can be solved by the organic nanoparticles because of their adaptability; nanoparticles can be used as adjustable vectors for viral targeting and selective medication delivery (Milovanovic et al. 2017a, b). Antiviral drugs such as chloroquine, lopinavir, ribavirin, ritonavir, and remdesivir have shown encouraging efficacy against SARS-CoV-2 (Li et al. 2020). Nanoencapsulation of antiviral medications may aid in the progress of safer therapeutics for SARS-CoV-2 and other viral infections. Although it is well recognized that nanotechnology-based drug delivery approach improves current therapies in medicine, it remains under investigation as shown in the SARS-CoV-2 pandemic (Uskokovic 2020). In conclusion, nanoparticles may play a crucial role in multiple phases of SARS-CoV-2 pathogenesis, given their ability to prevent viral adherence and their fusion with membrane during the entrance of virus. Furthermore, nanoencapsulated medications may be more effective in triggering intracellular pathways that produce permanent virus damage and limit transcription, translation, and replication of the virus (Mainardes and Diedrich 2020).

6.4.2 Biogenic and Non-biogenic Metallic Nanoparticles and Its Antiviral Efficiency

There are extensive groups of biological, chemical, and physical approaches to produce NPs (Khandel et al. 2018). Chemical routine production of metallic nanoparticles includes the bottom-up approach with procedures such as the polyol synthesis, sol-gel method, microemulsion, and hydrothermal synthesis. A well-defined size nanoparticles are produced by chemical approaches (Yu et al. 2008). The synthesis mechanism requires reducing metal salt ions by reducing agents or decay of metal salts with further energy in the existence of a stabilizer (Khandel et al. 2018). Despite the benefits of chemical synthesis, there may be significant drawbacks, such as the usage of hazardous and non-biodegradable compounds and NP unsuitability for biological purposes (Khandel et al. 2018; Patel et al. 2015).

The physical routine for the NPs production comprises techniques such as ultraviolet (UV) radiation, sonochemical, microwave irradiation, thermal decomposition, laser ablation, photochemical, and radical induction (Khandel et al. 2018; Dhand et al. 2015; Maduray and Parboosing 2020). In appropriately, the rich waste produced by physical approaches for NP production seems to be economically unfavorable (Dhand et al. 2015). Biosynthesis of metallic nanoparticles depends on the bottom-up method that enrolls unicellular and multicellular living organisms (e.g., actinomycetes, bacteria, viruses, fungus, yeast, algae, and plants) (Khandel et al. 2018; Pantidos 2014; Ingale and Chaudhari 2013). Biogenic nanoparticles are environmentally friendly, rapidly formed in large amounts, biocompatible, and of definite size and shape (Khandel et al. 2018; Shah et al. 2015).

Biological nanoparticles can attack drug-resistant viruses, and assisting in the progress of antiviral drugs. Recent literature by El-Sheekh et al. (2020) demonstrates the inhibitory efficiency of cyanobacterial synthesized Ag_2O|AgO-NPs and gold-NPs for the replication of the Herpes Simplex (HSV-1) virus. The results revealed a 90% decrease in cytopathic effect of Herpes Simplex virus using Ag_2O|AgO nanoparticles and gold nanoparticles at 31.25 μL, with a higher decrease rate (49.23%) using Ag_2O|AgO-nanoparticles than gold nanoparticles (42.75%). Additionally, the antiviral activity of biosynthesized Ag nanoparticles was demonstrated against Herpes Simplex Virus (HSV-1), Hepatitis virus-10, and Coxsackie B4 virus. The antiviral mode of action of biosynthesized silver nanoparticles has not been detected, but most probably the antiviral activity of them is attributed to the blocking virus's entrance into host cells by binding the viral envelope glycoproteins. SNPs also may exhibit their antiviral activity through interaction with viral genetic material or via interfering with viral replication pathways (Haggag et al. 2019).

The metallic nanoparticles' antiviral action is based on competitive interaction with cell receptors and viral envelope rupture (Rai et al. 2016). The viral infection is dependent on the virus's entrance and adherence to host cells via the interaction of virus's surface constituents through ligands and proteins of the cellular membrane. The most effective technique for generating novel antiviral medications is to hamper the contacts between virus's ligand and cellular membrane, hence preventing virus adherence and entrance into cells. By analyzing the mode of action of metallic

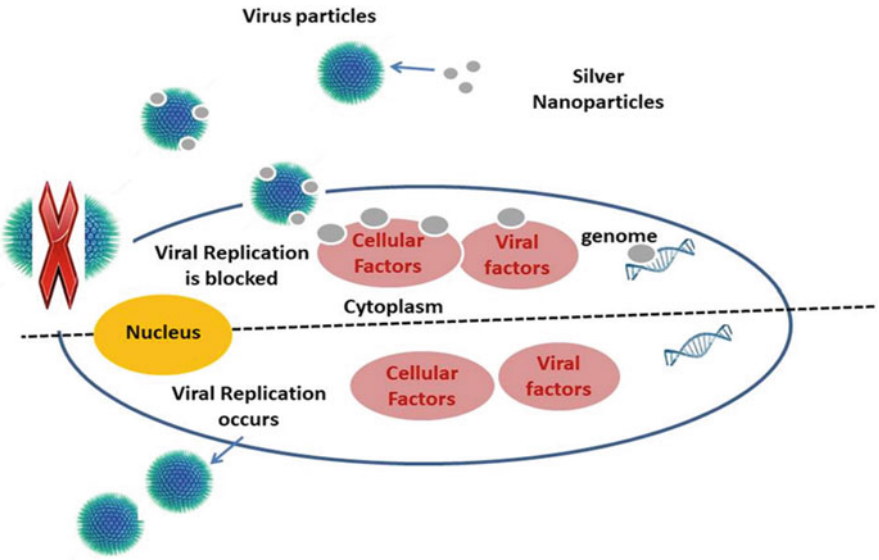

Fig. 6.2 The antiviral mechanism of silver nanoparticles. (Conducted by Heba S. Abbas)

nanoparticles in microorganisms, silver nanoparticles have emerged as one of the most promising antiviral agents (Salleh et al. 2020). Figure 6.2 showed the antiviral mechanism of silver nanoparticles.

The intricacy of viral structures may lead to a lack of understanding of the mode of nanoparticles' actions toward pathogenic viruses. Silver nanoparticles interact with the viruses in two ways: (1) Silver nanoparticles bind to the virus's outer coat, preventing viral adherence to the receptors of the cell, and (2) the silver nanoparticles attach to the virus's genetic material, preventing the virus from replicating or propagating within the host cells (Salleh et al. 2020). Table 6.2 demonstrates the antiviral activity of biogenic and non-biogenic silver nanoparticles against some respiratory viral infections. Also there is a hypothesis that silver nanoparticles inhibit SARS-CoV-2 binding to the cell receptor through binding to its spike glycoprotein and the releasing of silver decreases pH resulting in virus denaturation (Salleh et al. 2020).

Furthermore, the antiviral efficiency of zinc oxide nanoparticles and polyethylene glycol coated zinc oxide nanoparticles was evaluated against HINI influenza virus. Ghaffari et al. (2019) found that polyethylene glycol coating enhances the potential antiviral activity of the zinc oxide nanoparticles compared to non-coated zinc oxide nanoparticles. Polyethylene glycol (PEG) covered zinc oxide nanoparticles with 200 g/mL concentration hinders at the percentage of around 92% in replicates of the DNA genomic of HSV-1 and it lessens virus titer as well (Tavakoli et al. 2018). The hydroxyl group-ZnNPs, oleic acid modified-ZnNPs, and chitosan-ZnNPs have antiviral action against herpes simplex virus type-1 (Farouk and Shebl 2018). Also, it

Table 6.2 The antiviral activity of biogenic and non-biogenic silver nanoparticles against some respiratory viruses

Respiratory viruses infections	Biogenic silver nanoparticles	Size of nanoparticles (nm)[a]	Mode of action	References
Human parainfluenza virus type 3	Mycosynthesized silver nanoparticles (biogenic)	4–31	Interfere with virus–cell interactions	Gaikwad et al. (2013)
H1N1 influenza A	Chitosan-coated silver nanoparticle (biogenic)	3.5, 6.5, and 12.9	Interfere with viral glycoprotein, and prevent the contact of virus with the host cell	Mori et al. (2013)
Respiratory syncytial virus (RSV)	Polyvinylpyrrolidone-coated silver nanoparticles (non-biogenic)	10	Bind with gp120 glycoprotein and prevent the adherence of virus to the host cell	Morris et al. (2019)
Adenovirus type 3 (Ad3)	Silver nanoparticles (non-biogenic)	11.4	Interfere with the genomic material of the virus and destroy the virus	Chen et al. (2013)

[a] *nm* nanometer

was known that intracellular Zn^{2+} concentration inhibited the replication of Nidovirus and many platforms of RNA viruses (Ishida 2019).

In recent study, Kumar et al. (2019) evaluated the antiviral efficacy of iron oxide nanoparticles against the PR8-H1N1 strain, and they recommended it as a powerful influenza virus inhibitor with an eightfold decline in the viral RNA. Also, Lin et al. (2017) examined the antiviral characteristics of selenium nanoparticles and zanamivir coated selenium nanoparticles against H1N1 influenza virus, and their findings showed that the zanamivir coating had greater antiviral activity than non-coated selenium nanoparticles.

Similarly, Li et al. (2017) investigated the higher antiviral properties of oseltamivir coated selenium nanoparticles against the H1N1 influenza. With oseltamivir, selenium nanoparticles were modified to form more stable and compact globular nanocomposites.

Inhibitory activity of oseltamivir-coated selenium nanoparticles is attributed to suppressing the activity of hemagglutinin (HA) glycoprotein, which is found on the surface of the virus and responsible for combining receptors containing sialic acid on the host cells, and neuraminidase (NA) glycoprotein, which assists the linkage between sialic acid and HA to be cleaved to let the virus enter the host cells. Therefore, oseltamivir-coated selenium nanoparticles prevent the viral fusion and entry into host cells. The underlying molecular mechanisms verified that oseltamivir-coated selenium nanoparticles significantly suppressed the expression levels of PARP, caspase 3, p53 and increased the level of AKt. This indicates that oseltamivir-coated selenium nanoparticles depressed H1N1-induced host cells

apoptosis. Oseltamivir-coated selenium nanoparticles markedly decreased ROS generation compared to oseltamivir and selenium nanoparticles as well. Therefore, additional antiviral properties against multidrug resistance may be provided by the oseltamivir-coated selenium nano system to prospective selenium species (Li et al. 2017).

Gold nano-rods (AuNRs) have been widely used in biomedical applications as they can greatly improve therapeutic efficacy of drugs through delivering them to target tissues in an efficient way. Their surface can be easily modified with biocompatible materials as well which makes them ideal drug delivery systems. Additionally, AuNRs have tunable surface plasmon and photo thermal properties that provide them with worthy photo acoustic and photo thermal properties. They also have been used as nanocarriers for chemotherapeutic agents giving effective combined chemo-photo thermal therapy. Recently, AuNRs should be recognized as prospective biocompatible target-specific antiviral-drug carriers. For instance, AuNRs were developed for delivery of ssRNA immune activator for inhibition of seasonal and pandemic flu viral replication (Chakravarthy et al. 2010). The AuNRs have been used in developing antiviral therapy for combatting Middle East respiratory syndrome corona virus (MERS-CoV) by having the HR1 peptide inhibitor called pregnancy induced hypertension (PIH) immobilized in gold nano-rods. As the fusion between MERS-COV envelope and host cell membrane is mediated by S2 subunit of Spike protein of the viral envelope and since the process of drawing host cell membrane and viral envelope is initiated by the 6-helix bundle (6-HB) that is formed after binding of HR1 and HR2, which are two of three major domains of S2 subunit, takes place, PIH α-helix peptide was recognized as HR1 peptide inhibitor using the docking based virtual screening based on HR2 sequence. It was found that PIH mimics the HR2 conformation which means it can be used to inhibit HR1 and block the formation of 6-HB. Therefore, fusion between viral envelope and host cell membrane won't take place and viral genome won't be released into the host cells.

In spite of PIH alone showed effective inhibitory activity, it suffered major drawbacks like other peptides. These drawbacks include poor metabolic stability and bioavailability. PIH-modified gold nano-rods (PIH-AuNRs) exhibited more efficient inhibitory activity besides improving bio-stability and biocompatibility that leaded to improved physical and pharmaceutical profiles than those of PIH alone (Huang et al. 2019).

As per the previous research, nanomaterials developed with various forms and structures show unique benefits for practice in antiviral therapy, notably; nanometric diameter that allows drug delivery across resistant barriers, high surface area to volume ratios for the inclusion of large drug loads and enhanced efficiency, surface functionalization availability that facilitates passage across cellular membranes and improves stability as well as bioavailability, antiviral activity against several viruses due to bio-mimetic properties, high specificity, enhanced drug delivery and controlled drug release to target tissues, reduced drug resistance, possibility for personalized therapy and last but not least low incidence of drug adverse effects upon using nan-based therapeutics (Cojocaru et al. 2020).

Moreover, bio-functionalization of gold nanoparticles with seaweed *Sargassum wightii* extract was applied to achieve more efficient drug delivery to target cells. Antiviral activity of seaweed-gold nanoparticles against Herpes Simplex Virus (HSV) was assessed by the lessening of cytopathic effect (CPE) caused by HSV in a dose-dependent mode. It was found that 10 and 25 μL of seaweed-gold nanoparticles decreased HSV-1 and HSV-2 CPE by 70%. According to literature, functionalized gold nanoparticles have size-dependent interface and the capability to inhibit the adhering and virus's entrance, and this was the accountable for their antiviral efficiency (Dhanasezhian et al. 2019).

Recently, it was known that copper (Cu) abolishes the propagation tendency of SARS-CoV, influenza, and other respiratory viruses, having high prospective decontamination in hospitals, communities, and households (Cortes and Zuñiga 2020; Raha et al. 2020). Copper oxide nanoparticles (CuO-NPs) have antiviral activity against HCV by directing the adhering of HCV to hepatic cells and the entrance of the virus. Thus, it can be used as a nontoxic drug for HCV infected patients (Hang et al. 2015). In addition CuO-NPs are related with a noteworthy antiviral effectiveness against HSV-1 at nontoxic concentration to host cells causing 83% inhibition in virus titer (Tavakoli and Hashemzadeh 2019). Moreover Cu surfaces scored less time of stable infective SARS-CoV and SARS-CoV-2 as compared with the surfaces of plastic and steel (Van Doremalen et al. 2020) and thus, Cunano coating can decrease viral transmission (Pemmada et al. 2020). Cu-NPs were used with other materials for synthesizing a face mask that may protect from COVID-19 infection (Ahmed et al. 2020a, b).

6.4.3 The Antiviral Activity of Organic Nanoparticles

6.4.3.1 Polymeric Nanoparticles

Polymeric nanoparticles were created after liposomes in order to increase their stability and medicinal payload. They are compact colloidal nanoparticles with a size of less than 500 nm that are formed of a biocompatible polymeric medium comprised of artificial or natural origin polymers (Zazo et al. 2016). Polymeric nanoparticles may be more stable than liposomes in biological liquids and under storage circumstances due to their configuration. Polymeric nanoparticles can be made via a variety of techniques, including solvent evaporation, spontaneous emulsification, solvent diffusion, and polymerization (Lembo et al. 2018). They could be loaded with lipophilic and hydrophilic medicines, and several chemical methods, such as covalent chemistry and hydrophobic interactions, have been recommended. Above the threshold micelle concentration and temperature, the polymers unexpectedly form micellar configurations, forming hydrophobic aggregate polymeric chains (Zazo et al. 2016). Polymers, however, converted insoluble in liquids and behave like inactive ingredients below the above-mentioned precarious limits. They have piqued the interest of researchers as nanosized drug delivery systems, not only because of their several advantages (Cagel et al. 2017).

In the viral therapy, although, the conventional treatments against influenza virus infections were considered to target the proteins of the virus, the development of viral variants carrying drug-resistant transformations could hinder the progress of pathogen-targeting antivirals. For this reason, growing efforts have been directed towards developing host-targeted antiviral agents which act by controlling host aspects involved in viral replication. Since targeted antiviral approaches do not employ a choosy pressure on the target pathogen, they may be less liable to strain variations and mutations. Vacuolar ATPases (V-ATPases), which are abundant proton pumps found in the endo-membrane structure of all eukaryotic cells, have been identified as target for blocking virus entry into host cells due to their critical role in allowing viral entry by V-ATPase-mediated endosomal acidification. A lot of V-ATPase inhibitors have been developed such as plecomacrolide bafilomycin and diphyllin. However, their clinical application is limited by toxicity concerns and delivery challenges associated with their poor water solubility. Therefore, poly (ethylene glycol)-block-poly(lactide-coglycolide) (PEG-PLGA-)-based polymeric nanoparticle system was developed to encapsulate bafilomycin and diphyllin in its hydrophobic polymeric core. The drug-loaded nanoparticles achieved sustained drug release kinetics over 3 days and were proficiently taken up by various types of cell lines. The nanoparticulate V-ATPase inhibitors exhibited diminished cytotoxicity, enhanced antiviral activity as well as increased therapeutic index in comparison with free diphyllin and bafilomycin drugs. Treatment with diphyllin nanoparticles in a mouse model of sublethal influenza challenge exhibited good tolerability and achieved reduced body weight loss and viral load in the lungs. Additionally, diphyllin nanoparticle treatment offered a significant survival advantage following a lethal influenza viral challenge. Moreover, host-targeted treatment by diphyllin-loaded nanoparticles can be applied to multiple strains of influenza viruses as a broad-spectrum antiviral (Hu et al. 2018).

6.4.3.2 Carbon-Based Nanomaterials as Antivirals

It has been reported that carbon-based nanomaterials have potent antiviral properties. Carbon quantum dots (CQDs) can be synthesized using several simple and inexpensive methods with a very small diameter and efficient water dispersion to be used in several therapeutic applications. Furthermore, they have excellent optical properties that facilitate in vivo tracking, and they are known for lacking signs of toxicity in animals as well. CQDs resulting from citric acid/ethylene diamine then conjugated with boronic acid functions showed highly effective anti-HCoV-229E coronavirus behavior by interaction with the S protein of human coronavirus and therefore blocking its entry into host cells (Łoczechin et al. 2019).

Antiviral cationic carbon dots based on curcumin have been developed as multi-site inhibitors for enteric coronavirus. Although curcumin (CCM) has been reported to have antiviral activity against several viruses, it could not be widely applied in its pure form due to poor solubility and bioavailability. Encapsulating CCM in inorganic-based carriers has been widely used to overcome these two problems. This method could overcome the poor solubility and bioavailability of CCM without causing significant improvement on its antiviral activity. It was relatively tedious and

time-consuming as well. Therefore, another method was developed to improve solubility, bioavailability, and antiviral activity of curcumin.

Curcumin was applied as a precursor to prepare cationic carbon dots (CCM-CDs) with antiviral properties using one-step method. The effectiveness of (CCM-CDs) was studied using porcine epidemic diarrhea virus (PEDV) as corona virus typical. As-prepared CCM-CDs treatment was found to effectively hinder PEDV proliferation compared to corporate CDs (EDA-CDs). The CCM-CDs modify the configuration of viral surface protein which leads to blocking the viral entry. They also suppress the production of negative-strand RNA in virus, budding of the virus and the accumulation of reactive oxygen species (ROS) by PEDV. Moreover, CCM-CDs inhibit viral propagation by activating the generation of interferon-stimulating genes (ISGs) and pro-inflammatory cytokines (Ting et al. 2018).

6.4.3.3 Lactoferrin Loaded Nanoparticles as Antivirals

As long as the recommended first-line highly active antiretroviral therapy (HAART) for HIV/AIDS has been known to be a mixture of one non-nucleoside reverse transcriptase, and two nucleoside/nucleotide reverse transcriptase inhibitors, a mixture of Zidovudine (AZT), Efavirenz (EFV), and Lamivudine (3TC) is one of the commonly used main line treatment. Patient needs to take this regimen in a fixed schedule which may cause several adverse effects and health complications as the prolonged use of these drugs has been reported to cause different toxicities like cardio-toxicity and erythrocyte toxicity. From this perspective, a formulation of lactoferrin nanoparticles loaded with triple drug combination of zidovudine, efavirenz, and lamivudine has been developed with enhanced bioavailability, improved pharmacokinetic profile and minimal drug-associated toxicity over the free drugs. Lactoferrin is a pleiotropic particle with wide-ranging practical activities including anti-HIV activity. Lactoferrin nanoparticles exhibit high drug loading capacity and provide the loaded drugs with the advantage of bypassing first pass metabolism which leads to reduced drug dose and therefore reduced drug-associated toxicity (Kumar et al. 2016).

Lactoferrin nanoparticles were prepared using sol-oil protocol. In this protocol, an equal amount (3.33 mg) of drugs was dissolved separately in 100 μL of dimethyl sulfoxide solvent, and then different concentrations of lactoferrin were solvated in 500 μL of phosphate buffer (1×) saline (pH 7.4) separately. The incubation of drugs and protein solutions were on ice for 1 h and then mixed with 25 mL of olive oil. Further, they were sonicated for 15 min at 4 °C using an ultrasonic homogenizer. Samples were then instantly transferred into liquid nitrogen for 15 min and incubated on ice for 4 h. Centrifugation of the formed particles at 6000 rpm for half an hour was taken place. The oil containing supernatant was thrown away and the ice-cold di-ethyl ether washed pellet suspended in phosphate buffer for the next experiment (Kumar et al. 2016).

First-line antiretroviral therapy nanoparticles (FLART-NP) enter the cells slowly and reach the maximum level at 4 h, then become maintained at a constant level for long period until a significant decline takes place over a period of 8 h. This suggests that lactoferrin NPs undergo exocytosis after release of its payload making no

burden on the cells by the delivery vehicle. The maintenance period also delivers longer period for the drugs to perform against HIV existing inside macrophages leading to enhanced antiviral activity. Lactoferrin NPs show pH dependent drug release with maximum drug release in the endosomal pH (pH 5.0) and minimal release in the physiological pH (pH 7.4) which indicates that there is no drug release in extracellular condition, thus targeted drug release and reduced drug-related toxicity have been achieved. This is also supported by the microscopic analysis that shows characteristic surface projections/depressions that maintain lactoferrin's structural features which may be involved in recognition and receptor binding on the target tissue. With FLART-NP, liver and kidney damage has been completely abolished which confirms the advantage of targeted drug delivery (Kumar et al. 2016).

6.4.3.4 Silica Nanocarriers as Antivirals

Mesoporous Si-NP of 2–50 nm size are frequently used in drug delivery systems against viruses these particles can protect drug till reach the specific site besides improving its solubility and stability and enhancing drug circulation time and controlled release (LaBauve et al. 2018). Si-NP is a stable biocompatible that can carry and pass RNA/DNA molecules through the cells and protect them from degradation via nuclear enzymes which is an important step for development viral vaccine (Tarn et al. 2013). Moreover these particles can pass into the cells without damaging the cells membrane compared to lipid based delivery systems also do not produce inflammation at the site of injection or systemic side effects (Mehta et al. 2020). Also, De Souza et al. (2016) showed that functionalized Si-NP can be used as antiviral drug against HIV that can interact specifically with viral envelope at nontoxic concentrations to mammalian cells and prevent virus to enter the cells. The Si-NP associated with didodecyldimethylammonium bromide has virucidal activity against H1N1 (Capeletti et al. 2018).

Recently AbouAitah et al. (2020) showed that Si-NP-(NH2)-(shikimic acid)-(quercetin) displayed both an antiviral against H5N1 and anti-inflammatory effect by inhibition of cytokines (TNF-α, IL-1β) and nitric oxide production in rat model. As the biggest challenge facing COVID-19 vaccine development is confirming that the host cell receives the introduced genetic material this can be achieved using viral vector or nanotailored delivery system to promote the synthesis of spike protein. Thus functionalized Si-NP can propose a possibly nontoxic and active delivery structure for DNA/RNA vaccines and may be suitable in the pursuit for a COVID-19 vaccine. Recently a new Si-NP based delivery system for COVID19 is synthesized and is under trial called Nuvec® (Theobald 2020).

Finally, nanoparticles prevent the viral propagation or blocking the virus' entrance in to the host cell through their several interfaces with glycoprotein receptor and/or viral coat, these can hinder the viral propagation in the host cell. Nanoparticles are novel antimicrobial agents owing to unique chemical and physical features with their high surface area. The viral replication and assembly in the intracellular compartment of an infected cell require host cellular and viral factors for progeny virion production. The mechanisms of interaction between nanoparticles

with these aspects are the crucial to an efficient viral propagation inhibition (Dos Santos et al. 2014).

The mechanism of viral infection comprises attachment, penteration, replication, and budding. Blocking or suppressing any of these steps is the antiviral functional nanoparticles. There are many antiviral functional nanoparticles mechanisms. We will mention these mechanisms, as promising therapeutic strategies (Chen and Liang 2020). The early steps of virus entry are sites of action of the inhibitor, because of its accessibility and extracellular location making it attractive therapeutic strategy (Dos Santos et al. 2014). Consequently, the well-designed nanoparticles can be used as a wide-ranging antiviral agent by suppressing the attachment of the virus. The highly conserved target of viral attachment ligands (VALs) heparan sulfate proteoglycans is mimicked by a series of antiviral nanoparticles with long and flexible linkers which designed by Stellacci's group. In vitro nanomolar irreversible activity of these nanoparticles on papilloma virus, herpes simplex virus, dengue, respiratory syncytial virus, and lenti virus achieved efficient prevention of viral attachment (Cagno et al. 2018). The second way of viral suppression is hindering their dissemination and host cells entrance by varying the cell surface membrane and protein structures. These can be achieved by interaction of nanostructure with viruses and changing their capsid protein structure to reduce its virulence and entry into the host cell (Chen and Liang 2020). Haag and his collaborators prevent the glycoprotein coat of the vesicular stomatitis virus and the interaction of baby hamster kidney cells (Donskyi et al. 2018). Therefore, blocking between viruses and host cells is efficient therapeutic strategy to conquer viral infections. The third strategy to prevent viral infection is inhibiting the viral replication. By suppressing the expression of certain enzymes require for the viral DNA or RNA replication, these destroy the viral replication inside the host cell. As known, the offspring of a mother virus is more virulent. Therefore, the inhibition of virus budding and its excretion from host cells are another antiviral functional nanoparticle mechanism. This through prevent viral binding and reduce the number of viral offspring resulting in decreasing its virulence (Chen and Liang 2020).

6.5 Conclusion

Because of the virus's unusual behavior and particular viral metabolic activities, developing an effective management plan is difficult. If SARS-CoV-2 undergoes a genetic change, like the influenza virus did, this might be a limiting step in controlling viral transmission. Finally, nanomaterials offer numerous tools for use as nanotherapeutics against respiratory viral infections. The antiviral efficiency of metallic nanoparticles is a significant and potentially solution to the present SARS-CoV-2 pandemic; nanoparticles are capable of interfacing with virus particles. As a result, additional research in the chemistry and biology of nanotechnology is needed to design multifunctional nanoparticles that may be used as drug nanocarriers.

Funding No funding.

Availability of Data The datasets generated or analyzed during the review are available from the corresponding author on reasonable request.

Competing Interests "The authors declare that they have no competing interests".

Author Contribution Heba S. Abbas was responsible for sharing the idea, determining the points and objectives, writing introduction abstract, conclusion, sharing in nanoparticles parts, reviewing, rearrange for whole review and draw Fig. 6.2. Hosam Saleh wrote the classification of viruses and draw Fig. 6.1. Esraa M. M. Mohammad and Ebthal F. M. Elzayat wrote respiratory viruses part. Also, Hala A. Abdelgaid wrote SARS-CoV2 threat, and nanoparticles, Amira S. H. Mohamed shared in writing nanoparticles, Amany Y. El-Sayed shared in writing the antiviral mechanism of nanoparticles, Noha M. Gamil wrote the toxicity of antiviral drugs, and Salma E. S. Ismail wrote organic nanoparticles.

References

AbouAitah K et al (2020) Virucidal action against avian influenza H5N1 virus and immunomodulatory effects of nanoformulations consisting of mesoporous silica nanoparticles loaded with natural prodrugs. Int J Nanomedicine 15:5181

Aderibigbe BA (2017) Metal-based nanoparticles for the treatment of infectious diseases. Molecules 22(8):1370

Ahmed MK, Afifi M, Uskoković V (2020a) Protecting healthcare workers during COVID-19 pandemic with nanotechnology: a protocol for a new device from Egypt. J Infect Public Health 13(9):1243–1246. https://doi.org/10.1016/j.jiph.2020.07.015. Epub 2020 Aug 3

Ahmed MK, Afifi M et al (2020b) Protecting healthcare workers during COVID-19 pandemic with nanotechnology: a protocol for a new device from Egypt. J Infect Public Health 13(9): 1243–1246

Badgujar CK, Ram AH, Zanzany R et al (2020) Remdesivir for COVID-19: a review of pharmacology, mechanism of action, in-vitro activity and clinical use based on available case studies. J Drug Deliv Therap 10(4-s):264–270

Betakova T, Nermut MV, Hay AJ (1996) The NB protein is an integral component of the membrane of influenza B virus. J Gen Virol 77(Pt 11):2689–2694. https://doi.org/10.1099/0022-1317-77-11-2689

Boncristiani HF, Criado MF, Arruda E (2009) Respiratory viruses. In: Encyclopedia of microbiology, pp 500–518

Branche AR, Falsey AR (2016) Parainfluenza virus infection. Semin Respir Crit Care Med 37(4): 538–554. https://doi.org/10.1055/s-0036-1584798

Brister JR, Ako-Adjei D, Bao Y et al (2014) NCBI viral genomes resource. Nucleic Acids Res 2014:1. https://doi.org/10.1093/nar/gku1207

Cagel M, Tesan FC, Bernabeu E et al (2017) Polymeric mixed micelles as nanomedicines: achievements and perspectives. Eur J Pharm Biopharm 113:211–228. pii: S0939-6411(16) 30694-4

Cagno V, Androzzi PD, Alicarnasso M et al (2018) Broad-spectrum non-toxic antiviral nanoparticles with a virucidal inhibition mechanism. Nat Mater 17(2):195–203

Capeletti LB et al (2018) Silica nanoparticle applications in the biomedical field. In: Smart nanoparticles for biomedicine. Elsevier, Amsterdam, pp 115–129

Chakravarthy KV et al (2010) Gold nanorod delivery of an SsRNA immune activator inhibits pandemic H1N1 influenza viral replication. Proc Natl Acad Sci U S A 107(22):10172–10177. https://doi.org/10.1073/pnas.0914561107

Chen YH, Lai HJ (2013) Acute hemorrhagic colitis after oral administration of oseltamivir for influenza. Gastrointest Endosc 77(6):976. https://doi.org/10.1016/j.gie.2013.02.005

Chen L, Liang J (2020) An overview of functional nanoparticles as novel emerging antiviral therapeutic agents. Mater Sci Eng C 112:110924
Chen N, Zheng Y, Yin J et al (2013) Inhibitory effects of silver nanoparticles against adenovirus type 3 in vitro. J Virol Methods 193:470–477
Cojocaru F-D et al (2020) Nanomaterials designed for antiviral drug delivery transport across biological barriers. Pharmaceutics 12(2):171. https://doi.org/10.3390/pharmaceutics12020171
Cortes AA, Zuñiga JM (2020) The use of copper to help prevent transmission of SARS-coronavirus and influenza viruses. A general review. Diagn Microbiol Infect Dis 98:115176
Das C et al (2020) Silver-based nanomaterials as therapeutic agents against coronaviruses: a review. Int J Nanomedicine 15:9301
De Souza E, Silva JM et al (2016) Viral inhibition mechanism mediated by surface-modified silica nanoparticles. ACS Appl Mater Interfaces 8(26):16564
Dhama K, Pawaiya RVS, Chakrabort S et al (2014) Coronavirus infection in equines: a review. Asian J Anim Vet Adv 9:164–176
Dhanasezhian A, Srivani S, Govindaraju K et al (2019) Anti-Herpes Simplex Virus (HSV-1 and HSV-2) activity of biogenic gold and silver nanoparticles using seaweed Sargassum wightii. Indian J Geo Mar Sci 48:1252–1257
Dhand C, Dwivedi N, Loh XJ et al (2015) Methods and strategies for the synthesis of diverse nanoparticles and their applications: a comprehensive overview. RSC Adv 5(127): 105003–105037
Donskyi I, Druke M, Silberreis K et al (2018) Interactions of fullerene-polyglycerol sulfates at viral and cellular interfaces. Small 14(17):1800189
Dos Santos CA, Seckler MM, Ingle AP et al (2014) Silver nanoparticles: therapeutical uses toxicity, and safety issues. J Pharm Sci 103(7):1931–1944
El-Sheekh MM, Shabaan MT, Hassan L et al (2020) Antiviral activity of algae biosynthesized silver and gold nanoparticles against Herps Simplex (HSV-1) virus in vitro using cell-line culture technique. Int J Environ Health Res. https://doi.org/10.1080/09603123.2020.1789946
Fang RH, Kroll AV, Gao W et al (2018) Cell membrane coating nanotechnology. Adv Mater 30. https://doi.org/10.1002/adma.201706759
Farouk F, Shebl RI (2018) Comparing surface chemical modifications of zinc oxide nanoparticles for modulating their antiviral activity against herpes simplex virus type-1. Int J Nanopart Nanotechnol 4(021):1–14
Fluhmann B, Ntai I, Borchard G et al (2018) Nanomedicines: the magic bullets reaching their target? Eur J Pharm Sci 128:73–80
Gaikwad S, Ingle A, Gade A, Incoronato N, Russo L, Galdiero S, Galdiero M (2013) Antiviral activity of mycosynthesized silver nanoparticles against herpes simplex virus and human parainfluenza virus type 3. Int J Nanomedicine 8(1):4303–4314. https://doi.org/10.2147/IJN.S50070
Ghaffari H, Tavakoli A, Moradi A et al (2019) Inhibition of H1N1 influenza virus infection by zinc oxide nanoparticles: another emerging application of nanomedicine. J Biomed Sci 26:70. https://doi.org/10.1186/s12929-019-0563-4
Goossens H (2009) Antibiotic consumption and link to resistance. Clin Microbiol Infect 15(3): 12–15. https://doi.org/10.1111/j.1469-0691.2009.02725.x
Guisado-Macías JA, Rodríguez FF, Méndez-Sánchez F et al (2012) Oseltamivir-related psychiatric manifestations. Actas Espanolas de Psiquiatria 40(1):46–48. https://europepmc.org/article/med/22344496
Gulati S, Sachdeva M, Bhasin KK (2018) Emerging applications of nanoparticles: biomedical and environmental. AIP Conf Proc 1953:030213. https://doi.org/10.1063/1.5032548
Guo H, Wan X, Niu F et al (2019) Evaluation of antiviral effect and toxicity of total flavonoids extracted from Robinia pseudoacacia cv. idaho. Biomed Pharmacother 118:109809. https://doi.org/10.1016/j.biopha.2019.109335

Gurunathan S, Qasim M, Choi Y et al (2020a) Antiviral potential of nanoparticles-can nanoparticles fight against coronaviruses? Nanomaterials (Basel, Switzerland) 10(9):1645. https://doi.org/10.3390/nano10091645

Gurunathan S et al (2020b) Antiviral potential of nanoparticles—can nanoparticles fight against coronaviruses? Nanomaterials 10(9):1645

Haas LE, Thijsen SF, van Elden L et al (2013) Human metapneumovirus in adults. Viruses 5(1): 87–110. https://doi.org/10.3390/v5010087

Haggag EG, Elshamy AM, Rabeh MA et al (2019) Antiviral potential of green synthesized silver nanoparticles of Lampranthus coccineus and Malephora lutea. Int J Nanomedicine 14:6217–6229. https://doi.org/10.2147/IJN.S214171

Hang X, Peng H, Song H et al (2015) Antiviral activity of cuprous oxide nanoparticles against hepatitis C virus in vitro. J Virol Methods 222:150–157

Hofmann M, Kleine-Weber H, Krüger N et al (2020) The novel coronavirus 2019 (2019-nCoV) uses the SARS-coronavirus receptor ACE2 and the cellular protease TMPRSS2 for entry into target cells. Mol Biol. https://doi.org/10.1101/2020.01.31.929042

Hu C-MJ et al (2018) Antiviral efficacy of nanoparticulate vacuolar ATPase inhibitors against influenza virus infection. Int J Nanomedicine 13:8579–8593. https://doi.org/10.2147/ijn.s185806

Huang X et al (2019) Novel gold nanorod-based HR1 peptide inhibitor for Middle East respiratory syndrome coronavirus. ACS Appl Mater Interfaces 11(22):19799–19807. https://doi.org/10.1021/acsami.9b04240

Ingale AG, Chaudhari AN (2013) Biogenic synthesis of nanoparticles and potential applications: an eco-friendly approach. J Nanomed Nanotechol 4(165):1–7

Ishida T (2019) Review on the role of Zn^{2+} ions in viral pathogenesis and the effect of Zn^{2+} ions for host cell-virus growth inhibition. Am J Biomed Sci Res 2:28–37

Jacobs SE, Lamson DM, St George K (2013) Human rhinoviruses. Clin Microbiol Rev 26(1): 135–162. https://doi.org/10.1128/CMR.00077-12

Kaufman HL, Kohlhapp FJ, Zloza A (2015) Oncolytic viruses: a new class of immunotherapy drugs. Nat Rev Drug Discov 14:642–664

Kerry RG, Malik S, Redda YT (2019) Nano-based approach to combat emerging viral (NIPAH virus) infection. Nanomedicine 18:196–220

Keyser LA, Karl M, Nafziger AN et al (2000) Comparison of central nervous system adverse effects of amantadine and rimantadine used as sequential prophylaxis of influenza A in elderly nursing home patients. Arch Intern Med 160(10):1485–1488. https://doi.org/10.1001/archinte.160.10.1485

Khandel P, Yadaw RK, Soni DK, Kanwar L, Shahi SK (2018) Biogenesis of metal nanoparticles and their pharmacological applications: present status and application prospects. J Nanostruct Chem 8(3):217–254

Kiso M, Mitamura K, Sakai-Tagawa Y et al (2004) Resistant influenza A viruses in children treated with oseltamivir: descriptive study. Lancet 364(9436):759–765. https://doi.org/10.1016/S0140-6736(04)16934-1

Körner RW, Söderlund-Venermo M, van Koningsbruggen-Rietschel S et al (2011) Severe human bocavirus infection, Germany. Emerg Infect Dis 17(12):2303–2305. https://doi.org/10.3201/eid1712.110574

Kumar P et al (2016) Triple drug combination of zidovudine, efavirenz and lamivudine loaded lactoferrin nanoparticles: an effective nano first-line regimen for HIV therapy. Pharm Res 34(2): 257–268. https://doi.org/10.1007/s11095-016-2048-4

Kumar R, Nayak M, Sahoo GC et al (2019) Iron oxide nanoparticles based antiviral activity of H1N1 influenza a virus. J Infect Chemother 25(5):325–329

Kupferschmidt K, Cohen J (2020) Race to find COVID-19 treatments accelerates. Science 367(6485):1412–1413

Kutter JS, Spronken MI, Fraaij PL et al (2018) Transmission routes of respiratory viruses among humans. Curr Opin Virol 28:142–151

LaBauve AE et al (2018) Lipid-coated mesoporous silica nanoparticles for the delivery of the ML336 antiviral to inhibit encephalitic alphavirus infection. Sci Rep 8(1):1–13
Lei L, Sun RWY, Chen R et al (2008) Silver nanoparticles inhibit hepatitis B virus replication. Antivir Ther 13:252–262
Lembo D, Donalisio M, Civra A et al (2018) Nanomedicine formulations for the delivery of antiviral drugs: a promising solution for the treatment of viral infections. Expert Opin Drug Deliv 15(1):93–114. https://doi.org/10.1080/17425247.2017.1360863
Li B, Armstead AL (2011) Nanomedicine as an emerging approach against intracellular pathogens. Int J Nanomedicine 6:3281. https://doi.org/10.2147/ijn.s27285
Li Y, Lin Z, Guo M et al (2017) Inhibitory activity of selenium nanoparticles functionalized with oseltamivir on H1N1 influenza virus. Int J Nanomedicine 12:5733–5743. https://doi.org/10.2147/ijn.s140939
Li H, Liu S, Yu X, Tang S et al (2020) Coronavirus disease 2019 (COVID-19): current status and future perspective. Int J Antimicrob Agents 55:105951
Lin Z, Li Y, Guo M et al (2017) Inhibition of H1N1 influenza virus by selenium nanoparticles loaded with zanamivir through p38 and JNK signaling pathways. RSC Adv 7(56):35290–35296
Łoczechin A et al (2019) Functional carbon quantum dots as medical countermeasures to human coronavirus. ACS Appl Mater Interfaces 46:42964–42974. https://doi.org/10.1021/acsami.9b15032
Lozano R, Naghavi M, Foreman K et al (2012) Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 380:2095–2128
Lu R, Zhao X, Li J et al (2020) Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet 395(10224):565–574
Maduray K, Parboosing R (2020) Metal nanoparticles: a promising treatment for viral and Arboviral infections. Biol Trace Elem Res 199(8):3159–3176. https://doi.org/10.1007/s12011-020-02414-2
Mainardes RM, Diedrich C (2020) The potential role of nanomedicine on COVID-19 therapeutics. Ther Deliv 11(7):411–414. https://doi.org/10.4155/tde-2020-0069
Malathi VG, Renuka DP (2019) ssDNA viruses: key players in global virome. Virusdisease. https://doi.org/10.1007/s13337-019-00519-4
Matamoros T, Álvarez M, Barrioluengo V et al (2011) Reverse transcriptase and retroviral replication. IntechOpen, Rijeka, Croatia. https://doi.org/10.5772/21660
McClellan K, Perry CM (2001) Oseltamivir: a review of its use in influenza. Drugs 61(2):263–283. https://doi.org/10.2165/00003495-200161020-00011. Erratum in: Drugs 61(6):775
Mehranfar A, Izadyar M (2020) Theoretical design of functionalized gold nanoparticles as antiviral agents against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). J Phys Chem Lett 11:10284–10289
Mehta M et al (2020) Advanced drug delivery systems can assist in targeting coronavirus disease (COVID-19): a hypothesis. Med Hypotheses 144:110254
Michalet X, Pinaud FF, Bentolila LA et al (2005) Quantum dots for live cells, in vivo imaging, and diagnostics. Science 307:538–544
Milovanovic M, Arsenijevic A, Milovanovic J et al (2017a) Nanoparticles in antiviral therapy. In: Antimicrobial nanoarchitectonics: from synthesis to applications, 1st edn. Elsevier Inc., Amsterdam, pp 383–410
Milovanovic M, Arsenijevic A, Milovanovic J et al (2017b) Nanoparticles in antiviral therapy. In: Antimicrobial nanoarchitectonics. Elsevier, Amsterdam, pp 383–410. https://doi.org/10.1016/B978-0-323-52733-0.00014-8
Modrow S, Falke D, Truyen U et al (2013) Viruses with single-stranded, positive-sense RNA genomes. In: Molecular virology. Springer, Berlin. https://doi.org/10.1007/978-3-642-20718-1_14
Mori Y, Ono T, Miyahira Y et al (2013) Antiviral activity of silver nanoparticle/chitosan composites against H1N1 influenza A virus. Nanoscale Res Lett 8:93

Morris D, Ansar M, Speshock J (2019) Antiviral and immunomodulatory activity of silver nanoparticles in experimental RSV infection. Viruses 11:732
National Center for Immunization and Respiratory Diseases (NCIRD), Division of Viral Diseases (2021). https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/symptoms.html
Pantidos N (2014) Biological synthesis of metallic nanoparticles by bacteria, fungi and plants. J Nanomed Nanotechnol 5(5):1–10
Patel P et al (2015) Plant-based synthesis of silver nanoparticles and their characterization. In: Nanotechnology and plant science. Springer, New York, pp 271–288
Patra JK, Das G, Fraceto LF et al (2018) Nano based drug delivery systems: recent developments and future prospects. J Nanobiotechnol 16:1–33
Pemmada R et al (2020) Science-based strategies of antiviral coatings with viricidal properties for the COVID-19 like pandemics. Materials 13(18):4041
Prasad M, Lambe UP, Brar B et al (2018) Nanotherapeutics: an insight into healthcare and multi-dimensional applications in medical sector of the modern world. Biomed Pharmacother 97: 1521–1537
Rafiei S, Rezatofighi SE, Ardakani MR et al (2016) Gold nanoparticles impair foot-and-mouthdisease virus replication. IEEE Trans Nanobiosci 15:34–40
Raha S et al (2020) Is copper beneficial for COVID-19 patients? Med Hypotheses 2020:109814
Rai M, Deshmukh SD, Ingle AP et al (2016) Metal nanoparticles: the protective nanoshield against virus infection. Crit Rev Microbiol 42:46–56
Salleh A, Naomi R, Utami ND et al (2020) The potential of silver nanoparticles for antiviral and antibacterial applications: a mechanism of action. Nanomaterials 10(8):1566. https://doi.org/10.3390/nano10081566
Sandegren L, Andersson DI (2009) Bacterial gene amplification: implications for the evolution of antibiotic resistance. Nat Rev Microbiol 7:578–588. https://doi.org/10.1038/nrmicro2174
Schoeman D, Fielding BC (2019) Coronavirus envelope protein: current knowledge. Virol J 16:69
Shafagati N, Williams J (2018) Human metapneumovirus - what we know now. F1000Res 7:135. https://doi.org/10.12688/f1000research.12625.1
Shah M, Fawcett D, Sharma S et al (2015) Green synthesis of metallic nanoparticles via biological entities. Materials 8(11):7278–7308
Sharmin S, Rahaman MM, Sarkar C et al (2021) Nanoparticles as antimicrobial and antiviral agents: a literature-based perspective study. Heliyon 7:e06456. https://doi.org/10.1016/j.heliyon.2021.e06456
Simón-Talero M, Buti M, Esteban R (2012) Severe anaemia related to oseltamivir during treatment of chronic hepatitis C: a new drug interaction? J Viral Hepat 19:14–17. https://doi.org/10.1111/j.1365-2893.2011.01521.x
Singh L, Kruger HG, Maguire GEM (2017) The role of nanotechnology in the treatment of viral infections. Ther Adv Infect Dis 4(4):105–131
Stephen BJ, Suchanti S, Mishra R et al (2020) Cancer nanotechnology in medicine: a promising approach for cancer detection and diagnosis. Crit Rev Ther Drug Carrier Syst 37(4):375–405. https://doi.org/10.1615/CritRevTherDrugCarrierSyst.2020032634
Sumbria D, Berber E, Mathayan M et al (2021) Virus infections and host metabolism—can we manage the interactions? Front Immunol 11. https://doi.org/10.3389/fimmu.2020.594963
Tarn D et al (2013) Mesoporous silica nanoparticle nanocarriers: biofunctionality and biocompatibility. Acc Chem Res 46(3):792–801
Tavakoli A, Hashemzadeh MS (2019) Inhibition of herpes simplex virus type 1 by copper oxide nanoparticles. J Virol Methods 275:113688. https://doi.org/10.1016/j.jviromet.2019.113688. Epub 2019 Jul 2
Tavakoli A, Ataei-Pirkooh A, Sadeghi GM et al (2018) Polyethylene glycol-coated zinc oxide nanoparticle: an efficient nanoweapon to fight against herpes simplex virus type 1. Nanomedicine 13:2675–2690
Theobald N (2020) Emerging vaccine delivery systems for COVID-19: functionalised silica nanoparticles offer a potentially safe and effective alternative delivery system for DNA/RNA

vaccines and may be useful in the hunt for a COVID-19 vaccine. Drug Discov Today 25(9): 1556–1558
Ting D et al (2018) Multisite inhibitors for enteric coronavirus: antiviral cationic carbon dots based on curcumin. ACS Appl Nano Mater 10:5451–5459. https://doi.org/10.1021/acsanm.8b00779
Uskokovic V (2020) Why have nanotechnologies been underutilized in the global uprising against the coronavirus pandemic? Nanomedicine (Lond). https://doi.org/10.2217/nnm-2020-0163. Epub ahead of print
Van Doorn HR, Yu H (2020) Viral respiratory infections. In: Hunter's tropical medicine and emerging infectious diseases. Elsevier, Amsterdam, pp 284–288. https://doi.org/10.1016/B978-0-323-55512-8.00033-8
Van Doremalen N et al (2020) Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. N Engl J Med 382(16):1564–1567
Von Nussbaum F, Brands M, Hinzen B et al (2006) Antibacterial natural products in medicinal chemistrydexodus or revival? Angew Chem Int Ed 45(31):5072e129
Walsh EE, Peterson DR, Falsey AR (2008) Human metapneumovirus infections in adults: another piece of the puzzle. Ann Intern Med 168:2489–2496. https://doi.org/10.1001/archinte.168.22.2489
Wan Y, Shang J, Graham R et al (2020) Receptor recognition by the novel coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS coronavirus. J Virol 94: e00127–e00120
Wang J (2020) Fast identification of possible drug treatment of coronavirus disease-19 (COVID-19) through computational drug repurposing study. J Chem Inf Model 60(6):3277–3286
WHO (2022) Coronavirus Update, 83,527,714 confirmed cases and 1,819,905 deaths from COVID-19 virus outbreak. WHO, Geneva. https://covid19.who.int/. Accessed 5 Jan 2021
Wolf Y, Kazlauskas D, Iranzo J (2018) Origins and evolution of the global RNA virome. mBio 9: e02329–e02318. https://doi.org/10.1128/mBio.02329-18
World Health Organization (2003) Consensus document on the epidemiology of severe acute respiratory syndrome (SARS). World Health Organization, Geneva. hdl:10665/70863
World Health Organization (WHO). World Health Statistics (2016) Monitoring health for the SDGs sustainable development goals. World Health Organization, Geneva, Switzerland
Wrapp D, Wang N, Corbett KS et al (2020) Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science 367:1260–1263
Wu F, Zhao S, Yu B et al (2020a) A new coronavirus associated with human respiratory disease in China. Nature 579:265–269
Wu Y, Ho W, Huang Y et al (2020b) SARS-CoV-2 is an appropriate name for the new coronavirus. Lancet 395(10228):949–950
Yan R et al (2020) Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. Science 367(6485):1444–1448
Yarnall AJ, Burn DJ (2012) Amantadine-induced myoclonus in a patient with progressive supranuclear palsy. Age Ageing 41(5):695–696. https://doi.org/10.1093/ageing/afs043
Yu C-H, Tam K, Tsang ES (2008) Chemical methods for preparation of nanoparticles in solution. Handb Metal Phys 5:113–141
Zazo H, Colino CI, Lanao JM (2016) Current applications of nanoparticles in infectious diseases. J Control Release 224:86–102
Zhou P, Yang X-L, Wang X-G et al (2020) A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 579:270–273
Zhou B, Thao TTN, Hoffmann D et al (2021) SARS-CoV-2 spike D614G change enhances replication and transmission. Nature 592:122–127. https://doi.org/10.1038/s41586-021-03361-1
Zhu J-D, Meng W, Wang X-J et al (2015) Broad-spectrum antiviral agents. Front Microbiol 6. https://doi.org/10.3389/fmicb.2015.00517/abstract

Application of Nanoparticles to Invasive Fungal Infections

7

Samuel Rodrigues dos Santos Junior, Andre Correa Amaral, and Carlos Pelleschi Taborda

Abstract

The present chapter provides an overview of the applications of nanoparticles in Medical Mycology. Nanoparticles possess sizes between 1 and 400 nm with different chemical and physical properties. The aim of the present chapter is to describe studies and applications with drugs and peptides into different types of nanoparticles with the objective of reducing fungal infection by stimulation of the immune system or acting directly on the fungus.

Keywords

Nanoparticles · Systemic mycosis · Fungal infections · Medical Mycology · *Candida* · *Aspergillus* · *Cryptococcus* · *Paracoccidioides*

S. R. dos Santos Junior
Department of Microbiology, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, SP, Brazil
e-mail: samuelmicrobio@usp.br

A. C. Amaral
Nano & Biotechnology Laboratory, Department of Biotechnology, Institute of Tropical Pathology and Public Health, Federal University of Goias, Goiania, GO, Brazil
e-mail: andre_amaral@ufg.br

C. P. Taborda (✉)
Department of Microbiology, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, SP, Brazil

Institute of Tropical Medicine of Sao Paulo, Department of Dermatology, Faculty of Medicine, University of Sao Paulo, Sao Paulo, SP, Brazil
e-mail: taborda@usp.br

S. Hameed, S. Rehman (eds.), *Nanotechnology for Infectious Diseases*,
https://doi.org/10.1007/978-981-16-9190-4_7

7.1 Introduction

Fungi can be found in different habitats on the planet (Blackwell 2011), and it is assumed that there are about five million species of fungi distributed around the globe (Kohler et al. 2015; Fisher et al. 2020), of which about 100,000 are known and only a few hundred are pathogenic to humans (Blackwell 2011; Kohler et al. 2015). Fungi are mostly saprophytes and can be found in the soil or be part of the microbiota of plants and animals (Casadevall 2005; Casadevall and Damman 2020). These microorganisms can become pathogenic and cause disease to the host (Pires et al. 2014; Casadevall 2018; Chaturvedi et al. 2018).

Mycoses are caused by different fungi (Table 7.1), which are classified into opportunistic or "true pathogenic fungi" (Staab and Wong 2014). Opportunistic fungi are those that take advantage of biological or environmental changes to colonize the host (Lionakis and Kontoyiannis 2003; Schmiedel and Zimmerli 2016; Taborda et al. 2018; Sanguinetti et al. 2019; Dantas et al. 2021). These mycoses are usually associated with alterations in the host's immune response and may occur mostly as systemic disease. The most common fungal genus involved are *Aspergillus* sp., *Candida* sp., *Cryptococcus* sp., *Pneumocystis* sp., and *Fusarium* sp. (Schmiedel and Zimmerli 2016).

Although infections caused by true pathogenic fungi do not necessarily depend on changes in the host's immune response, these diseases are usually more severe in immunocompromised patients. These mycoses are classified as cutaneous, subcutaneous, and systemic (Aditya et al. 2005; Degreef 2008; Vermout et al. 2008; Pires et al. 2014; Laniosz and Wetter 2014). Cutaneous, or superficial mycoses, are the most common mycoses among the population and are caused by a wide variety of fungi (Aditya et al. 2005; Degreef 2008; Vermout et al. 2008; Pires et al. 2014; Laniosz and Wetter 2014). Subcutaneous and systemic mycoses are caused by fungi of the genus *Sporothrix* sp., *Blastomyces* sp., *Coccidioides* sp., *Histoplasm*a sp., and

Table 7.1 Mycoses and their etiological agents

Pathogenic potential	Form	Gender	Mycosis
Opportunistic	Mycelium	*Aspergillus*	Aspergillosis
		Fusarium	Fusarioses
		Cryptococcus	Cryptococcosis
	Yeast	*Candida*	Candidiasis (systemic and cutaneous)
		Pneumocystis	Pneumocystosis
True pathogens	Dimorphic fungi	*Blastomyces*	Blastomycosis
		Coccidioides	Coccidioidomycosis
		Histoplasma	Histoplasmosis
		Sporothrix	Sporotrichosis
		Paracoccidioides	Paracoccidioidomycosis

Paracoccidioides sp., among others (Staab and Wong 2014; Kohler et al. 2015; Fisher et al. 2016, 2020; Almeida et al. 2019; Dantas et al. 2021).

Since the 1980s, there has been a significant increase in the number of cases of systemic mycoses (Casadevall 2018; Sanguinetti et al. 2019; Firacative 2020; Dantas et al. 2021). This is related to the increasing number of people with some type of immunodeficiency, such as those caused by the human immunodeficiency virus (HIV), use of compounds such as broad-spectrum antibiotics or corticosteroids, drugs to prevent organ transplant rejection, hematopoietic diseases, and cancer (Lionakis and Kontoyiannis 2003; Enoch et al. 2006, 2017; Streinu-Cercel 2012; Schmiedel and Zimmerli 2016; Taborda et al. 2018; Sanguinetti et al. 2019; Firacative 2020; Dantas et al. 2021).

It is also important to highlight the importance of the progressive devastation of tropical forests changing the entire balance of nature (Taborda et al. 2018). Considering the debilitated condition of the patients, systemic mycoses are difficult to be treated and usually lead to the death of the patient (Enoch et al. 2006, 2017; Streinu-Cercel 2012; Firacative 2020).

7.2 Antifungal Therapy

The therapeutic approach for mycoses (Table 7.2) is based on azole compounds, pyrimidine analogues, polyenes, and echinocandins (Gubbins and Anaissie 2009; Shoham et al. 2010; Laniosz and Wetter 2014; McManus 2015; Scorzoni et al. 2017). These molecules (Fig. 7.1) can be used topically in the treatment of cutaneous and subcutaneous mycoses, presenting low toxicity and orally or parenterally against systemic mycoses, causing significant toxic effects (Aditya et al. 2005; Enoch et al. 2006; Degreef 2008; Gubbins and Anaissie 2009; Shoham et al. 2010; Staab and

Table 7.2 Antifungal compounds and targets of action

Antifungal family	Target	Name	Route of administration
Azoles	14-α-demethylase (Ergosterol biosynthesis)	Ketoconazole	Oral/IV
		Fluconazole	Oral/IV
		Itraconazole	Oral/IV
		Voriconazole	Oral/IV
		Posaconazole	Oral/IV
Polyenes	Membrane ergosterol	Nystatin	Topical
		Amphotericin B	Oral/IV
Pyrimidine analogues	DNA/RNA	5-Fluocytosine	Oral
Echinocandins	β-1,3-glucan synthase (β-glucan biosynthesis)	Caspofungin	IV
		Anidulafungin	IV
		Micafungin	IV

IV intravenous

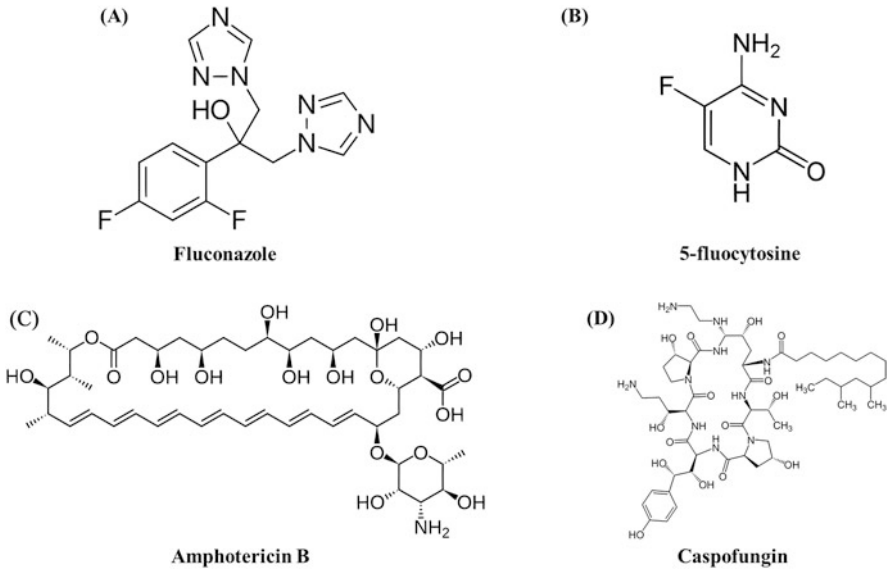

Fig. 7.1 Leading antifungal compounds used to treat invasive mycosis; (**a**) Fluconazole, (**b**) 5-fluocytosine, (**c**) Amphotericin B, (**d**) Caspofungin

Wong 2014; Pires et al. 2014; Laniosz and Wetter 2014; McManus 2015; Schmiedel and Zimmerli 2016; Scorzoni et al. 2017; Sanguinetti et al. 2019).

7.2.1 Azole Compounds

The main azole compounds are ketoconazole, fluconazole (Fig. 7.1a), itraconazole, voriconazole, and posaconazole. The mechanism of action of these compounds occurs by interference in ergosterol synthesis by inhibition of the enzyme 14-α-demethylase P450 cytochrome-dependent. This inhibition prevents the conversion of lanosterol into ergosterol, leading to dysfunction of the fungus plasma membrane and accumulation of toxic compounds to the cell (Gubbins and Anaissie 2009; Shoham et al. 2010; Laniosz and Wetter 2014; McManus 2015; Scorzoni et al. 2017).

7.2.2 Pyrimidine Analogous

The 5-fluocytosine (Fig. 7.1b) is the only compound of the molecules analogous to pyrimidine. It is administered orally and has low toxicity (Gubbins and Anaissie 2009; Shoham et al. 2010; Laniosz and Wetter 2014; McManus 2015; Scorzoni et al. 2017). The 5-fluocytosine is transported into the fungal cell facilitated by the enzyme cytosine permease and then converted to 5-fluorouracil by the cytosine deaminase,

causing interruption of DNA and or RNA synthesis (Gubbins and Anaissie 2009; Shoham et al. 2010; Laniosz and Wetter 2014; McManus 2015; Scorzoni et al. 2017).

7.2.3 Polyenes

Nystatin and Amphotericin B are antifungals of the polyene class, the latter being considered the gold standard drug in the treatment of mycosis, used orally or parenterally. This drug interacts with ergosterol, a component of the fungal membrane which corresponds to the cholesterol present in the human membranes. This interaction results in rupture of the fungus plasma membrane (Gubbins and Anaissie 2009; Shoham et al. 2010; Laniosz and Wetter 2014; McManus 2015; Scorzoni et al. 2017).

The mechanism of action of Amphotericin B (Fig. 7.1c) is based on its binding to ergosterol forming pores that lead to membrane depolarization and extravasation of cytosol content. However, although in lesser intensity, Amphotericin B is also able to interact with cholesterol and, therefore, presents a high level of toxicity, mainly for the kidneys (Amaral et al. 2009; Souza et al. 2015).

In an attempt to circumvent the toxic adverse effects of this drug, different drug formulations have been proposed to reduce these problems. Drug delivery formulation in nanoscale such as Amphotericin B lipid complex (Abelcet®), Amphotericin B colloidal dispersion (Amphotec®), and Amphotericin B liposomal (AmBisome®) are safer than the conventional Amphotericin B deoxycholate (Fungizone®) (Gubbins and Anaissie 2009; Amaral et al. 2009; Shoham et al. 2010; Souza et al. 2015).

7.2.4 Echinocandins

Echinocandins are synthetic lipopeptides representing the most modern group of antifungals. The main drugs in this class are caspofungin (Fig. 7.1d), micafungin, and anidulafungin. The mechanism of action of these drugs is by inhibiting the synthesis of β1,3-glucan, a molecule essential to the cell wall composition of the fungus and, since it is not present in animal cells, causes lower toxicity to the patient. The inhibition in the synthesis of this cell component causes an increase in cell wall permeability leading to its subsequent rupture (Gubbins and Anaissie 2009; Shoham et al. 2010; Laniosz and Wetter 2014; McManus 2015; Scorzoni et al. 2017).

7.3 Nanoformulations for Antifungal Therapy

The successful use of Amphotericin B within nanoparticles has motivated the development of different nanoscale formulations for classical drugs and molecules with antifungal effects (Table 7.3). There are important advantages to develop these formulations on a nanoscale. Considering the possibility of using biodegradable

Table 7.3 Examples of nanoparticles development for invasive fungal infections

Pathogen	Class of nanoparticle	Material	Active compounds	Tests in vivo (+) or in vitro (++)	Reference
Candida	Polymeric	Chitosan	Amphotericin B	+/++	Park et al. (2017)
		Chitosan	Miconazole/ Farnesol	+/++	Fernandes Costa et al. (2019)
		PLGA	Amphotericin B	+/++	Liu et al. (2014)
		Chitosan	Fluconazole	+/++	Rençber et al. (2016)
		Eudragit S100	Osthol/Fluconazole	+/++	Li et al. (2018)
		Poloxamer P407/P188	Amphotericin B	+/++	Ci et al. (2018)
		Alginate	Miltefosine	+/++	Spadari et al. (2019)
		Poly-ε-caprolactone	2-Amino-thiophene	++	Neves et al. (2020)
		MPEG-PCL	Amphotericin B	+/++	Zhang et al. (2017)
		Chitosan-PLGA	Natamycin/ Clotrimazole	+/++	Cui et al. (2021)
	Lipid	Solid lipid	Amphotericin B	+/++	Riaz et al. (2020)
		Cholesterol conjugated	CG3R6TAT	+/++	Xu et al. (2011b)
		Solid lipid	Miconazole	+/++	Aljaeid and Hosny (2016)
		Cubosome	Amphotericin B	+/++	Xu et al. (2014)
		Solid lipid	Eugenol	+/++	Garg and Singh (2011)
		Solid lipid	Uncharacterized	+/++	Vidal Bonifácio et al. (2015)
		Encochleated	Amphotericin B	+/++	Santangelo et al. (2000)
Aspergillus	Polymeric	PLGA	Amphotericin B	+/++	Van de Ven et al. (2012)
		Chitosan	Voriconazole	+/++	Kaur et al. (2021)
		B-polymethacrylic	Amphotericin B	+/++	Shirkhani et al. (2015)
Cryptococcus	Polymeric	Poly-ε-caprolactone	2-Amino-thiophene	++	Neves et al. (2020)
		Alginate	Miltefosine	+/++	Spadari et al. (2019)
		PLA-b-PEG	Amphotericin B	+	Ren et al. (2009)

		Polybutylcyanoacrylate	Amphotericin B	+	Xu et al. (2011a)
	Metallic/Lipid	Pd@Ag	Amphotericin B	++	Zhang et al. (2016)
		Encochleated	Amphotericin B	+	Lu et al. (2019)
		Lipid core nanocapsules	Amiodarone	+/++	Oliveira et al. (2021)
		Cholesterol conjugated	CG3R6TAT	+/++	Wang et al. (2010)
Paracoccidioides	Polymeric	PLGA	scFv Mab against gp43	+	Jannuzzi et al. (2018)
			Amphotericin B	+	Amaral et al. (2009)
			P10 peptide	+	Amaral et al. (2010)
		Chitosan	P10 peptide	+/++	Rodrigues Dos Santos Junior et al. (2020)
	Metallic/Lipid	Fe_3O_4	Amphotericin B	+/++	Saldanha et al. (2016)
		Nanoemulsion	2-Hydroxychalcone	+	Medina-Alarcón et al. (2020)
		Solid lipid	Dodecyl gallate	+/++	de Singulani et al. (2018)

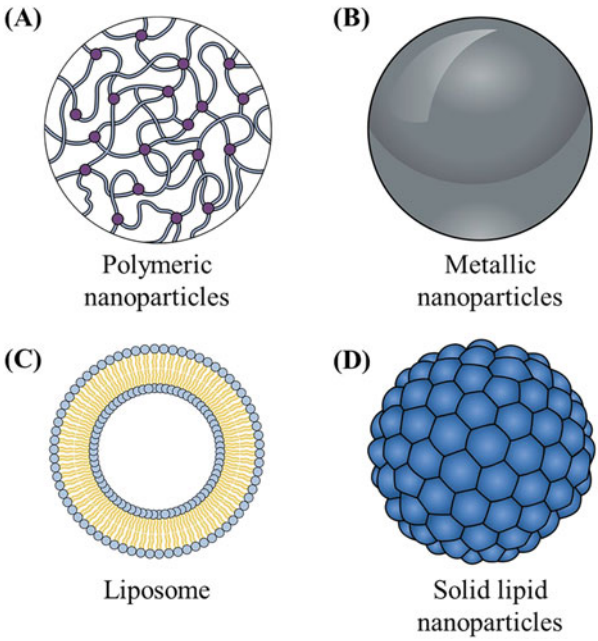

Fig. 7.2 Most studied nanoparticles for treating invasive fungal infections. (**a**) Polymeric nanoparticles, (**b**) Metallic nanoparticles, (**c**) Liposomes, (**d**) Solid lipids nanoparticles. (Image created using the Mind the graph website)

materials, it is possible to promote the sustained release of compounds associated with nanoparticles. Next, instead of applying multiple doses or high concentrations of the drug to reach the therapeutic level, a large amount of the antifungal can be encapsulated in the nanoparticles to be released over time (Couvreur and Vauthier 2006; Peek et al. 2008; das Neves et al. 2010; Etheridge et al. 2013; Thorley and Tetley 2013).

It is also possible to direct the drugs to act in specific treatment locations, linking molecules on the surface of these structures that present tropism by specific tissues or organs of the body (Csaba et al. 2009; Gupta and Vyas 2012; Gregory et al. 2013; Melkoumov et al. 2013; Nazarian et al. 2014; Zhao et al. 2014; Yan et al. 2014; Sharma et al. 2015; Spadari et al. 2019; Riaz et al. 2020). Drugs or molecules can also be protected against degradation by enzymes present in the biological environment. These nanoscale drug delivery systems can be prepared using a variety of nanoparticles (Fig. 7.2), such as polymers, metals, and phospholipids (Csaba et al. 2009; Gupta and Vyas 2012; Gregory et al. 2013; Melkoumov et al. 2013; Nazarian et al. 2014; Zhao et al. 2014; Yan et al. 2014; Sharma et al. 2015; Spadari et al. 2019; Riaz et al. 2020).

7.3.1 Polymeric Nanoparticles

Polymeric nanoparticles (Fig. 7.2a) are prepared based on different types of polymers, both natural and synthetic (Carcaboso et al. 2004; Csaba et al. 2009;

Garcia-Fuentes and Alonso 2012; Bolhassani et al. 2014; Ahmed and Aljaeid 2016). The physical and chemical properties of nanoparticles are defined according to the characteristics of the materials used in its preparation, such as concentration of the monomers and/or polymers, charge, and hydrophilicity (Carcaboso et al. 2004; Csaba et al. 2009; Garcia-Fuentes and Alonso 2012; Bolhassani et al. 2014; Ahmed and Aljaeid 2016). The polymers most commonly used, but not restricted, to prepare polymeric nanoparticles are chitosan, polylactic acid (PLA), polyglycolic acid (PGA), poly (lactic acid-*co*-glycolic acid) (PLGA), and alginate (Carcaboso et al. 2004; Csaba et al. 2009; Garcia-Fuentes and Alonso 2012; Bolhassani et al. 2014; Ahmed and Aljaeid 2016).

7.3.2 Metallic Nanoparticles

Metallic nanoparticles (Fig. 7.2b) are very small structures that can be obtained using metals such as gold, silver, titanium, zinc, copper, and iron (Mody et al. 2010; Schröfel et al. 2014; Govindrao Jamkhande et al. 2019; Marinescu et al. 2020). These types of nanoparticles are widely studied because of their chemical and physical characteristics that can be used in nanocarriers of different types of molecules, including antimicrobials and bioactive molecules (Schröfel et al. 2014; Marinescu et al. 2020). The main mechanism of action of metallic nanoparticles with antimicrobial action is the interference of these nanoparticles in the cellular homeostasis, and the disruption of the flow of electrons inside and around the cell's membranes, leading the cells to apoptosis (Mody et al. 2010; Schröfel et al. 2014; Aderibigbe 2017).

7.3.3 Lipid Nanoparticles

Lipid nanoparticles can be classified into liposomes/micelle (Fig. 7.2c), solid lipid nanoparticles (Fig. 7.2d), and nanoemulsions (Gupta and Vyas 2012). The liposomes/micelles are spherical with an aqueous or oily nucleus in a lipid shell. Depending on the phospholipid used in the preparation of these nanoparticles, micelles can be formed, which contain only a lipid layer or liposomes with a double lipid layer, mimicking a biological membrane (Lambros et al. 1998; Bolhassani et al. 2014; De Serrano and Burkhart 2017; Zhang et al. 2017). Solid lipid nanoparticles are a class of lipid blocks forming a matrix and may be fluid or rigid depending on the type of lipid used (Jain et al. 2010; Gupta and Vyas 2012; Vaghasiya et al. 2013). In the same category, nanoemulsions, which are dispersions of two immiscible solutions, widely used for topical applications (Mirza et al. 2013; Soriano-Ruiz et al. 2019; do Carmo Silva et al. 2020).

7.4 Systemic Mycoses and Nanotechnology

Despite their medical relevance and the need for more efficient and less toxic therapy based on the clinical situation of the patient, the researchers are focused on developing a more sophisticated therapy for systemic mycoses (Dantas et al. 2021).

7.4.1 *Candida* sp.

Candidiasis are fungal diseases responsible for causing various types of infections: superficially such as onychomycosis, candidiasis related to sexually transmitted infections (STIs), and the systemic candidiasis, which is the most severe form and occurs when the fungus spreads and colonizes the host through the circulatory system leading to sepsis and death (Enoch et al. 2006; Giri and Kindo 2012; Quindós 2014; Pappas et al. 2018; Sanguinetti et al. 2019; Firacative 2020).

Candida sp. are saprophytic microorganisms that colonize the epithelial microbiota of animals. The onset of systemic candidiasis occurs when there is a lesion of the epithelial tissue allowing the contact of the fungus with the bloodstream (Giri and Kindo 2012; Quindós 2014; Scorzoni et al. 2017; Pappas et al. 2018). One of the main causes of systemic candidiasis is the contamination in hospitals, making systemic candidiasis one of the main nosocomial fungal infections with a high mortality rate along with aspergillosis (Enoch et al. 2006; Giri and Kindo 2012; Quindós 2014; Pappas et al. 2018).

There are about 15 species of Candida with pathogenic potential; *C. albicans* is the main species causing candidiasis, followed by *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, and *C. krusei*. The pathogenic importance varies according to the geographical location and factors such as the main antifungal compound used to combat fungal infections (Giri and Kindo 2012; Quindós 2014; Scorzoni et al. 2017; Pappas et al. 2018). In recent years, a species that has been attracting the attention of researchers is the newly identified *C. auris*, which presented resistance to several types of antifungal compounds (Giri and Kindo 2012; Quindós 2014; Scorzoni et al. 2017; Pappas et al. 2018; Singh et al. 2020).

The main antifungals used in the treatment of invasive candidiasis are fluconazole, Amphotericin B, and echinocandins, but due to its toxicity, the use of nanoparticles is highly recommended (Giri and Kindo 2012; Quindós 2014; Scorzoni et al. 2017; Pappas et al. 2018).

7.4.1.1 Polymeric Nanoparticles

Xiaolong Tang et al. 2014 prepared a formulation for the use of Amphotericin B with PLGA nanoparticles, improving bioavailability and reducing toxicity and fungal load in mice models (Tang et al. 2014).

Seda Rençber et al. 2016 prepared a formulation of fluconazole within chitosan nanoparticles for the treatment of oral candidiasis in rabbit, and they demonstrated that it is possible to use this treatment for local application due to the bioadhesive characteristics of chitosan (Rençber et al. 2016).

Seong-Cheol Park et al. 2017 prepared a combination of Amphotericin B and the antifungal peptide histatin 5 within chitosan nanoparticles targeting the nanoparticle to the fungus cell, reducing Amphotericin B toxicity and fungal load in mice models (Park et al. 2017).

Peipei Zhang et al. 2017 showed that an Amphotericin B formulation in MPEG-PCL (poly(ethylene poly(ε-caprolactone))) used in starch tablets where able to reduce oral candidiasis and Amphotericin B toxicity in mice models (Zhang et al. 2017).

Lin-peng Li et al. 2018 evaluated the *Cnidium monnier* secondary metabolite Osthol interaction with fluconazole for evaluation of the metabolite fungicidal capacity in fluconazole-resistant strains. The delivery of the osthol was made by Eudragit S100 nanoparticles. They showed a reduction of the fungal load of the fluconazole resistant strains in mice models (Li et al. 2018).

Tianyuan Ci et al. 2018 prepared a new formulation made by nanosuspensions of Amphotericin B in a p407/P188 poloxamer thermogel for the treatment of vulvovaginal candidiasis in mice. The formulation demonstrated a better efficiency, gradually releasing the Amphotericin B and reducing the fungal load when compared with a commercial Amphotericin B effervescent tablet used as a control (Ci et al. 2018).

Adelaide Fernandes Costa et al. 2019 used chitosan nanoparticles as a farnesol and miconazole carrier to treat mice vulvovaginal candidiasis, showing reduced fungal proliferation and tissue inflammation (Fernandes Costa et al. 2019).

Cristina de Castro Spadari et al. 2019 prepared a new formulation of the redirected compound miltefosine within alginate nanoparticles. They showed that the formulation was able to reduce the miltefosine toxicity and the fungal load of the *Candida* yeast in mice models (Spadari et al. 2019).

Wendell Wons Neves et al. 2020 prepared a formulation of the secondary metabolite 2-amino-thiophene (6CN10) in 2-hydroxy nanocarriers propyl-β-cyclodextrin within nanocapsules or nanospheres of poly-ε-caprolactone to access biofilm reduction and fungal susceptibility. The formulations worked against *Cryptococcus* and not against *Candida* (Neves et al. 2020).

Xiaoming Cui et al. 2021 created a formulation with Natamycin and Clotrimazole in PLGA and chitosan nanoparticles to treat keratitis caused by *Candida*. They showed an increase in the solubility, bioavailability, and a reduction of toxic effect and reduction of the infection in rabbits (Cui et al. 2021).

7.4.1.2 Lipid Nanoparticles

Kaijin Xu et al. 2011 showed a reduction of the fungal load by using an antimicrobial peptide conjugated with cholesterol molecules for the automatic formation of lipopeptide nanoparticles to treat meningitis caused by *Candida* in rabbit (Xu et al. 2011b).

Anuj Garg and Sanjay Kumar Singh, 2011 demonstrated an increase of the solubilization, bioavailability, and antifungal activity of the eugenol when formulated in solid lipid nanoparticles associated with a carbopol matrix for the treatment of oral candidiasis in immunosuppressed mice (Garg and Singh 2011).

Zhiwen Yang et al. 2014 prepared a formulation of Amphotericin B in cubosomes (lipid nanoparticles in cubic form) that increased the bioavailability of the compound by oral administration showing reduction of fungal load only in the kidney of the animals (Xu et al. 2014).

Bruna Vidal Boniface et al. 2015 showed that a formulation of hydroethanolic extracts of *Astronium* in solid lipid nanoparticles reduced the fungal load in the treatment of vulvovaginal candidiasis in comparison with free extract and Amphotericin B in mice models (Vidal Bonifácio et al. 2015).

Bader Mubarak Aljaeid Khaled and Mohamed Hosny 2016 prepared a formulation of miconazole in solid lipid nanoparticles for oral application, increasing the solubility of the compound and reducing the fungal load in comparison to the encapsulated formulation available in the market used as a control in mice models (Aljaeid and Hosny 2016).

Amina Riaz et al. 2020 prepared a formulation of Amphotericin B in a lipid nanocarrier that showed reduction of the Amphotericin B toxicity and reduction of the cutaneous leishmaniasis and vulvovaginal candidiasis in mice models (Riaz et al. 2020).

Rosaria Santangelo et al. 2000 prepared an Amphotericin B formulation in curly lipid nanoparticles (Encochleated) for oral administration and evaluation of the bioavailability and toxicity of the compound. They demonstrated an increase in the survival rate and reduction of the fungal load in mice models of systemic candidiasis (Santangelo et al. 2000).

7.4.2 *Aspergillus* sp.

Aspergillosis is a fungal disease and may present mild manifestations such as allergies, and in severe cases, such as invasive aspergillosis. The host immunological condition is a determining factor driving the level of severity of the infection (Enoch et al. 2006; Zmeili and Soubani 2007; Kousha et al. 2011; Thompson and Patterson 2011; Streinu-Cercel 2012; Kosmidis and Denning 2015; Schmiedel and Zimmerli 2016; Firacative 2020).

Aspergillus sp. is a ubiquitous fungus, being found everywhere by transposing virtually all types of barriers. Due to its presence in any given place, several cases of nosocomial infections by *Aspergillus* are reported annually, and these numbers continue to grow as more people become immunocompromised due to increased cases of cancer, organ transplants autoimmune diseases, and immunodeficiencies conditions such as AIDS (Zmeili and Soubani 2007; Kousha et al. 2011; Thompson and Patterson 2011; Kosmidis and Denning 2015).

The frequent fungus causing invasive aspergillosis is *A. fumigatus*, and in most cases, infection occurs when fungal particles are inhaled and subsequently deposited in the lower part of the respiratory tract of the patient (Zmeili and Soubani 2007; Kousha et al. 2011; Thompson and Patterson 2011; Kosmidis and Denning 2015). As occurs in the treatment for candidiasis and other systemic mycosis, the main compounds used in the treatment of invasive aspergillosis are fluconazole,

Amphotericin B and echinocandins, but due to its toxicity, the use of nanoparticles is once again highly recommended (Zmeili and Soubani 2007; Kousha et al. 2011; Thompson and Patterson 2011; Kosmidis and Denning 2015).

7.4.2.1 Polymeric Nanoparticles

Helene Van de Ven et al. 2012—Preparation of a formulation of Amphotericin B in PLGA nanoparticles and nanosuspensions to reduce the toxic effects of the compound in addition to evaluating the efficacy of the formulation in reducing fungal load in mice models (Van de Ven et al. 2012).

Khojasteh Shirkhani et al. 2015 prepared an Amphotericin B formulation in polymethacrylate nanoparticles for prophylactic administration in immunosuppressed mice to mimic organ transplant-related immunosuppression. The trials showed that the formulation presented low toxicity and great efficacy in reducing fungal infection in mice models (Shirkhani et al. 2015).

Ranjot Kaur et al. 2021 developed a voriconazole solution in chitosan nanoparticles functionalized with dipalmitoylphosphatidylcholine (DPPC) for nebulization, allowing greater retention and bioavailability of the formulation in the lung. In vitro assays have demonstrated efficacy reducing the fungal load, and in vivo assays have demonstrated the retention capacity and greater bioavailability of the compound in mice models (Kaur et al. 2021).

7.4.3 *Cryptococcus* sp.

Cryptococcosis is a globally disseminated systemic fungal disease (Srikanta et al. 2014; Gushiken et al. 2021). The main organs affected by cryptococcosis are the lungs and brain due to the tropism that the fungus presents to the central nervous system (CNS) (Casadevall et al. 2018). The spread of *Cryptococcus* sp. through the body results from its main virulence mechanisms, corresponding to the presence of a polysaccharide capsule and production of melanin (Casadevall et al. 2018; Colombo et al. 2019; Cordero et al. 2020; Kuttel et al. 2020). Cryptococcosis is a disease that affects hosts with some type of immunodeficiency, mostly related to AIDS (Casadevall et al. 2018; Colombo et al. 2019; Cordero et al. 2020; Kuttel et al. 2020).

This co-infection dynamic was widely reported in the 1970s and 1980s during the dissemination of AIDS cases (Srikanta et al. 2014; May et al. 2016; Khatun et al. 2020).

Genus *Cryptococcus* are divided into two species complexes: *C. neoformans and C. gattii*; within these two complexes there are the species *C. neoformans and C. deneoformans* belonging to the neoformans complex and the species *C. gattii*, *C. deuterogattii*, *C. bacillisporus*, *C. tetragattii* and a hybrid species not yet classified (Danesi et al. 2021; Wang 2021; Montoya et al. 2021).

These species differ from each other due to the different serotypes, which are classified according to the polysaccharide composition of the capsule (Zaragoza et al. 2009; Casadevall et al. 2019; Crawford et al. 2020). The *Cryptococcus* capsule consists of different branches and basically two amounts of sugars:

glucuronoxylomannan (GXM) and galactoxilomanana (GalXM). Due to these variations of sugars and branches, it is possible to classify the strains and species of *Cryptococcus* in five different serotypes: A, B, C, D, and AD (Zaragoza et al. 2009; Casadevall et al. 2019; Crawford et al. 2020).

Another important virulence factor is the ability of *Cryptococcus* to produce melanin. Melanin is associated with the fungus ability to resist to environmental stresses and escape from the human immune system (Chrissian et al. 2020; Cordero et al. 2020; de Sousa et al. 2021).

The main antifungals for the treatment of cryptococcosis are the polyene compounds such as Amphotericin B, pyrimidine-like analogue compounds such as 5-fluorocytosine and azole compounds, such as fluconazole, but due to its toxicity, the use of nanoparticles is once again highly recommended (Gushiken et al. 2021).

7.4.3.1 Polymeric Nanoparticles

Tianbin Ren et al. 2009 prepared an Amphotericin B formulation in PLA-b-PEG poly(lactic acid)-b-poly(ethylene glycol) nanoparticles, functionalized with polysorbate 80, that allowed greater bioavailability of the compound in the central nervous system through the passage of nanoparticles by the blood–brain barrier, reducing the toxicity of the compound and the fungal load in mice models (Ren et al. 2009).

Nan Xu et al. 2011 created a formulation of Amphotericin B in b-polybutylcyanoacrylate nanoparticles functionalized with Polysorbate 80 that also allowed greater bioavailability of the compound in the central nervous system through the passage of nanoparticles by the blood–brain barrier, reducing the toxicity of the compound and the fungal load in mice models (Xu et al. 2011a).

Cristina de Castro Spadari et al. 2019 prepared a new formulation of the redirected compound miltefosine within alginate nanoparticles. They showed that the formulation was able to reduce the miltefosine toxicity and the fungal load of the *Cryptococcus* yeast in mice models (Spadari et al. 2019).

Wendell Wons Neves et al. 2020 prepared a formulation of the secondary metabolite 2-amino-thiophene (6CN10) in 2-hydroxy nanocarriers propyl-β-cyclodextrin within nanocapsules or nanospheres of poly-ε-caprolactone to access biofilm reduction and fungal susceptibility. The formulations worked against *Cryptococcus* and not against *Candida* (Neves et al. 2020).

7.4.3.2 Metallic Nanoparticles

Chao Zhang et al. 2016 evaluated the combination of the fungicidal action of metallic nanoparticles with Amphotericin B and showed synergism of the formulation in mice models (Zhang et al. 2016).

7.4.3.3 Lipid Nanoparticles

Huaying Wang et al. 2010 used antimicrobial peptides conjugated with cholesterol molecules for the automatic formation of lipopeptide nanoparticles for the treatment of Cryptococcosis meningitis. They showed a reduction of the fungal load in the brain due to the nanoparticles cross of the blood–brain barrier in rabbits (Wang et al. 2010).

Ruying Lu et al. 2019 prepared a formulation of Amphotericin B in curly lipid nanoparticles (Encochleated) for oral administration of the compound. They demonstrated increasing in the bioavailability and toxicity reduction of the compound and showed efficiency of the formulation against Cryptococcosis meningitis in association with 5-fluocytosine in mice model (Lu et al. 2019).

Natália Kronbauer Oliveira et al. 2021 prepared a formulation of amiodarone (blocker of calcium channels) in lipid nucleus nanoparticles for intranasal and or intraperitoneal application. They showed that this compound only reduced the infection and fungal load when inside the nanoparticle in mice models (Oliveira et al. 2021).

7.4.4 *Paracoccidioides* sp.

Paracoccidioidomycosis (PCM) is a systemic mycosis that primarily affects the lungs and may later disseminate throughout the body (Travassos and Taborda 2012). PCM is caused by dimorphic fungi of the genus *Paracoccidioides*, and five species of this fungus are currently recognized, *P. brasiliensis strictu* sensu, *P. americana*, *P. restrepiensis*, *P. venezuelensis*, *P. lutzii* (Turissini et al. 2017).

This fungus presents at room temperature around 24 °C, a filamentous and saprophytic characteristic (Travassos and Taborda 2012). The most frequent form of infection of humans and animals by the fungus is by inhaling conidia or fungal propagules that settle primarily in the lungs and can spread throughout the body through the lymphatic system, with the increase of temperature around 37 °C, the fungus changes from the mycelial to pathogenic yeast form (Amaral et al. 2010; De Melo et al. 2014; Gegembauer et al. 2014).

These fungi can be found in almost all Latin American countries, from Mexico to Argentina, excluding Suriname, Chile, and the Caribbean islands (Palmeiro et al. 2005).

PCM can be classified as: PCM infection or disease, in which PCM infection does not present clinical manifestations and PCM disease is classified into two clinical forms: juvenile/acute and subacute or adult/chronic (Shikanai-Yasuda et al. 2017). When the disease manifests in the juvenile phase it has a systemic characteristic and has no distinction between the sexes of the hosts. In the adult form, the disease manifests mainly in the lungs and especially in men between 20 and 50 years old (Palmeiro et al. 2005; Restrepo et al. 2012; Taborda et al. 2021).

The treatment of PCM occurs in two phases. The first consists of an initial attack treatment to quickly control the infection and the second phase consists of a treatment to inhibit the proliferation of the remaining fungi to prevent the recurrence of the disease (Shikanai-yasuda et al. 2006).

The main drugs for the treatment of PCM are Amphotericin B, sulfadiazine, and itraconazole (Shikanai-Yasuda et al. 2017). Although these compounds are very effective in the treatment of PCM and other mycoses, they present important adverse effects to be observed: common headaches, gastric disorders, and rashes until

nephrotoxicity in the case of Amphotericin B (Borges et al. 2014; Taborda et al. 2021).

Nanotechnology has been demonstrating a role of great importance to improve the adverse effects because it allows not only to reduce the concentration of the drug used, but also to allow sustained release (Amaral et al. 2009).

7.4.4.1 Polymeric Nanoparticles

Andre Correa Amaral et al. 2009 prepared a formulation of Amphotericin B in PLGA nanoparticles functionalized with DMSA (dimercaptosuccinic acid), a molecule that presents tropism by the lungs, showing reduction of the toxic effects of the compound and reducing fungal load in mice models (Amaral et al. 2009).

Polymeric Nanoparticles Used in Vaccines

Andre Correa Amaral et al. 2010 produced a formulation composed of the complexation of an immunomodulatory peptide in PLGA nanoparticles associated with chemotherapy compounds (Bactrim) for the development of a therapeutic vaccine against PCM. They reduced the fungal load in mice models (Amaral et al. 2010).

Grasielle Pereira Jannuzzi et al. 2018 developed a vaccine formulation composed of the complexation of the variable fractions of the light and heavy chains (scFv) of a mimetic antibody to Gp43 in PLGA nanoparticles. They showed a reduction in fungal load and an increase of IFN-g and IL-12 cytokine production by using the vaccine as a prophylactic or therapeutic treatment for PCM in mice models (Jannuzzi et al. 2018).

Santos Junior et al. 2020 produced a vaccine formulation composed of the complexation of an immunomodulatory peptide in chitosan nanoparticles for the development of an intranasal therapeutic vaccine against PCM. They showed that the intranasal vaccine was effective, reducing mice lung fungal load (Rodrigues Dos Santos Junior et al. 2020).

7.4.4.2 Metallic Nanoparticles

Camila Arruda Saldanha et al. 2016 prepared a formulation of Amphotericin B within magnetite nanoparticles (Fe_3O_4) functionalized in a lipid bilayer of uric acid. They showed reduction of toxic effects of the compound and an increase of the effectiveness of the formulation in reducing fungal load in mice models (Saldanha et al. 2016).

7.4.4.3 Lipid Nanoparticles

Junya de Lacorte Singulani et al. 2018 created a formulation of dodecyl gallate (secondary metabolite) in solid lipid nanoparticles. They demonstrated an increase bioavailability and toxicity reduction of the compound and reduction of the fungal infection in the treatment of PCM in mice models (de Singulani et al. 2018).

Kaila Medina-Alarcón et al. 2020 prepared a formulation of 2-hydroxyccalcone (precursor of secondary metabolites) in a nanoemulsion, showing increase in the bioavailability and toxicity reduction of the compound, in addition to reducing the

viability of *Paracoccidioides brasiliensis* in mice models (Medina-Alarcón et al. 2020).

7.5 Conclusion

Nanotechnology has brought enormous advantages in drug treatment by reducing the cytotoxicity of antifungal agents or in the formulation of vaccines. An important point that we highlight is that most studies are still in the experimental phase. We believe that nanotechnology may soon be included in clinical trials and its effectiveness will be proven.

References

Aderibigbe B (2017) Metal-based nanoparticles for the treatment of infectious diseases. Molecules 22:1370. https://doi.org/10.3390/molecules22081370

Aditya KG, Jennifer ER, Melody C, Elizabeth AC (2005) Dermatophytosis: the management of fungal infections. Skinmed 4:305–310. https://doi.org/10.1111/j.1540-9740.2005.03435.x

Ahmed TA, Aljaeid BM (2016) Preparation, characterization, and potential application of chitosan, chitosan derivatives, and chitosan metal nanoparticles in pharmaceutical drug delivery. Drug Des Devel Ther 10:483–507. https://doi.org/10.2147/DDDT.S99651

Aljaeid B, Hosny KM (2016) Miconazole-loaded solid lipid nanoparticles: formulation and evaluation of a novel formula with high bioavailability and antifungal activity. Int J Nanomedicine 11:441. https://doi.org/10.2147/IJN.S100625

Almeida F, Rodrigues ML, Coelho C (2019) The still underestimated problem of fungal diseases worldwide. Front Microbiol 10:1–5. https://doi.org/10.3389/fmicb.2019.00214

Amaral AC, Bocca AL, Ribeiro AM et al (2009) Amphotericin B in poly(lactic-co-glycolic acid) (PLGA) and dimercaptosuccinic acid (DMSA) nanoparticles against paracoccidioidomycosis. J Antimicrob Chemother 63:526–533. https://doi.org/10.1093/jac/dkn539

Amaral AC, Marques AF, Muñoz JE et al (2010) Poly(lactic acid-glycolic acid) nanoparticles markedly improve immunological protection provided by peptide P10 against murine paracoccidioidomycosis. Br J Pharmacol 159:1126–1132. https://doi.org/10.1111/j.1476-5381.2009.00617.x

Blackwell M (2011) The fungi: 1, 2, 3 . . . 5.1 million species? Am J Bot 98:426–438. https://doi.org/10.3732/ajb.1000298

Bolhassani A, Javanzad S, Saleh T et al (2014) Polymeric nanoparticles potent vectors for vaccine delivery targeting cancer and infectious diseases. Hum Vaccines Immunother 10:321–323. https://doi.org/10.4161/hv.26796

Borges SRC, Da Silva GMS, Da Costa Chambela M et al (2014) Itraconazole vs. trimethoprim-sulfamethoxazole: a comparative cohort study of 200 patients with paracoccidioidomycosis. Med Mycol 52:303–310. https://doi.org/10.1093/mmy/myt012

Carcaboso AM, Hernández RM, Igartua M et al (2004) Potent, long lasting systemic antibody levels and mixed Th1/Th2 immune response after nasal immunization with malaria antigen loaded PLGA microparticles. Vaccine 22:1423–1432. https://doi.org/10.1016/j.vaccine.2003.10.020

Casadevall A (2005) Fungal virulence, vertebrate endothermy, and dinosaur extinction: is there a connection? Fungal Genet Biol 42:98–106. https://doi.org/10.1016/j.fgb.2004.11.008

Casadevall A (2018) Fungal diseases in the 21st century: the near and far horizons. Pathog Immun 3:183. https://doi.org/10.20411/pai.v3i2.249

Casadevall A, Damman C (2020) Updating the fungal infection-mammalian selection hypothesis at the end of the Cretaceous Period. PLoS Pathog 16:e1008451. https://doi.org/10.1371/journal.ppat.1008451

Casadevall A, Coelho C, Alanio A (2018) Mechanisms of Cryptococcus neoformans-mediated host damage. Front Immunol 9:1–8. https://doi.org/10.3389/fimmu.2018.00855

Casadevall A, Coelho C, Cordero RJB et al (2019) The capsule of Cryptococcus neoformans. Virulence 10:822–831. https://doi.org/10.1080/21505594.2018.1431087

Chaturvedi V, Bouchara J-P, Hagen F et al (2018) Eighty years of mycopathologia: a retrospective analysis of progress made in understanding human and animal fungal pathogens. Mycopathologia 183:859–877. https://doi.org/10.1007/s11046-018-0306-1

Chrissian C, Camacho E, Fu MS et al (2020) Melanin deposition in two Cryptococcus species depends on cell-wall composition and flexibility. J Biol Chem 295:1815–1828. https://doi.org/10.1074/jbc.RA119.011949

Ci T, Yuan L, Bao X et al (2018) Development and anti-Candida evaluation of the vaginal delivery system of amphotericin B nanosuspension-loaded thermogel. J Drug Target 26:829–839. https://doi.org/10.1080/1061186X.2018.1434660

Colombo AC, Rella A, Normile T et al (2019) Cryptococcus neoformans glucuronoxylomannan and sterylglucoside are required for host protection in an animal vaccination model. MBio 10:1–22. https://doi.org/10.1128/mBio.02909-18

Cordero RJB, Camacho E, Casadevall A (2020) Melanization in Cryptococcus neoformans requires complex regulation. MBio 11:1–4. https://doi.org/10.1128/mBio.03313-19

Couvreur P, Vauthier C (2006) Nanotechnology: intelligent design to treat complex disease. Pharm Res 23(7):1417–1450

Crawford CJ, Cordero RJB, Guazzelli L et al (2020) Exploring Cryptococcus neoformans capsule structure and assembly with a hydroxylamine-armed fluorescent probe. J Biol Chem 295:4327–4340. https://doi.org/10.1074/jbc.RA119.012251

Csaba N, Garcia-Fuentes M, Alonso MJ (2009) Nanoparticles for nasal vaccination. Adv Drug Deliv Rev 61:140–157. https://doi.org/10.1016/j.addr.2008.09.005

Cui X, Li X, Xu Z et al (2021) Fabrication and characterization of chitosan/poly(lactic-co-glycolic acid) core-shell nanoparticles by coaxial electrospray technology for dual delivery of natamycin and clotrimazole. Front Bioeng Biotechnol 9:1–13. https://doi.org/10.3389/fbioe.2021.635485

Danesi P, Falcaro C, Schmertmann LJ et al (2021) Cryptococcus in wildlife and free-living mammals. J Fungi 7:29. https://doi.org/10.3390/jof7010029

Dantas KC, Mauad T, de André CDS et al (2021) A single-centre, retrospective study of the incidence of invasive fungal infections during 85 years of autopsy service in Brazil. Sci Rep 11:3943. https://doi.org/10.1038/s41598-021-83587-1

das Neves J, Amiji MM, Bahia MF, Sarmento B (2010) Nanotechnology-based systems for the treatment and prevention of HIV/AIDS. Adv Drug Deliv Rev 62:458–477. https://doi.org/10.1016/j.addr.2009.11.017

De Melo Teixeira M, Theodoro RC, Freire F et al (2014) Paracoccidioides lutzii sp. nov.: biological and clinical implications. Med Mycol 52:19–28. https://doi.org/10.3109/13693786.2013.794311

De Serrano LO, Burkhart DJ (2017) Liposomal vaccine formulations as prophylactic agents: design considerations for modern vaccines. J Nanobiotechnol 15:1–23. https://doi.org/10.1186/s12951-017-0319-9

de Singulani JL, Scorzoni L, Lourencetti NMS et al (2018) Potential of the association of dodecyl gallate with nanostructured lipid system as a treatment for paracoccidioidomycosis: in vitro and in vivo efficacy and toxicity. Int J Pharm 547:630–636. https://doi.org/10.1016/j.ijpharm.2018.06.013

de Sousa HR, de Frazão S, de Oliveira Júnior GP et al (2021) Cryptococcal virulence in humans: learning from translational studies with clinical isolates. Front Cell Infect Microbiol 11:1–8. https://doi.org/10.3389/fcimb.2021.657502

Degreef H (2008) Clinical forms of dermatophytosis (ringworm infection). Mycopathologia 166: 257–265. https://doi.org/10.1007/s11046-008-9101-8

do Carmo Silva L, Miranda MACM, de Freitas JV et al (2020) Antifungal activity of Copaíba resin oil in solution and nanoemulsion against Paracoccidioides spp. Braz J Microbiol 51:125–134. https://doi.org/10.1007/s42770-019-00201-3

Enoch DA, Ludlam HA, Brown NM (2006) Invasive fungal infections: a review of epidemiology and management options. J Med Microbiol 55:809–818. https://doi.org/10.1099/jmm.0.46548-0

Enoch DA, Yang H, Aliyu SH, Micallef C (2017) The changing epidemiology of invasive fungal infections. In: Pediatric research. Springer, New York, pp 17–65

Etheridge ML, Campbell SA, Erdman AG et al (2013) The big picture on nanomedicine: the state of investigational and approved nanomedicine products. Nanomedicine 9:1–14. https://doi.org/10.1016/j.nano.2012.05.013

Fernandes Costa A, Evangelista Araujo D, Santos Cabral M et al (2019) Development, characterization, and in vitro–in vivo evaluation of polymeric nanoparticles containing miconazole and farnesol for treatment of vulvovaginal candidiasis. Med Mycol 57:52–62. https://doi.org/10.1093/mmy/myx155

Firacative C (2020) Invasive fungal disease in humans: are we aware of the real impact? Mem Inst Oswaldo Cruz 115:1–9. https://doi.org/10.1590/0074-02760200430

Fisher MC, Gow NAR, Gurr SJ (2016) Tackling emerging fungal threats to animal health, food security and ecosystem resilience. Philos Trans R Soc B Biol Sci 371:20160332. https://doi.org/10.1098/rstb.2016.0332

Fisher MC, Gurr SJ, Cuomo CA et al (2020) Threats posed by the fungal kingdom to humans, wildlife, and agriculture. MBio 11:1–17. https://doi.org/10.1128/mBio.00449-20

Garcia-Fuentes M, Alonso MJ (2012) Chitosan-based drug nanocarriers: where do we stand? J Control Release 161:496–504. https://doi.org/10.1016/j.jconrel.2012.03.017

Garg A, Singh S (2011) Enhancement in antifungal activity of eugenol in immunosuppressed rats through lipid nanocarriers. Colloids Surf B Biointerfaces 87:280–288. https://doi.org/10.1016/j.colsurfb.2011.05.030

Gegembauer G, Araujo LM, Pereira EF et al (2014) Serology of paracoccidioidomycosis due to Paracoccidioides lutzii. PLoS Negl Trop Dis 8:e2986. https://doi.org/10.1371/journal.pntd.0002986

Giri S, Kindo A (2012) A review of Candida species causing blood stream infection. Indian J Med Microbiol 30:270–278. https://doi.org/10.4103/0255-0857.99484

Govindrao Jamkhande P, Ghule NW, Haque Bamer A, Kalaskar MG (2019) Metal nanoparticles synthesis: an overview on methods of preparation, advantages and disadvantages, and applications. J Drug Deliv Sci Technol. https://doi.org/10.1016/j.jddst.2019.101174

Gregory AE, Titball R, Williamson D (2013) Vaccine delivery using nanoparticles. Front Cell Infect Microbiol 3:13. https://doi.org/10.3389/fcimb.2013.00013

Gubbins PO, Anaissie EJ (2009) Antifungal therapy. In: Clinical mycology. Elsevier, Amsterdam, pp 161–195

Gupta M, Vyas SP (2012) Development, characterization and in vivo assessment of effective lipidic nanoparticles for dermal delivery of fluconazole against cutaneous candidiasis. Chem Phys Lipids 165:454–461. https://doi.org/10.1016/j.chemphyslip.2012.01.006

Gushiken AC, Saharia KK, Baddley JW (2021) Cryptococcosis. Infect Dis Clin N Am 35:493–514. https://doi.org/10.1016/j.idc.2021.03.012

Jain S, Jain S, Khare P et al (2010) Design and development of solid lipid nanoparticles for topical delivery of an anti-fungal agent. Drug Deliv 17:443–451. https://doi.org/10.3109/10717544.2010.483252

Jannuzzi GP, de Araujo Souza N, Françoso KS et al (2018) Therapeutic treatment with scFv–PLGA nanoparticles decreases pulmonary fungal load in a murine model of paracoccidioidomycosis. Microbes Infect 20:48–56. https://doi.org/10.1016/j.micinf.2017.09.003

Kaur R, Dennison SR, Burrow AJ et al (2021) Nebulised surface-active hybrid nanoparticles of voriconazole for pulmonary Aspergillosis demonstrate clathrin-mediated cellular uptake, improved antifungal efficacy and lung retention. J Nanobiotechnol 19:19. https://doi.org/10.1186/s12951-020-00731-1

Khatun F, Toth I, Stephenson RJ (2020) Immunology of carbohydrate-based vaccines. Adv Drug Deliv Rev 165–166:117–126. https://doi.org/10.1016/j.addr.2020.04.006

Kohler JR, Casadevall A, Perfect J (2015) The spectrum of fungi that infects humans. Cold Spring Harb Perspect Med 5:a019273. https://doi.org/10.1101/cshperspect.a019273

Kosmidis C, Denning DW (2015) The clinical spectrum of pulmonary aspergillosis. Thorax 70:270–277. https://doi.org/10.1136/thoraxjnl-2014-206291

Kousha M, Tadi R, Soubani AO (2011) Pulmonary aspergillosis: a clinical review. Eur Respir Rev 20:156–174. https://doi.org/10.1183/09059180.00001011

Kuttel MM, Casadevall A, Oscarson S (2020) Cryptococcus neoformans capsular GXM conformation and epitope presentation: a molecular modelling study. Molecules 25:2651. https://doi.org/10.3390/molecules25112651

Lambros MP, Schafer F, Blackstock R, Murphy JW (1998) Liposomes, a potential immunoadjuvant and carrier for a cryptococcal vaccine. J Pharm Sci 87:1144–1148. https://doi.org/10.1021/js9704184

Laniosz V, Wetter D (2014) What's new in the treatment and diagnosis of dermatophytosis? Semin Cutan Med Surg 33:136–139. https://doi.org/10.12788/j.sder.0110

Li L, Wang X, Zhang J-Y et al (2018) Antifungal activity of osthol in vitro and enhancement in vivo through Eudragit S100 nanocarriers. Virulence 9:555–562. https://doi.org/10.1080/21505594.2017.1356503

Lionakis MS, Kontoyiannis DP (2003) Glucocorticoids and invasive fungal infections. Lancet 362:1828–1838. https://doi.org/10.1016/S0140-6736(03)14904-5

Lu R, Hollingsworth C, Qiu J et al (2019) Efficacy of oral encochleated amphotericin B in a mouse model of cryptococcal meningoencephalitis. MBio 10:e00724–e00719. https://doi.org/10.1128/mBio.00724-19

Marinescu L, Ficai D, Oprea O et al (2020) Optimized synthesis approaches of metal nanoparticles with antimicrobial applications. J Nanomater. https://doi.org/10.1155/2020/6651207

May RC, Stone NRH, Wiesner DL et al (2016) Cryptococcus: from environmental saprophyte to global pathogen. Nat Rev Microbiol 14:106–117. https://doi.org/10.1038/nrmicro.2015.6

McManus DS (2015) Antifungal drugs. In: Side effects of drugs annual, 1st edn. Elsevier B.V, Amsterdam, pp 307–319

Medina-Alarcón KP, de Singulani JL, Dutra LA et al (2020) Antifungal activity of 2-′-hydroxychalcone loaded in nanoemulsion against Paracoccidioides spp. Future Microbiol 15:21–33. https://doi.org/10.2217/fmb-2019-0095

Melkoumov A, Goupil M, Louhichi F et al (2013) Nystatin nanosizing enhances in vitro and in vivo antifungal activity against Candida albicans. J Antimicrob Chemother 68:2099–2105. https://doi.org/10.1093/jac/dkt137

Mirza MA, Ahmad S, Mallick MN et al (2013) Development of a novel synergistic thermosensitive gel for vaginal candidiasis: an in vitro, in vivo evaluation. Colloids Surf B Biointerfaces 103:275–282. https://doi.org/10.1016/j.colsurfb.2012.10.038

Mody V, Siwale R, Singh A, Mody H (2010) Introduction to metallic nanoparticles. J Pharm Bioallied Sci 2:282. https://doi.org/10.4103/0975-7406.72127

Montoya MC, Magwene PM, Perfect JR (2021) Associations between Cryptococcus genotypes, phenotypes, and clinical parameters of human disease: a review. J Fungi 7:260. https://doi.org/10.3390/jof7040260

Nazarian S, Gargari SLM, Rasooli I et al (2014) A PLGA-encapsulated chimeric protein protects against adherence and toxicity of enterotoxigenic Escherichia coli. Microbiol Res 169:205–212. https://doi.org/10.1016/j.micres.2013.06.005

Neves WW, Neves RP, Macêdo DPC et al (2020) Incorporation of 2-amino-thiophene derivative in nanoparticles: enhancement of antifungal activity. Braz J Microbiol 51:647–655. https://doi.org/10.1007/s42770-020-00248-7

Oliveira NK, Frank LA, Squizani ED et al (2021) New nanotechnological formulation based on amiodarone-loaded lipid core nanocapsules displays anticryptococcal effect. Eur J Pharm Sci 162:105816. https://doi.org/10.1016/j.ejps.2021.105816

Palmeiro M, Cherubini K, Yurgel LS (2005) Paracoccidioidomicose – Revisão da Literatura. Sci Medica 15:274–278

Pappas PG, Lionakis MS, Arendrup MC et al (2018) Invasive candidiasis. Nat Rev Dis Prim 4: 18026. https://doi.org/10.1038/nrdp.2018.26

Park S-C, Kim Y-M, Lee J-K et al (2017) Targeting and synergistic action of an antifungal peptide in an antibiotic drug-delivery system. J Control Release 256:46–55. https://doi.org/10.1016/j.jconrel.2017.04.023

Peek LJ, Middaugh CR, Berkland C (2008) Nanotechnology in vaccine delivery. Adv Drug Deliv Rev 60:915–928. https://doi.org/10.1016/j.addr.2007.05.017

Pires CAA, da Cruz NFS, Lobato AM et al (2014) Clinical, epidemiological, and therapeutic profile of dermatophytosis. An Bras Dermatol 89:259–264. https://doi.org/10.1590/abd1806-4841.20142569

Quindós G (2014) Epidemiology of candidaemia and invasive candidiasis. A changing face. Rev Iberoam Micol 31:42–48. https://doi.org/10.1016/j.riam.2013.10.001

Ren T, Xu N, Cao C et al (2009) Preparation and therapeutic efficacy of polysorbate-80-coated amphotericin B/PLA-b-PEG nanoparticles. J Biomater Sci Polym Ed 20:1369–1380. https://doi.org/10.1163/092050609X12457418779185

Rençber S, Karavana SY, Yılmaz FF et al (2016) Development, characterization, and in vivo assessment of mucoadhesive nanoparticles containing fluconazole for the local treatment of oral candidiasis. Int J Nanomedicine 11:2641. https://doi.org/10.2147/IJN.S103762

Restrepo A, Gómez BL, Tobón A (2012) Paracoccidioidomycosis: Latin America's own fungal disorder. Curr Fungal Infect Rep 6:303–311. https://doi.org/10.1007/s12281-012-0114-x

Riaz A, Hendricks S, Elbrink K et al (2020) Preparation and characterization of nanostructured lipid carriers for improved topical drug delivery: evaluation in cutaneous Leishmaniasis and vaginal candidiasis animal models. AAPS PharmSciTech 21:185. https://doi.org/10.1208/s12249-020-01717-w

Rodrigues Dos Santos Junior S, Lopes K, da Silva F, Santos Dias L et al (2020) Intranasal vaccine using P10 peptide complexed within chitosan polymeric nanoparticles as experimental therapy for paracoccidioidomycosis in murine model. J Fungi 6:160. https://doi.org/10.3390/jof6030160

Saldanha CA, Garcia MP, Iocca DC et al (2016) Antifungal activity of amphotericin B conjugated to nanosized magnetite in the treatment of paracoccidioidomycosis. PLoS Negl Trop Dis 10: e0004754. https://doi.org/10.1371/journal.pntd.0004754

Sanguinetti M, Posteraro B, Beigelman-Aubry C et al (2019) Diagnosis and treatment of invasive fungal infections: looking ahead. J Antimicrob Chemother 74:ii27–ii37. https://doi.org/10.1093/jac/dkz041

Santangelo R, Paderu P, Delmas G et al (2000) Efficacy of oral cochleate-amphotericin B in a mouse model of systemic candidiasis. Antimicrob Agents Chemother 44:2356–2360. https://doi.org/10.1128/AAC.44.9.2356-2360.2000

Schmiedel Y, Zimmerli S (2016) Common invasive fungal diseases: an overview of invasive candidiasis, aspergillosis, cryptococcosis, and Pneumocystis pneumonia. Swiss Med Wkly 146:w14281. https://doi.org/10.4414/smw.2016.14281

Schröfel A, Kratošová G, Šafařík I et al (2014) Applications of biosynthesized metallic nanoparticles-a review. Acta Biomater. https://doi.org/10.1016/j.actbio.2014.05.022

Scorzoni L, de Paula e Silva AC, Marcos CM et al (2017) Antifungal therapy: new advances in the understanding and treatment of mycosis. Front Microbiol 08:1–23. https://doi.org/10.3389/fmicb.2017.00036

Sharma R, Agrawal U, Mody N, Vyas SP (2015) Polymer nanotechnology based approaches in mucosal vaccine delivery: challenges and opportunities. Biotechnol Adv 33:64–79. https://doi.org/10.1016/j.biotechadv.2014.12.004

Shikanai-Yasuda MA, de Queiroz F, Filho T et al (2006) Consenso em paracoccidioidomicose Guideliness in paracoccidioidomycosis. Rev Soc Bras Med Trop 39:297–310

Shikanai-Yasuda MA, Mendes RP, Colombo AL et al (2017) Brazilian guidelines for the clinical management of paracoccidioidomycosis. Rev Soc Bras Med Trop 50:715–740. https://doi.org/10.1590/0037-8682-0230-2017

Shirkhani K, Teo I, Armstrong-James D, Shaunak S (2015) Nebulised amphotericin B-polymethacrylic acid nanoparticle prophylaxis prevents invasive aspergillosis. Nanomed Nanotechnol Biol Med 11:1217–1226. https://doi.org/10.1016/j.nano.2015.02.012

Shoham S, Groll AH, Walsh TJ (2010) Antifungal agents. In: Infectious diseases, 3rd edn. Elsevier, Amsterdam, pp 1477–1489

Singh DK, Tóth R, Gácser A (2020) Mechanisms of pathogenic Candida species to evade the host complement attack. Front Cell Infect Microbiol 10:94. https://doi.org/10.3389/fcimb.2020.00094

Soriano-Ruiz JL, Calpena-Capmany AC, Cañadas-Enrich C et al (2019) Biopharmaceutical profile of a clotrimazole nanoemulsion: evaluation on skin and mucosae as anticandidal agent. Int J Pharm 554:105–115. https://doi.org/10.1016/j.ijpharm.2018.11.002

Souza ACO, Nascimento AL, de Vasconcelos NM et al (2015) Activity and in vivo tracking of Amphotericin B loaded PLGA nanoparticles. Eur J Med Chem 95:267–276. https://doi.org/10.1016/j.ejmech.2015.03.022

Spadari CC, da Silva de Bastiani FWM, Lopes LB, Ishida K (2019) Alginate nanoparticles as non-toxic delivery system for miltefosine in the treatment of candidiasis and cryptococcosis. Int J Nanomedicine 14:5187–5199. https://doi.org/10.2147/IJN.S205350

Srikanta D, Santiago-Tirado FH, Doering TL (2014) Cryptococcus neoformans: historical curiosity to modern pathogen. Yeast 31:47–60. https://doi.org/10.1002/yea.2997

Staab JF, Wong B (2014) Fungal infections, systemic. In: Reference module in biomedical sciences, 4th edn. Elsevier, Amsterdam, pp 341–361

Streinu-Cercel A (2012) Invasive fungal infections. GERMS 2:35. https://doi.org/10.11599/germs.2012.1011

Taborda CP, Buccheri R, Benard G et al (2018) Paracoccidioides spp. and Histoplasma capsulatum: current and new perspectives for diagnosis and treatment. Curr Top Med Chem 18:1333–1348. https://doi.org/10.2174/1568026618666181002112231

Taborda CP, Travassos LR, Benard G (2021) Paracoccidioidomycosis. In: Encyclopedia of mycology. Elsevier, Amsterdam, pp 654–675

Tang X, Zhu H, Sun L et al (2014) Enhanced antifungal effects of amphotericin B-TPGS-b-(PCL-ran-PGA) nanoparticles in vitro and in vivo. Int J Nanomedicine 9:5403. https://doi.org/10.2147/IJN.S71623

Thompson G, Patterson T (2011) Pulmonary aspergillosis: recent advances. Semin Respir Crit Care Med 32:673–681. https://doi.org/10.1055/s-0031-1295715

Thorley AJ, Tetley TD (2013) New perspectives in nanomedicine. Pharmacol Ther 140:176–185. https://doi.org/10.1016/j.pharmthera.2013.06.008

Travassos LR, Taborda CP (2012) Paracoccidioidomycosis vaccine. Hum Vaccine Immunother 8:1450–1453. https://doi.org/10.4161/hv.21283

Turissini DA, Gomez OM, Teixeira MM et al (2017) Species boundaries in the human pathogen *Paracoccidioides*. Fungal Genet Biol 106:9–25. https://doi.org/10.1016/j.fgb.2017.05.007

Vaghasiya H, Kumar A, Sawant K (2013) Development of solid lipid nanoparticles based controlled release system for topical delivery of terbinafine hydrochloride. Eur J Pharm Sci 49:311–322. https://doi.org/10.1016/j.ejps.2013.03.013

Van de Ven H, Paulussen C, Feijens PB et al (2012) PLGA nanoparticles and nanosuspensions with amphotericin B: potent in vitro and in vivo alternatives to Fungizone and AmBisome. J Control Release 161:795–803. https://doi.org/10.1016/j.jconrel.2012.05.037

Vermout S, Tabart J, Baldo A et al (2008) Pathogenesis of dermatophytosis. Mycopathologia 166: 267–275. https://doi.org/10.1007/s11046-008-9104-5

Vidal Bonifácio B, dos Santos Ramos MA, Silva P et al (2015) Nanostructured lipid system as a strategy to improve the anti-Candida albicans activity of Astronium sp. Int J Nanomedicine 10: 5081. https://doi.org/10.2147/IJN.S79684

Wang P (2021) Genetic transformation in Cryptococcus species. J Fungi 7:56. https://doi.org/10.3390/jof7010056

Wang H, Xu K, Liu L et al (2010) The efficacy of self-assembled cationic antimicrobial peptide nanoparticles against Cryptococcus neoformans for the treatment of meningitis. Biomaterials 31:2874–2881. https://doi.org/10.1016/j.biomaterials.2009.12.042

Xu N, Julin G, Yuanjie Z et al (2011a) Efficacy of intravenous amphotericin B-polybutylcyanoacrylate nanoparticles against cryptococcal meningitis in mice. Int J Nanomedicine 6:905. https://doi.org/10.2147/IJN.S17503

Xu K, Wang H, Liu L et al (2011b) Efficacy of CG3R6TAT nanoparticles self-assembled from a novel antimicrobial peptide for the treatment of Candida albicans meningitis in rabbits. Chemotherapy 57:417–425. https://doi.org/10.1159/000330855

Xu P, Yang Z, Chen M et al (2014) Evaluating the potential of cubosomal nanoparticles for oral delivery of amphotericin B in treating fungal infection. Int J Nanomedicine 9:327. https://doi.org/10.2147/IJN.S54967

Yan L, Yang Y, Zhang W, Chen X (2014) Advanced materials and nanotechnology for drug delivery. Adv Mater. https://doi.org/10.1002/adma.201305683

Zaragoza O, Rodrigues ML, De Jesus M et al (2009) Chapter 4 The capsule of the fungal pathogen Cryptococcus neoformans. In: Advances in applied microbiology, 1st edn. Elsevier Inc., Amsterdam, pp 133–216

Zhang C, Chen M, Wang G et al (2016) Pd@Ag Nanosheets in combination with amphotericin B exert a potent anti-cryptococcal fungicidal effect. PLoS One 11:e0157000. https://doi.org/10.1371/journal.pone.0157000

Zhang P, Yang X, He Y et al (2017) Preparation, characterization and toxicity evaluation of amphotericin B loaded MPEG-PCL micelles and its application for buccal tablets. Appl Microbiol Biotechnol 101:7357–7370. https://doi.org/10.1007/s00253-017-8463-6

Zhao K, Zhang Y, Zhang X et al (2014) Chitosan-coated poly(lactic-co-glycolic) acid nanoparticles as an efficient delivery system for Newcastle disease virus DNA vaccine. Int J Nanomedicine 9: 4609–4619. https://doi.org/10.2147/IJN.S70633

Zmeili OS, Soubani AO (2007) Pulmonary aspergillosis: a clinical update. QJM 100:317–334. https://doi.org/10.1093/qjmed/hcm035

Nanomaterials in the Diagnosis and Treatment of Leishmaniasis

8

Fayyaz Rasool, Shaheer Hasan Khan, Abdulaziz S. Alouffi, Sri Krishna Jayadev Magani, and Abdur Rub

Abstract

Leishmaniasis is caused by protozoan parasite, *Leishmania* spp. It has been classified as one of the major neglected tropical diseases. More than one million new cases of leishmaniasis and 20,000 to 30,000 deaths are reported annually throughout the world. Parasite exploits the host-immune defence system through different ways for its survival and progression of the disease. The currently available diagnostic procedures for leishmaniasis are tedious, painful and less sensitive. In addition to this the available drugs against it have the problem of toxicity, resistance and high cost. Nonetheless, with the up-coming trends and current advances in nanotechnology, it has become very much possible to develop highly sensitive, rapid, non-invasive and less expensive diagnostic procedures for early detection as well as effective and cheap treatment for

F. Rasool
Infection and Immunity Lab, Department of Biotechnology, Jamia Millia Islamia (A Central University), New Delhi, India

Department of Life Sciences, School of Natural Sciences, Shiv Nadar University, Noida, Uttar Pradesh, India

S. H. Khan · A. Rub (✉)
Infection and Immunity Lab, Department of Biotechnology, Jamia Millia Islamia (A Central University), New Delhi, India
e-mail: arub@jmi.ac.in

A. S. Alouffi
King Abdulaziz City for Science and Technology, Riyadh, Saudi Arabia

S. K. J. Magani
Department of Life Sciences, School of Natural Sciences, Shiv Nadar University, Noida, Uttar Pradesh, India

S. Hameed, S. Rehman (eds.), *Nanotechnology for Infectious Diseases*,
https://doi.org/10.1007/978-981-16-9190-4_8

infectious diseases including leishmaniasis. Here, we discussed the recent developments in nanomedicine for the diagnosis and treatment of leishmaniasis.

Keywords

Nanoparticles · Leishmaniasis · Nanomedicine · Nanovaccines · Drug delivery

Abbreviations

AmB	Amphotericin B
DDS	Drug delivery system
NPs	Nanoparticle
PCR	Polymerase chain reaction
VL	Visceral Leishmaniasis

8.1 Introduction

Leishmaniasis is a group of diseases with a wide range of clinical manifestations that ranges from self-healing cutaneous ulcers to severe form of visceral disease and sometimes it can even be fatal (Torres-Guerrero et al. 2017). Leishmaniasis counts on number three in the list of challenging vector borne diseases. It is a tropical disease which is neglected by pharmaceutical industries due to its more prevalence in poor population. According to world health organization (WHO), leishmaniasis is reported in nearly 100 countries however it is rarely observed in Polar as well as Australian sub-continent. It is considered under neglected tropical diseases and prevalent in tropical, sub-tropical and temperate regions (Pace 2014; Burza et al. 2018; Leishmaniasis 2021). About 0.2–0.4 million new cases of visceral leishmaniasis (VL) are reported per year across the world. Most of the cases are seen in India, Bangladesh, Sudan and South America. Globally about 0.7–1.2 million new cases of cutaneous leishmaniasis are annually registered, 85% of which are seen in Asia, South America (especially Brazil and Peru), South Africa, Sudan, Algeria and Costa Rica (Burza et al. 2018; Georgiadou et al. 2015). Ten percent of new cases of Post kala azar dermal leishmaniasis is reported in Indian Sub-continent and 50% of it are reported in East Africa (Georgiadou et al. 2015).

Leishmania spp. are the members of digenetic parasites which complete their life cycle in two different hosts. First in the sand fly, that acts as a primary invertebrate host vector and second the mammals that acts as a secondary vertebrate host. It exists as a flagellated, motile promastigote form in the alimentary canal (midgut) of primary host, i.e., sand fly. It is the infective stage that is meta cyclic promastigote form, that gets transmitted to mammalian host during bite, whereas it exists in a non-motile amastigote form within the phagolysosomes of the mammalian macrophages (Späth et al. 2003) (Fig. 8.1).

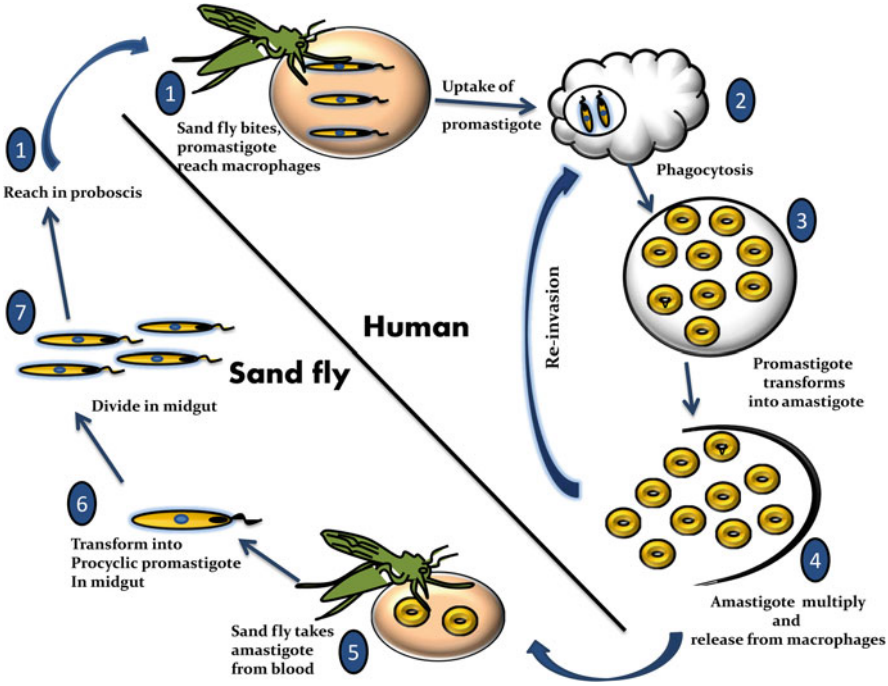

Fig. 8.1 Life cycle of *Leishmania* spp. Sandfly bite results in transfer of promastigote to blood circulation and finally macrophages, where it gets transformed into amastigote and multiply. When macrophage ruptures amastigote either infect other cell or reaches in sandfly on its bite. Amastigotes transform to promastigote and then divide in midgut, finally reaches proboscis and then to human host in the form of metacyclic promastigote

The battle between the host and the parasite went on, from the bite of sandfly to the final internalization inside macrophage. Occurrence of disease depends on the ability of parasites to evade or get eradicated by the host-immune system. All the components of host-immune defence system, i.e., complement system, chemokines and cytokines, neutrophils, NK cells, etc. which act against parasite get failed to complete their work successfully (Gupta et al. 2013). Parasites evade or exploit host-immune mechanism in various ways which help it to persist in the host cell. It modifies host cell signalling pathways, phagocytic cells entry, and chemokine and cytokine secretion which lead to the alteration of immune cell activation and trafficking at the site of infection (Gupta et al. 2013). There are various treatment options till date but all have one or more drawback like high cost, resistance, toxicity, etc. Thus, leishmaniasis is worldwide problem and recent therapeutic approaches have failed to provide effective cure.

8.2 Available Chemotherapeutic Drugs for Leishmaniasis

8.2.1 Pentavalent Antimonial

Primarily used antimonial as a drug was trivalent antimonials (Sb III), but later on pentavalent antimonials (SbV) were found effective for VL (Kala azar). Meglumine antimoniate (Glucantime) and sodium stibogluconate (Pentostam) are pentavalent antimonials which are currently in Practice (Ouellette et al. 2004). To act against on parasite amastigotes, SbV must have to cross the host cell and penetrate the phagolysosomal membrane. For SbV to be active it must highly likely to be transformed into trivalent (SbIII) form (Fig. 8.1). It is seen that amastigote parasite not a promastigote reduces SbV to SbIII (Shaked-Mishan et al. 2001), other study recommended that SbV activation occurs in macrophage not in leishmania parasite (Roberts and Rainey 1993). Possibly the activation may occur in both ways and also many other mechanisms might be responsible for it. Thus, for a parasite to become resistant one of the means could be loss of reduction/activation. SbV eradicates the parasite in both direct and indirect way. It activates the macrophages to destroy the intracellular parasite when present in infected macrophage, but by reaching the intracellular parasite, SbV gets activated to SbIII and kills it directly by restraining trypanothione reductase (Krauth-Siegel and Comini 2008).

8.2.2 Pentamidine

Aromatic diamidines are first used in African trypanosomiasis treatment and in 1939 its activity against leishmania infection is seen (Dietze et al. 2001). It was used as second line of drug in SbV resistant patient but it has side effects like low efficacy and high toxicity (hypotension, cardiac, gastrointestinal, diabetes mellitus), additional use of this drug is abandoned (Das et al. 2001). Its mechanism of action was also least studied. Polyamine and arginine transporters are helpful in pentamidine entry inside the cell (Basselin et al. 2000, 2002), however entry of pentamidine inside a parasite takes place by transporter (triangle) not yet identified. Mitochondria of pentamidine resistant parasite expels the drug out of the cell by ABC transporter (Coelho et al. 2003), however mitochondria of sensitive parasite accumulates the drug in it (Basselin et al. 2002), enhancing the effectiveness of Complex II respiratory chain inhibitor of mitochondria. It also acts on parasite DNA thus obstruct the transcription and replication at mitochondrial level (Mishra et al. 2007) and also inhibits active transport system (No 2016) (Fig. 8.2).

8.2.3 Amphotericin B

Amphotericin B, an important antifungal drug was recommended in the fungal infection. Primarily Amphotericin B was proposed for treatment of Sbv resistance patient in India (Thakur et al. 1993; Jha et al. 1995). At present, it is used to treat VL

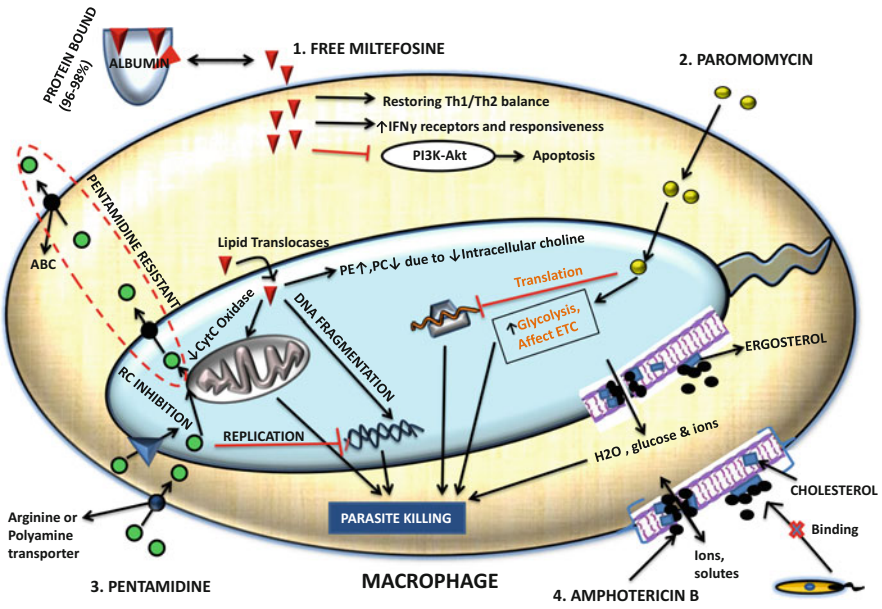

Fig. 8.2 Mechanism of action of different chemotherapeutic antileishmanial drugs

as a first line drug, due to continuous mounting unresponsiveness of SbV in endemic areas. AmB intermingles favourably with ergosterol, fungal membrane sterol and macrophage membrane cholesterol. Major membrane sterol present in *Leishmania* spp. is ergostane-based sterol (Goad et al. 1984), thus AmB is highly effective against *Leishmania* spp. AmB is inactive against organism lacking sterol in their cell membrane. Sodium deoxycholate, a formulation of AmB is initial parental preparation of AmB commercially available as Fungisome (Bristol-Myers-Squibb). Cell membrane cholesterol is essential for host–pathogen interaction, leading to parasite entry to the cell. Studies showed that when cholesterol depletion by methyl β-cyclodextrin (MβCD) treatment reduced *Leishmania* spp. infection and parasite load (Pucadyil et al. 2004; Rub et al. 2009), thus cholesterol is important for the parasite to infect as well as to survive inside the host. However, AmB interacts with host-membrane cholesterol and sequester it also, thus reduces the chance of parasite to infect macrophages. AmB is said to be a membrane active drug which also spans in parasite lipid bilayer with ergosterol and forms a pore (No 2016; Rub et al. 2009). These pores lead to cell death of parasites by the movement of ions and small solute out of it (No 2016; Rub et al. 2009) (Fig. 8.2). To avoid the drawback associated with Amphotericin B, new original polyene lipid-associated formulation, liposomal Amphotericin B has been introduced (Rub et al. 2013). Toxicity with AmB is due to its attachment to mammalian cells (kidney, erythrocytes, etc.) through sterol (Chattopadhyay and Jafurulla 2011), which is less in liposomal AmB. Major limitation of these formulations are high cost and least accessible to the rural patient (Sundar et al. 2010).

8.2.4 Miltefosine

Miltefosine or hexadecylphosphocholine is an alkyl phospholipid primarily build as an anti-tumour drug. Initially, miltefosine was known to be active in vitro against L. donovani (Croft et al. 1987). Later, it was shown highly effective in phase III trial against VL (Sundar and Rai 2002) alters the phospholipids biosynthesis and alkyl lipid metabolism though detailed mode of its action is not known yet (Lux et al. 2000). It is suggested that miltefosine provokes the parasite death through apoptosis (Paris et al. 2004). However, pathway related to apoptosis is not understood exactly (Zangger et al. 2002; Debrabant et al. 2003). Miltefosine increases IFN-γ response which helps in parasite killing. It also maintains Th1/Th2 balance thus maintaining cytotoxicity and parasite killing simultaneously (Zangger et al. 2002; Debrabant et al. 2003). Miltefosine attacks mitochondria and DNA of parasite to induce DNA fragmentation and killing of parasites (Fig. 8.2).

8.2.5 Paromomycin

Paromomycin binds with 30s ribosomal subunit, thus interferes with protein synthesis initiation. It fixes 30s–50s ribosomal complex at start codon of mRNA, thus abnormal initiation complex is assembled (Jha et al. 2005). In paromomycin resistant *L. donovani*, ribosomal protein was observed to be upregulated, which suggested protein synthesis inhibition as the site of paromomycin (No 2016). Paromomycin also affects glycolytic enzyme and respiratory chain of parasites (No 2016) (Fig. 8.2). Several side effects of paromomycin are already reported which restricted its usage (Dietze et al. 2001; Sherwood et al. 1994).

8.2.6 Sitamaquine

Sitamaquine 12 or 8-aminoquinoline analogue is another oral drug known as WR 6026, build up by Walter Reed Army Institute of Research (USA) in collaboration with GlaxoSmithKline (UK). India, Kenya and Brazil are countries in which complete clinical trials were done (Dietze et al. 2001; Sherwood et al. 1994; Wasunna et al. 2005) with cure rate ranging from 27% to 87%. Encouraging results were observed in animals against VL after 28 days of treatment (Dietze et al. 2001). Again due to several side effects its use was restricted (Carvalho et al. 2011).

8.2.7 Other New Antileishmanial Molecules

Combination therapy is newly introduced to overcome the side effects present in various drugs like high treatment time, high cost, resistant emergence, low efficacy rate, etc. (Olliaro et al. 2005). It also showed effective results in complicated conditions like HIV co-infection. Ideal combination drugs must show synergistic

and additive effect, i.e., paromomycin in combination with sodium stibogluconate is widely used in Sudan for VL treatment in 17 days (Melaku and Collin 2007). Knowledge of physiochemical and structural properties of antileishmanial compounds and their target sites along with advancement in molecular and computational biology led to the development of synthetic drugs with high efficacy (Liñares et al. 2006). Several specific inhibitors were designed which showed promising results in controlling the growth of parasites without harming the host (Santos et al. 2008). Mitochondria (Sen and Majumder 2008), kinetoplast (Motta 2008), topoisomerase (Das et al. 2008), cysteine protease (McKerrow et al. 1999), trypanothione reductase (Krauth-Siegel et al. 2003), fatty acid and sterol pathway (Krauth-Siegel et al. 2003; Majumder et al. 2012; Rub et al. 2013) are some of the potential targets which were exploited to develop the new drugs to treat leishmaniasis (Rana et al. 2014; Arish et al. 2015, 2016). Advantages of synthetic compounds are low cost, low treatment time, novelty and scale up, low resistance and low intellectual property complication (Newman 2008). Plant and plant products are potential source of antileishmanial compounds (Kashif et al. 2018; Tabrez et al. 2021; Rahman et al. 2021; Ali et al. 2021). Drug Discovery Research program and Tropical disease program (TDR/WHO) made precedence to the pharmacological investigation of plants (Chan-Bacab and Peña-Rodríguez 2001). Recent reports with remarkable antileishmanial activity have already been reviewed (Chan-Bacab and Peña-Rodríguez 2001). All the above treatment options have one or other drawbacks like high cost, resistance, toxicity and less efficacy. Therefore, further researches in this direction are required.

8.3 Conventional Methods for Detection of Leishmaniasis

The convention methods available for the detection of the leishmaniasis are as follows:

1. Microscopic examination and culture: Microscopic examinations of the parasites are performed to detect the infection.
2. Leishmania skin test: Skin test is performed in the infected persons.
3. KAtex: Latex agglutination test for detection of antigen in urine (Riera et al. 2004).
4. Serological methods: It is indirect fluorescent antibody test (IFAT) in which specificity and sensitivity are usually defined on the basis of parasitism and disease rather than infection (Dye et al. 1993).
5. Species identification: Real time PCR of 18S rRNA is done to detect all *Leishmania* species but not only those specific to given *Leishmania* species (Foulet et al. 2007).

8.4 Nanomaterials in the Diagnosis of Leishmaniasis

There is an increasing demand of diagnosis of this disease with specificity and reliability that is lacking with traditional methods. Now with the advent of nanotechnology, there is a great hope in the development of effective, sensitive and cheap diagnostic methods (Fig. 8.3). Nanoparticles are blessed with high surface area to volume ratio and their ability to interact one-to-one with biomolecules due to the fact that they have some unique optical and physiochemical properties. Novel techniques are now available to detect even a small quantity of biological sample without any amplification or high cost equipment's which are probably the main demerits of polymerase chain reaction based diagnostic methods (Andreadou et al. 2014). Margarita and Readou et al. designed and functionalized gold nanoparticles (AuNPs), for the detection of *Leishmania* spp. in clinical samples (Andreadou et al. 2014). AuNPs were synthesized and conjugated with four oligonucleotides probes for targeting it to kinetoplastid minicircle DNA of parasites. It was found that in the absence of complementary DNA in the clinical samples, the probe coated AuNPs precipitated under acidic environment causing a change in colour from red to purple. While in case of complementation between sample DNA and probe coated AuNPs, colour remains red. Therefore, this method was highly sensitive and reproducible (Andreadou et al. 2014). It is intracellular parasite which resides mainly in liver and spleen. Hence, its detection is crucial in diagnosis and for proper treatment of disease. Determination of parasite by counting followed by Giemsa staining is classical existing method to study the parasite load. Another group of workers introduced rapid, non-PCR and non-cell culture based detection method using salt triggered aggregation of AuNPs in the presence of extracted total RNA from infected macrophages and specific oligo-nucleotide probe specific to particular *Leishmania* species (Bose and Kumar 2016). It was also used to determine the parasitic load in macrophages. In case of complementarity of probe with isolated RNA, hybridization

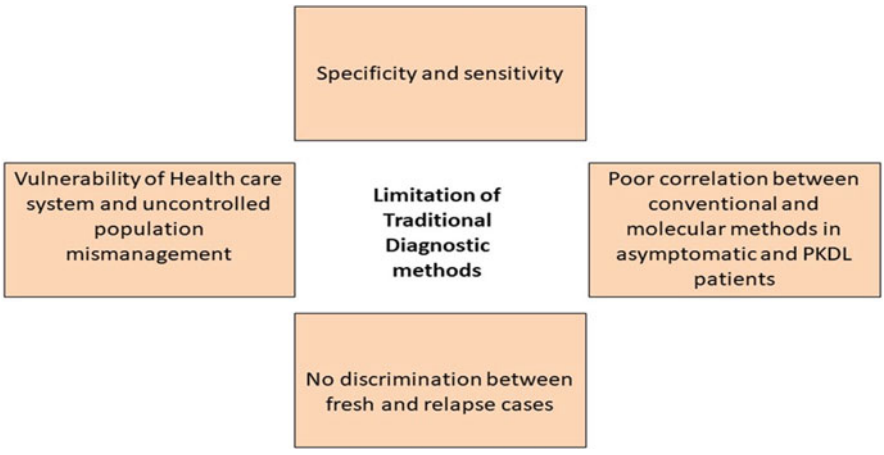

Fig. 8.3 Limitations of conventional diagnostic methods

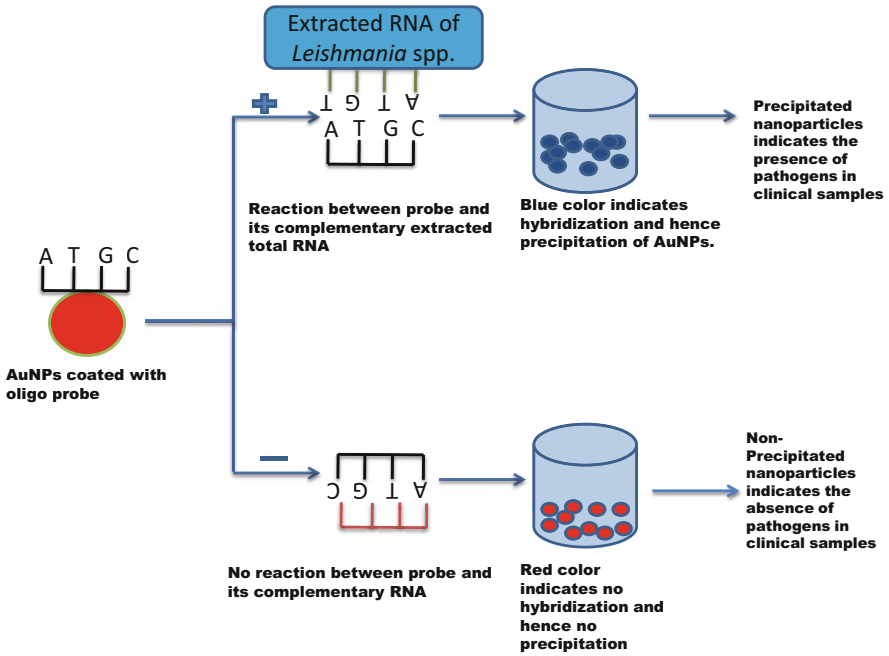

Fig. 8.4 Diagrammatic representation of gold nanoparticles (AuNPs) used for diagnosis of leishmaniasis

led to the formation of double strand which was unable to stabilize AuNPs from salt-triggered aggregation that resulted in production of blue color. This blue colour indicated the presence of amastigote RNA due to the fact that the probe was designed based on the characteristics of SSU rRNA. In absence of probe-RNA complementarity, however, AuNPs got stabilized by single stranded oligos restoring the red color. Hence, one can conclude that retention of red color is the indication of absence of amastigotes in total RNA extracted from macrophage (Bose and Kumar 2016) (Fig. 8.4).

In an another study by Welearegay et al., they functionalized copper nanoparticles (CuNPs) with organic ligand which was used to detect cutaneous leishmaniasis in human samples of expired breath (Welearegay et al. 2018). Volatile substances present in exhaled breath of a patient were probed on an array of smartly and specifically design chemical gaseous sensor. It was found that CuNPs with 2-mercaptobenzoxazole yielded 100% specificity, 100% sensitivity, and 100% accuracy in detecting cutaneous leishmaniasis (Welearegay et al. 2018). Many other studies in the development of nanoparticle based sensors are summarized in Table 8.1.

Table 8.1 Nanoparticle based diagnosis of leishmaniasis

Nanoparticle type	Modification of nanoparticle	Mechanism of diagnosis	References
Metallic NPs	Oligonucleotide functionalized gold nanoparticle (AuNPs)	Probe and complementary DNA binds to form a soluble complex or an aggregated complex if not bound.	Andreadou et al. (2014)
Metallic NPs	Salt triggered gold nanoparticles (AuNPs)	Double stranded was unable to stabilized AuNPs from salt triggered aggregation and in the absence of complementarity AuNPs was stabilized by ssRNA.	Bose and Kumar (2016)
Metallic NPs	2-Mercaptobenzoxazole functionalized copper nanoparticles (CuNPs)	Based on the analysis of volatile organic compound in exhaled breath of patient by an array of smartly and specifically designed chemical gas biosensors.	Welearegay et al. (2018)

8.5 Nanomaterials in the Therapy and Protection Against *Leishmania* spp. Infection

Due to special characteristics of nanoparticles like small size, customized surface, improved solubility, it has lots of potential to be used in the development of antileishmanial drugs as well as vaccines for the protection against *Leishmania* spp. infection.

8.5.1 Antileishmanial Nanoparticles and Nanopreparations

Despite all the good results, chemotherapeutic drugs have lots of cytotoxic effects due to requirement of higher dose for the elimination of parasites from the body. This problem was overcomed by nanotechnology which provided selective and preferential delivery of drugs of nanometer size. Targeted delivery of drugs through nanotechnology reduced several other problems like inadequate drug delivery, serious toxic effect and high cost. In nanomedicine the drug is entrapped in targeted carriers which enable the drug to act more efficiently with fewer side effects. In addition, nanomedicine control both biodistribution of drug and pharmacokinetics (Gupta et al. 2010). The main considerations before designing an ideal nanomedicine against leishmaniasis include (1) killing of parasites in the macrophages without harming healthy cells, (2) least loss of drug content and activity in reaching the target site through systemic circulation (Cho et al. 2008). To achieve this, active targeting shows better results rather than passive targeting. In passive targeting, antileishmanial drugs are entrapped in some colloids taken up by macrophages so that these colloidal vectors may take the drug to macrophages. However side effects of passive transport include (1) all drugs do not disperse efficiently, (2) random

delivery method makes it tough to control the process (Jain 1994). In active targeting, empathy ligands such as peptides, antibodies or small molecules are attached by covalent or conjugate linkage on the surface of nanocarriers. These empathy ligands interact with specific cell surface receptors to transfer the drugs with least loss of content, activity and least toxicity to healthy tissues. Different carriers used in nanotechnology for the development of drugs against leishmaniasis are liposomes (cholesterol vesicles), microspheres/nanospheres, microparticle/nanoparticle, emulsomes (solid fat nano emulsions) and noisome (non-ionic surfactant vesicles) (Gupta et al. 2016). Among these, liposomes were found as best due to the fact that the *Leishmania* spp. clears the liposomes within the macrophages itself (Gour 2009). Furthermore, liposomes with antileishmanial drugs are proved to reduce the toxicity of those drugs (Gupta et al. 2016). Gupta et al reviewed liposomes and microspheres to be more effectively used in this context (Date et al. 2007). As macrophage is the niche of several pathogens, its specific delivery system is very interesting field in nanomedicine nowadays. Surface of macrophages bears various receptors recognizing terminal mannose, glucose, galactose residues which enabled the sugar bearing liposomes to be act as good carrier of antileishmanial compounds (Date et al. 2007). In these sugar bearing liposomes, mannose-grafted liposomal form is more valuable (Das et al. 1990). Lipid nanospheres of AmB, one mannose coated and another mannose uncoated were developed (Veerareddy et al. 2004). It showed 94–95% reduction in parasite burden in spleen and liver, respectively. Immunoliposome (antibody coupled to liposomes) is another approach in drug delivery, in which antileishmanial activity of IgG coupled liposomes had increased to two- to threefold than free IgG (Shinjini Singh 2008). Several groups had published reports on different nanodrugs showing promising results (Table 8.2).

8.5.2 Nanomaterials in the Development of Vaccine Against Leishmaniasis

The use of chemotherapy against leishmaniasis results in the positive outcomes in the treatment regimes, but due to their adverse effects, limited efficacy, drug induced resistance and the need of prolong regimes switches to immunotherapy which was proven to be an effective therapy against this parasite. There is no vaccine till date for the treatment of leishmaniasis in human. Many attempts have been made by different groups of scientists to develop the nano-based vaccines against leishmaniasis (Fig. 8.5 and Table 8.3).

For instance, first generation vaccine was synthesized in Iran, which consisted of dead parasites mixed with Bacillus Calmette-Guerin (BCG) (Sharifi et al. 1998). Its major drawback was low efficacy up to 54% (Sharifi et al. 1998). In continuation with this research, they used antigen component of parasite which were naïve fraction purified from parasite or artificial antigens synthesized by DNA recombinant technology. In third generation they included genes coding for a protective antigen which was cloned into a vector containing a promoter from eukaryote. It was found that there was reduction in parasite load of about 68% and 59% in second and

Table 8.2 Antileishmanial nanodrugs and nanopreparations

Nano system used	Core drug	Study	Reference
PLGA nanosphere	AmB	PLGA nanoparticle with AmB was found significantly effective against leishmaniasis in vivo and in vitro with profound immune-modulatory effect.	Costa Lima et al. (2014)
Anhydrous gel	Buparvaquone	Reduce lesion size and decrease the parasite burden by nearly 34%.	Garnier et al. (2007)
Anhydrous gel	Sitamaquine	Inhibited *L. major* parasites significantly at very low concentration.	Garnier et al. (2006)
Apolipoprotein-stabilized phospholipid bilayer disk	AmB	*Decreased the L. major* infection in BALB/c VL model.	Nelson et al. (2006)
PLGA and albumin microsphere	AmB	Inhibited the growth of promastigotes and amastigotes forms of *L. infantum.*	Ordóñez-Gutiérrez et al. (2007)
PLGA	Kinetoplast membrane protein-11 (KMP 11)	Showed the production of protective antibodies against leishmaniasis.	Santos et al. (2012)
Silver nanoparticle	Silver nanoparticle	It was found effective on *L. major* pro- and amastigotes.	Dolat et al. (2015)
Lipid nanoparticle	Miltefosine	This formulation reduced the cytotoxicity of miltefosine with enhanced antileishmanial effects.	da Gama Bitencourt et al. (2016)
Calcium phosphate nanoparticle	AmB	It was found significantly effective against leishmaniasis in vivo and in vitro.	Chaurasia et al. (1985)
Albumin nanoparticle	Meglumine antimonite	Effective on *L. major* promastigote with low cytotoxicity.	Barazesh et al. (2018)
BSA nanoparticle	AmB	Effective on *L. amazonensis* in vitro and in vivo model of infection with lower toxicity.	Casa et al. (2018)
Mesoporous silica nanoparticles (MSNs)	Pentamidine	It was found more effective and less cytotoxic than parental pentamidine.	Peretti et al. (2018)
PLGA nanoparticle	Pentamidine	Reduced parasitic burden in vivo with less toxicity than pure drug.	Valle et al. (2019)
Solid lipid nanoparticles (HPCD modified)	Paromomycin and AmB	Reduced the growth of *L. donovani*.	Parvez et al. (2020)

third generation vaccines, respectively, in preclinical models. But it failed in the clinical trials finally (Palatnik-de-Sousa 2008). The first recombinant antigen used for this vaccine therapy against leishmaniasis was a surface protein GP63. This is membrane protease present in the promastigotes of all species though it was not

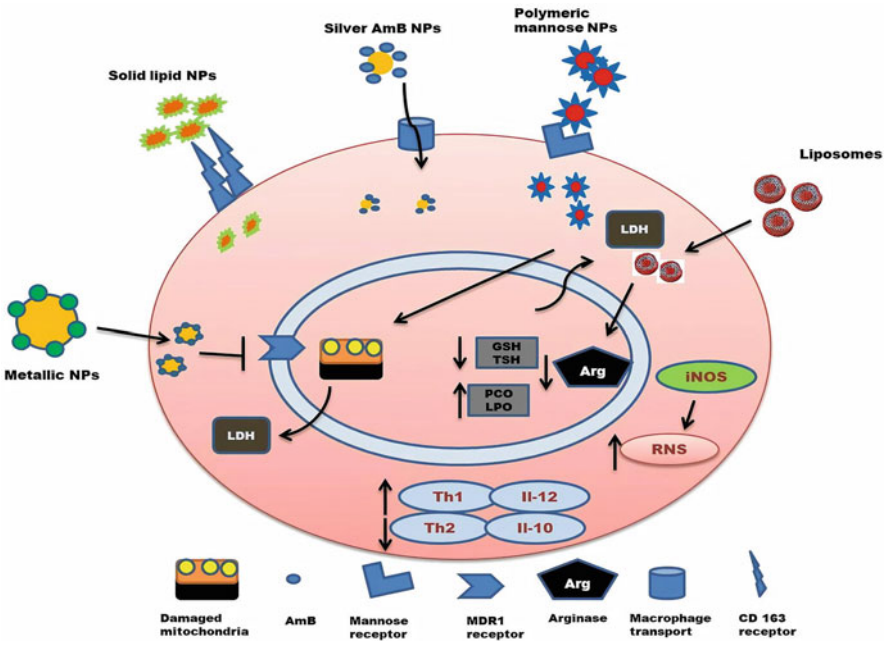

Fig. 8.5 Different nanovaccine under development against leishmaniasis and its mechanism of action

Table 8.3 Nanomaterials as nanovaccines against leishmaniasis

Nanovaccine type	Component of *Leishmania* spp. used	Immunity induced	Reference
Polymeric	*Leishmania* superoxide dismutase B1(SODB1)	Cell-mediated immunity	Danesh-Bahreini et al. (2011)
Polymeric	*Leishmania infantum* antigens (sLiAg) and monophosphoryl lipid A (MPLA)	Both innate and cell mediated	Margaroni et al. (2017)
Polymeric	*Leishmania infantum* cysteine peptidase A, histone H1, and kinetoplastid membrane protein 11	Cell-mediated immunity	Athanasiou et al. (2017)
Polymeric	Encapsulated lipophosphoglycan molecule (LPG) and/or soluble leishmanial antigens (SLA)	Macrophage activation and immunity against visceral leishmaniasis	Tosyali et al. (2021)
Polymeric	TSA (Thiol-specific-Antioxidant)	Induction of cytokines and humoral immunity against cutaneous leishmaniasis	Zarrati et al. (2016)

found up to the mark in clinical trials due to their low immunogenic properties (Spitzer et al. 1999). In another study recombinant antigen used was known as *Leishmania* spp. superoxide dismutase B1 (SODB1) for the development of nanovaccine. This antigen was 195 amino acid long protein with a molecular weight of 21,287 Da. However, the single use of peptides has a drawback of low immunogenicity, while incorporating it with some adjuvants led to enhanced immunogenicity up to 82% (Dolat et al. 2015). So, chitosan was used as a drug delivery system for encapsulating SODB1 antigen via ionic gelation method. It was found that this system enhanced the immunogenicity towards cell-mediated immunity as well as humoral immunity (Danesh-Bahreini et al. 2011). In another study, Poly (D,L-lactide-co-glycolide) PLGA nanoparticles were used as nanovaccine to increase the immunogenicity in a leishmaniasis model. These nanoparticles were surface-modified by a TNFα- mimicking eight amino acid peptide (p8), and then encapsulated with *Leishmania infantum* LiAg and MPLA antigens then further evaluated for immunogenicity. It showed antigen-specific humoral and cellular mediated immune response induction in mouse model (Margaroni et al. 2017). In another study, they designed synthetic long peptides, chimeric peptides, by using amino acid linkers and multiepitope peptides that contained HLA class I restricted (specific) epitopes of the *Leishmania infantum* proteins such as cysteine peptidase A (CPA), Histone H1 and kinetoplastid membrane protein 11 (KMP-11) followed by encapsulation with peptides into PLGA nanoparticles alone or in combination with the monophosphoryl lipid A(MPLA) as an adjuvant. In a nutshell, they differentially construct functionalized peptide based nanovaccine and then investigate its potential to stimulate dendritic cells (DCs). They found that there was prominent IL-12 production and $CD4^+$ and $CD8^+$ T cell activation (Chaurasia et al. 1985). Immunization of HLA A2.1 transgenic mice with this nanovaccine induced peptide specific IFN-γ producing $CD8^+$ T cells which marked an enhanced protection against *L. infantum* infection (Athanasiou et al. 2017). Tosyali et al. encapsulated lipophosphoglycan molecule (LPG), which is thought to be a most important immunogenic antigens of *Leishmania* parasite, into PLGA nanoparticles with autoclaved (ALA) or soluble leishmanial antigens (SLA). The formed nanoparticles were (SLA-LPG) PLGA nanoparticle, and (ALA-LPG) PLGA nanoparticle which had a size of 253 nm and 307 nm, respectively. These nanoparticles significantly triggered macrophages to synthesize excessive levels of IFN-γ and IL-12 cytokines and 80% protection against visceral leishmaniasis. It also enhanced Th1 cytokine response after nano vaccination. Hence, it can be concluded that these two nanoformulations have the capability to evoke immune responses against *Leishmania* spp. infection and may be used as the nanovaccines in near future (Tosyali et al. 2021).

In spite of design and trials of various preparations on murine models, no protective vaccine against leishmaniasis has been commercially produced yet (Ahmed et al. 2009). DNA vaccine is a novel therapy against leishmaniasis that has several benefits over traditional vaccines (Zadeh-Vakili et al. 2004). TSA is major recombinant protein homologue to eukaryotic thiol-specific-antioxidant protein with a molecular weight of 22.1 kDa and is composed of around 200 amino acids which was placed in the chromosomes number 15 (Monnerat et al. 2004). A

group of workers synthesized nanovaccine containing TSA recombinant plasmid and poly (methyl methacrylate) nanoparticles, which acted as an adjuvant. Finally, its immunogenicity was tested upon BALB/c mice. The results showed that the formed nanovaccine was capable of induction of cytokines and evoked specific antibody responses, parasite clearance in the spleen of *Leishmania major*-infected BALB/c mice. This study proved to be a significant synthesis of DNA based nanovaccines and could be a future vaccine against leishmaniasis (Zarrati et al. 2016).

8.6 Conclusion

Despite advancement in the medical science, there is still increase in frequency of new cases of leishmaniasis endemic as well as non-endemic areas. Arsenals against leishmaniasis still face problems like toxicity, high cost, unavailability as well as resistance. Silent parasite entry and its persistence in macrophages helped parasite to hide itself. The advancement in the development of new formulation in nanotechnology-based drug delivery system is a new hope for treating leishmaniasis by specific targeting. Nanotechnology helped to administer low dose of drug to specific target, thus proving itself superior than other strategies against leishmaniasis. Advancement in the nanotechnology overcomed the problems of the antileishmanial drugs like high cost, high toxicity and low bioavailability. Promising results were reported in the direction of the development of nanodrugs, diagnostic techniques and nanovaccines. We feel that nanotechnology-based diagnostic kits, drugs as well as vaccines against leishmaniasis will soon be approved and launched in the market with high efficacy, more sensitivity and low cost.

References

Ahmed SB, Touihri L, Chtourou Y, Dellagi K, Bahloul C (2009) DNA based vaccination with a cocktail of plasmids encoding immunodominant Leishmania (Leishmania) major antigens confers full protection in BALB/c mice. Vaccine 27:99–106. https://doi.org/10.1016/J.VACCINE.2008.10.013

Ali R, Tabrez S, Rahman F, Alouffi AS, Mohammed Alshehri B, Ayed Alshammari F, Alaidarous MA, Aziz Bin Dukhyil A (2021) Phytochemical analysis of Acacia nilotica and evaluation of its antileishmanial potential. PrePrints. https://doi.org/10.21203/rs.3.rs-120320/v2

Andreadou M, Liandris E, Gazouli M, Taka S, Antoniou M, Theodoropoulos G, Tachtsidis I, Goutas N, Vlachodimitropoulos D, Kasampalidis I, Ikonomopoulos J (2014) A novel non-amplification assay for the detection of Leishmania spp. in clinical samples using gold nanoparticles. J Microbiol Methods 96:56–61. https://doi.org/10.1016/J.MIMET.2013.10.011

Arish M, Husein A, Kashif M, Sandhu P, Hasnain SE, Akhter Y, Rub A (2015) Orchestration of membrane receptor signaling by membrane lipids. Biochimie 113:111–124. https://doi.org/10.1016/J.BIOCHI.2015.04.005

Arish M, Husein A, Kashif M, Saleem M, Akhter Y, Rub A (2016) Sphingosine-1-phosphate signaling: unraveling its role as a drug target against infectious diseases. Drug Discov Today 21:133–142. https://doi.org/10.1016/J.DRUDIS.2015.09.013

Athanasiou E, Agallou M, Tastsoglou S, Kammona O, Hatzigeorgiou A, Kiparissides C, Karagouni E (2017) A poly(lactic-co-glycolic) acid nanovaccine based on chimeric peptides from different Leishmania infantum proteins induces dendritic cells maturation and promotes peptide-specific IFNγ-producing CD8+ T cells essential for the protection against experimental visceral Leishmaniasis. Front Immunol 8. https://doi.org/10.3389/FIMMU.2017.00684

Barazesh A, Motazedian MH, Sattarahmady N, Morowvat MH, Rashidi S (2018) Preparation of meglumine antimonate loaded albumin nanoparticles and evaluation of its anti-leishmanial activity: an in vitro assay. J Parasit Dis 423(42):416–422. https://doi.org/10.1007/S12639-018-1018-7

Basselin M, Coombs GH, Barrett MP (2000) Putrescine and spermidine transport in Leishmania. Mol Biochem Parasitol 109:37–46. https://doi.org/10.1016/S0166-6851(00)00234-6

Basselin M, Denise H, Coombs GH, Barrett MP (2002) Resistance to pentamidine in Leishmania mexicana involves exclusion of the drug from the mitochondrion. Antimicrob Agents Chemother 46:3731. https://doi.org/10.1128/AAC.46.12.3731-3738.2002

Bose PP, Kumar P (2016) Visual assessment of parasitic burden in infected macrophage by plasmonic detection of leishmania specific marker RNA. Biochem Biophys Res Commun 480:81–86. https://doi.org/10.1016/J.BBRC.2016.10.005

Burza S, Croft SL, Boelaert M (2018) Leishmaniasis. Lancet 392:951–970. https://doi.org/10.1016/S0140-6736(18)31204-2

Carvalho L, Luque-Ortega JR, López-Martín C, Castanys S, Rivas L, Gamarro F (2011) The 8-aminoquinoline analogue sitamaquine causes oxidative stress in Leishmania donovani promastigotes by targeting succinate dehydrogenase. Antimicrob Agents Chemother 55:4204–4210. https://doi.org/10.1128/AAC.00520-11

Casa DM, Scariot DB, Khalil NM, Nakamura CV, Mainardes RM (2018) Bovine serum albumin nanoparticles containing amphotericin B were effective in treating murine cutaneous leishmaniasis and reduced the drug toxicity. Exp Parasitol 192:12–18. https://doi.org/10.1016/J.EXPPARA.2018.07.003

Chan-Bacab MJ, Peña-Rodríguez LM (2001) Plant natural products with leishmanicidal activity. Nat Prod Rep 18:674–688. https://doi.org/10.1039/B100455G

Chattopadhyay A, Jafurulla M (2011) A novel mechanism for an old drug: amphotericin B in the treatment of visceral leishmaniasis. Biochem Biophys Res Commun 416:7–12. https://doi.org/10.1016/J.BBRC.2011.11.023

Chaurasia M, Singh PK, Jaiswal AK, Kumar A, Pawar VK, Dube A, Paliwal SK, Chourasia MK (1985) Bioinspired calcium phosphate nanoparticles featuring as efficient carrier and prompter for macrophage intervention in experimental Leishmaniasis. Pharm Res. https://doi.org/10.1007/s11095-016-1985-2

Cho K, Wang X, Nie S, Chen ZG, Shin DM (2008) Therapeutic nanoparticles for drug delivery in cancer. Clin Cancer Res 14:1310–1316. https://doi.org/10.1158/1078-0432.CCR-07-1441

Coelho AC, Beverley SM, Cotrim PC (2003) Functional genetic identification of PRP1, an ABC transporter superfamily member conferring pentamidine resistance in Leishmania major. Mol Biochem Parasitol 130:83–90. https://doi.org/10.1016/S0166-6851(03)00162-2

Costa Lima SA, Silvestre R, Barros D, Cunha J, Baltazar MT, Dinis-Oliveira RJ, Cordeiro-da-Silva A (2014) Crucial CD8+ T-lymphocyte cytotoxic role in amphotericin B nanospheres efficacy against experimental visceral leishmaniasis. Nanomed Nanotechnol Biol Med 10:e1021–e1030. https://doi.org/10.1016/J.NANO.2013.12.013

Croft SL, Neal RA, Pendergast W, Chan JH (1987) The activity of alkyl phosphorylcholines and related derivatives against Leishmania donovani. Biochem Pharmacol 36:2633–2636. https://doi.org/10.1016/0006-2952(87)90543-0

da Gama Bitencourt JJ, Pazin WM, Ito AS, Barioni MB, de Paula Pinto C, Santos MA, Guimarães TH, Santos MR, Valduga CJ (2016) Miltefosine-loaded lipid nanoparticles: improving miltefosine stability and reducing its hemolytic potential toward erythtocytes and its cytotoxic effect on macrophages. Biophys Chem 217:20–31. https://doi.org/10.1016/J.BPC.2016.07.005

Danesh-Bahreini MA, Shokri J, Samiei A, Kamali-Sarvestani E, Barzegar-Jalali M, Mohammadi-Samani S (2011) Nanovaccine for leishmaniasis: preparation of chitosan nanoparticles containing Leishmania superoxide dismutase and evaluation of its immunogenicity in BALB/c mice. Int J Nanomedicine 6:835–842. https://doi.org/10.2147/IJN.S16805

Das N, Mahato SB, Naskar K, Ghosh DK, Basu MK (1990) Targeting of urea stibamine encapsulated in liposomes to reticuloendothelial system for the treatment of experimental leishmaniasis. Biochem Med Metab Biol 43:133–139. https://doi.org/10.1016/0885-4505(90)90018-V

Das VN, Ranjan A, Sinha AN, Verma N, Lal CS, Gupta AK, Siddiqui NA, Kar SK (2001) A randomized clinical trial of low dosage combination of pentamidine and allopurinol in the treatment of antimony unresponsive cases of visceral leishmaniasis. J Assoc Physicians India 49:609–613

Das BB, Ganguly A, Majumder HK (2008) DNA topoisomerases of Leishmania: the potential targets for anti-leishmanial therapy. Adv Exp Med Biol 625:103–115. https://doi.org/10.1007/978-0-387-77570-8_9

Date AA, Joshi MD, Patravale VB (2007) Parasitic diseases: liposomes and polymeric nanoparticles versus lipid nanoparticles. Adv Drug Deliv Rev 59:505–521. https://doi.org/10.1016/J.ADDR.2007.04.009

Debrabant A, Lee N, Bertholet S, Duncan R, Nakhasi HL (2003) Programmed cell death in trypanosomatids and other unicellular organisms. Int J Parasitol 33:257–267. https://doi.org/10.1016/S0020-7519(03)00008-0

Dietze R, Carvalho SF, Valli LC, Berman J, Brewer T, Milhous W, Sanchez J, Schuster B, Grogl M (2001) Phase 2 trial of WR6026, an orally administered 8-aminoquinoline, in the treatment of visceral leishmaniasis caused by Leishmania chagasi. Am J Trop Med Hyg 65:685–689. https://doi.org/10.4269/AJTMH.2001.65.685

Dolat E, Rajabi O, Salarabadi SS, Yadegari-Dehkordi S, Sazgarnia A (2015) Silver nanoparticles and electroporation: their combinational effect on Leishmania major. Bioelectromagnetics 36:586–596. https://doi.org/10.1002/BEM.21945

Dye C, Vidor E, Dereure J (1993) Serological diagnosis of leishmaniasis: on detecting infection as well as disease. Epidemiol Infect 110:647. https://doi.org/10.1017/S0950268800051074

Foulet F, Botterel F, Buffet P, Morizot G, Rivollet D, Deniau M, Pratlong F, Costa J-M, Bretagne S (2007) Detection and identification of Leishmania species from clinical specimens by using a real-time PCR assay and sequencing of the cytochrome b gene. J Clin Microbiol 45:2110. https://doi.org/10.1128/JCM.02555-06

Garnier T, Brown MB, Lawrence MJ, Croft SL (2006) In-vitro and in-vivo studies on a topical formulation of sitamaquine dihydrochloride for cutaneous leishmaniasis. J Pharm Pharmacol 58:1043–1054. https://doi.org/10.1211/JPP.58.8.0004

Garnier T, Mäntylä A, Järvinen T, Lawrence J, Brown M, Croft S (2007) In vivo studies on the antileishmanial activity of buparvaquone and its prodrugs. J Antimicrob Chemother 60:802–810. https://doi.org/10.1093/JAC/DKM303

Georgiadou SP, Makaritsis KP, Dalekos GN (2015) Leishmaniasis revisited: current aspects on epidemiology, diagnosis and treatment. J Transl Intern Med 3:43–50. https://doi.org/10.1515/JTIM-2015-0002

Goad LJ, Holz GG Jr, Beach DH (1984) Sterols of Leishmania species. Implications for biosynthesis. Mol Biochem Parasitol 10:161–170. https://doi.org/10.1016/0166-6851(84)90004-5

Gour J (2009) Nanomedicine and Leishmaniasis: future prospects. Dig J Nanomater Biostruct. https://www.researchgate.net/publication/237284963_Nanomedicine_and_Leishmaniasis_future_prospects. Accessed 30 Aug 2021

Gupta S, Pal A, Vyas SP (2010) Drug delivery strategies for therapy of visceral leishmaniasis. Expert Opin Drug Deliv 7:371–402. https://doi.org/10.1517/17425240903548232

Gupta G, Oghumu S, Satoskar AR (2013) Mechanisms of immune evasion in Leishmaniasis. Adv Appl Microbiol 82:155. https://doi.org/10.1016/B978-0-12-407679-2.00005-3

Gupta S, Sharma P, Gupta MK (2016) Leishmaniasis - drugs, nanotechnology based delivery systems and recent patents survey. Curr Nanomed 6:21–42. https://doi.org/10.2174/1877912306999151209160449

Jain RK (1994) Barriers to drug delivery in solid tumors. Sci Am 271:58–65. https://doi.org/10.1038/SCIENTIFICAMERICAN0794-58

Jha TK, Giri YN, Singh TK, Jha S (1995) Use of amphotericin B in drug-resistant cases of visceral leishmaniasis in North Bihar, India. Am J Trop Med Hyg 52:536–538. https://doi.org/10.4269/AJTMH.1995.52.536

Jha TK, Sundar S, Thakur CP, Felton JM, Sabin AJ, Horton J (2005) A phase II dose-ranging study of sitamaquine for the treatment of visceral leishmaniasis in India. Am J Trop Med Hyg 73:1005–1011

Kashif M, Tabrez S, Husein A, Arish M, Kalaiarasan P, Manna PP, Subbarao N, Akhter Y, Rub A (2018) Identification of novel inhibitors against UDP-galactopyranose mutase to combat leishmaniasis. J Cell Biochem 119:2653–2665. https://doi.org/10.1002/JCB.26433

Krauth-Siegel RL, Comini MA (2008) Redox control in trypanosomatids, parasitic protozoa with trypanothione-based thiol metabolism. Biochim Biophys Acta 1780:1236–1248. https://doi.org/10.1016/J.BBAGEN.2008.03.006

Krauth-Siegel RL, Meiering SK, Schmidt H (2003) The parasite-specific trypanothione metabolism of trypanosoma and leishmania. Biol Chem 384:539–549. https://doi.org/10.1515/BC.2003.062

Leishmaniasis (2021). https://www.who.int/news-room/fact-sheets/detail/leishmaniasis. Accessed 19 Sept 2021

Liñares GE, Ravaschino EL, Rodriguez JB (2006) Progresses in the field of drug design to combat tropical protozoan parasitic diseases. Curr Med Chem 13:335–360. https://doi.org/10.2174/092986706775476043

Lux H, Heise N, Klenner T, Hart D, Opperdoes FR (2000) Ether--lipid (alkyl-phospholipid) metabolism and the mechanism of action of ether--lipid analogues in Leishmania. Mol Biochem Parasitol 111:1–14. https://doi.org/10.1016/S0166-6851(00)00278-4

Majumder S, Dey R, Bhattacharjee S, Rub A, Gupta G, Bhattacharyya Majumdar S, Saha B, Majumdar S (2012) Leishmania-induced biphasic ceramide generation in macrophages is crucial for uptake and survival of the parasite. J Infect Dis 205:1607–1616. https://doi.org/10.1093/INFDIS/JIS229

Margaroni M, Agallou M, Athanasiou E, Kammona O, Kiparissides C, Gaitanaki C, Karagouni E (2017) Vaccination with poly(D,L-lactide-co-glycolide) nanoparticles loaded with soluble Leishmania antigens and modified with a TNFα-mimicking peptide or monophosphoryl lipid A confers protection against experimental visceral leishmaniasis. Int J Nanomedicine 12:6169–6184. https://doi.org/10.2147/IJN.S141069

McKerrow JH, Engel JC, Caffrey CR (1999) Cysteine protease inhibitors as chemotherapy for parasitic infections. Bioorg Med Chem 7:639–644. https://doi.org/10.1016/S0968-0896(99)00008-5

Melaku Y, Collin SM (2007) Treatment of kala-azar in southern Sudan using a 17-day regimen of sodium stibogluconate combined with paromomycin: a retrospective comparison with 30-day sodium stibogluconate monotherapy. Am J Trop Med Hyg 77(1):89–94

Mishra J, Saxena A, Singh S (2007) Chemotherapy of leishmaniasis: past, present and future. Curr Med Chem 14:1153–1169. https://doi.org/10.2174/092986707780362862

Monnerat S, Martinez-Calvillo S, Worthey E, Myler PJ, Stuart KD, Fasel N (2004) Genomic organization and gene expression in a chromosomal region of Leishmania major. Mol Biochem Parasitol 134:233–243. https://doi.org/10.1016/J.MOLBIOPARA.2003.12.004

Motta MC (2008) Kinetoplast as a potential chemotherapeutic target of trypanosomatids. Curr Pharm Des 14:847–854. https://doi.org/10.2174/138161208784041051

Nelson KG, Bishop JV, Ryan RO, Titus R (2006) Nanodisk-associated amphotericin B clears Leishmania major cutaneous infection in susceptible BALB/c mice. Antimicrob Agents Chemother 50:1238–1244. https://doi.org/10.1128/AAC.50.4.1238-1244.2006

Newman DJ (2008) Natural products as leads to potential drugs: an old process or the new hope for drug discovery? J Med Chem 51:2589–2599. https://doi.org/10.1021/JM0704090

No JH (2016) Visceral leishmaniasis: revisiting current treatments and approaches for future discoveries. Acta Trop 155:113–123. https://doi.org/10.1016/J.ACTATROPICA.2015.12.016

Olliaro PL, Guerin PJ, Gerstl S, Haaskjold AA, Rottingen JA, Sundar S (2005) Treatment options for visceral leishmaniasis: a systematic review of clinical studies done in India, 1980–2004. Lancet Infect Dis 5:763–774. https://doi.org/10.1016/S1473-3099(05)70296-6

Ordóñez-Gutiérrez L, Espada-Fernández R, Dea-Ayuela MA, Torrado JJ, Bolás-Fernandez F, Alunda JM (2007) In vitro effect of new formulations of amphotericin B on amastigote and promastigote forms of Leishmania infantum. Int J Antimicrob Agents 30:325–329. https://doi.org/10.1016/J.IJANTIMICAG.2007.05.013

Ouellette M, Drummelsmith J, Papadopoulou B (2004) Leishmaniasis: drugs in the clinic, resistance and new developments. Drug Resist Updat 7:257–266. https://doi.org/10.1016/J.DRUP.2004.07.002

Pace D (2014) Leishmaniasis. J Infect 69:S10–S18. https://doi.org/10.1016/J.JINF.2014.07.016

Palatnik-de-Sousa CB (2008) Vaccines for leishmaniasis in the fore coming 25 years. Vaccine 26:1709–1724. https://doi.org/10.1016/J.VACCINE.2008.01.023

Paris C, Loiseau PM, Bories C, Bréard J (2004) Miltefosine induces apoptosis-like death in Leishmania donovani promastigotes. Antimicrob Agents Chemother 48:852–859. https://doi.org/10.1128/AAC.48.3.852-859.2004

Parvez S, Yadagiri G, Gedda MR, Singh A, Singh OP, Verma A, Sundar S, Mudavath SL (2020) Modified solid lipid nanoparticles encapsulated with Amphotericin B and Paromomycin: an effective oral combination against experimental murine visceral leishmaniasis. Sci Rep 101(10):1–14. https://doi.org/10.1038/s41598-020-69276-5

Peretti E, Miletto I, Stella B, Rocco F, Berlier G, Arpicco S (2018) Strategies to obtain encapsulation and controlled release of pentamidine in mesoporous silica nanoparticles. Pharmaceutics 10:195. https://doi.org/10.3390/PHARMACEUTICS10040195

Pucadyil TJ, Tewary P, Madhubala R, Chattopadhyay A (2004) Cholesterol is required for Leishmania donovani infection: implications in leishmaniasis. Mol Biochem Parasitol 133:145–152. https://doi.org/10.1016/J.MOLBIOPARA.2003.10.002

Rahman F, Tabrez S, Ali R, Akand SK, Zahid M, Alaidarous MA, Alsaweed M, Alshehri BM, Banawas S, Bin Dukhyil AA, Rub A (2021) Virtual screening of natural compounds for potential inhibitors of sterol C-24 methyltransferase of Leishmania donovani to overcome leishmaniasis. J Cell Biochem 122:1216–1228. https://doi.org/10.1002/JCB.29944

Rana A, Rub A, Akhter Y (2014) Proteome-scale identification of outer membrane proteins in Mycobacterium avium subspecies paratuberculosis using a structure based combined hierarchical approach. Mol BioSyst 10:2329–2337. https://doi.org/10.1039/C4MB00234B

Riera C, Fisa R, Lopez P, Ribera E, Carrió J, Falcó V, Molina I, Gállego M, Portús M (2004) Evaluation of a latex agglutination test (KAtex) for detection of Leishmania antigen in urine of patients with HIV-Leishmania coinfection: value in diagnosis and post-treatment follow-up. Eur J Clin Microbiol Infect Dis 23:899–904. https://doi.org/10.1007/S10096-004-1249-7

Roberts WL, Rainey PM (1993) Antimony quantification in Leishmania by electrothermal atomic absorption spectroscopy. Anal Biochem 211:1–6. https://doi.org/10.1006/ABIO.1993.1223

Rub A, Dey R, Jadhav M, Kamat R, Chakkaramakkil S, Majumdar S, Mukhopadhyaya R, Saha B (2009) Cholesterol depletion associated with Leishmania major infection alters macrophage CD40 signalosome composition and effector function. Nat Immunol 10:273–280. https://doi.org/10.1038/NI.1705

Rub A, Arish M, Husain SA, Ahmed N, Akhter Y (2013) Host-lipidome as a potential target of protozoan parasites. Microbes Infect 15:649–660. https://doi.org/10.1016/J.MICINF.2013.06.006

Santos DO, Coutinho CE, Madeira MF, Bottino CG, Vieira RT, Nascimento SB, Bernardino A, Bourguignon SC, Corte-Real S, Pinho RT, Rodrigues CR, Castro HC (2008) Leishmaniasis treatment—a challenge that remains: a review. Parasitol Res 103:1–10. https://doi.org/10.1007/S00436-008-0943-2

Santos DM, Carneiro MW, de Moura TR, Fukutani K, Clarencio J, Soto M, Espuelas S, Brodskyn C, Barral A, Barral-Netto M, de Oliveira CI (2012) Towards development of novel immunization strategies against leishmaniasis using PLGA nanoparticles loaded with kinetoplastid membrane protein-11. Int J Nanomedicine 7:2115–2127. https://doi.org/10.2147/IJN.S30093

Sen N, Majumder HK (2008) Mitochondrion of protozoan parasite emerges as potent therapeutic target: exciting drugs are on the horizon. Curr Pharm Des 14:839–846. https://doi.org/10.2174/138161208784041024

Shaked-Mishan P, Ulrich N, Ephros M, Zilberstein D (2001) Novel intracellular SbV reducing activity correlates with antimony susceptibility in Leishmania donovani. J Biol Chem 276:3971–3976. https://doi.org/10.1074/JBC.M005423200

Sharifi I, FeKri AR, Aflatonian MR, Khamesipour A, Nadim A, Mousavi MR, Momeni AZ, Dowlati Y, Godal T, Zicker F, Smith PG, Modabber F (1998) Randomised vaccine trial of single dose of killed Leishmania major plus BCG against anthroponotic cutaneous leishmaniasis in Bam, Iran. Lancet (London, England) 351:1540–1543. https://doi.org/10.1016/S0140-6736(98)09552-X

Sherwood JA, Gachihi GS, Muigai RK, Skillman DR, Mugo M, Rashid JR, Wasunna KM, Were JB, Kasili SK, Mbugua JM et al (1994) Phase 2 efficacy trial of an oral 8-aminoquinoline (WR6026) for treatment of visceral leishmaniasis. Clin Infect Dis 19:1034–1039. https://doi.org/10.1093/CLINIDS/19.6.1034

Shinjini Singh MS (2008) Nanotechnology for Alzheimer's disease detection | Request PDF. https://www.researchgate.net/publication/256462786_Nanotechnology_for_Alzheimer's_disease_detection. Accessed 30 Aug 2021

Späth GF, Garraway LA, Turco SJ, Beverley SM (2003) The role(s) of lipophosphoglycan (LPG) in the establishment of Leishmania major infections in mammalian hosts. Proc Natl Acad Sci 100:9536–9541. https://doi.org/10.1073/PNAS.1530604100

Spitzer N, Patterson KK, Kipps DW (1999) Long-term protection of mice against Leishmania major with a synthetic peptide vaccine. Vaccine 17:1298–1300. https://doi.org/10.1016/S0264-410X(98)00363-6

Sundar S, Rai M (2002) Advances in the treatment of leishmaniasis. Curr Opin Infect Dis 15:593–598. https://doi.org/10.1097/00001432-200212000-00007

Sundar S, Chakravarty J, Agarwal D, Rai M, Murray HW (2010) Single-dose liposomal amphotericin B for visceral Leishmaniasis in India. N Engl J Med 362:504–512. https://doi.org/10.1056/NEJMOA0903627

Tabrez S, Rahman F, Ali R, Alouffi AS, Alshehri BM, Alshammari FA, Alaidarous MA, Banawas S, Bin Dukhyil AA, Rub A (2021) Assessment of the antileishmanial potential of Cassia fistula leaf extract. ACS Omega 6:2318–2327. https://doi.org/10.1021/ACSOMEGA.0C05629

Thakur CP, Sinha GP, Pandey AK, Barat D, Sinha PK (1993) Amphotericin B in resistant kala-azar in Bihar. Natl Med J India 6(2):57–60

Torres-Guerrero E, Quintanilla-Cedillo MR, Ruiz-Esmenjaud J, Arenas R (2017) Leishmaniasis: a review F1000Research, 6. https://doi.org/10.12688/F1000RESEARCH.11120.1

Tosyali OA, Allahverdiyev A, Bagirova M, Abamor ES, Aydogdu M, Dinparvar S, Acar T, Mustafaeva Z, Derman S (2021) Nano-co-delivery of lipophosphoglycan with soluble and autoclaved leishmania antigens into PLGA nanoparticles: evaluation of in vitro and in vivo immunostimulatory effects against visceral leishmaniasis. Mater Sci Eng C 120:111684. https://doi.org/10.1016/J.MSEC.2020.111684

Valle IV, Machado ME, Araújo CCB, da Cunha-Junior EF, Pacheco JS, Torres-Santos EC, da Silva LCRP, Cabral LM, do Carmo FA, Sathler PC (2019) Oral pentamidine-loaded poly(d,l-lactic-co-glycolic) acid nanoparticles: an alternative approach for leishmaniasis treatment. Nanotechnology 30:455102. https://doi.org/10.1088/1361-6528/AB373E

Veerareddy PR, Vobalaboina V (2004) Lipid-based formulations of amphotericin B. Drugs Today (Barc) 40:133–145. https://doi.org/10.1358/dot.2004.40.2.799425

Wasunna MK, Rashid JR, Mbui J, Kirigi G, Kinoti D, Lodenyo H, Felton JM, Sabin AJ, Albert MJ, Horton J (2005) A phase II dose-increasing study of sitamaquine for the treatment of visceral leishmaniasis in Kenya. Am J Trop Med Hyg 73:871–876

Welearegay TG, Diouani MF, Österlund L, Ionescu F, Belgacem K, Smadhi H, Khaled S, Kidar A, Cindemir U, Laouini D, Ionescu R (2018) Ligand-capped ultrapure metal nanoparticle sensors for the detection of cutaneous Leishmaniasis disease in exhaled breath. ACS Sens 3:2532–2540. https://doi.org/10.1021/ACSSENSORS.8B00759

Zadeh-Vakili A, Taheri T, Taslimi Y, Doustdari F, Salmanian AH, Rafati S (2004) Immunization with the hybrid protein vaccine, consisting of Leishmania major cysteine proteinases Type I (CPB) and Type II (CPA), partially protects against leishmaniasis. Vaccine 22:1930–1940. https://doi.org/10.1016/J.VACCINE.2003.11.014

Zangger H, Mottram JC, Fasel N (2002) Cell death in Leishmania induced by stress and differentiation: programmed cell death or necrosis? Cell Death Differ 9:1126–1139. https://doi.org/10.1038/SJ.CDD.4401071

Zarrati S, Mahdavi M, Tabatabaie F (2016) Immune responses in DNA vaccine formulated with PMMA following immunization and after challenge with Leishmania major. J Parasit Dis 40:427. https://doi.org/10.1007/S12639-014-0521-8

A Comprehensive Review on the Synthesis, Surface Decoration of Nanoselenium and Their Medical Applications

9

Heba S. Abbas, Maii M. Nagy, Walaa E. Hammam, Asmaa A. Abd El Fatah, Mai S. Abd-Elafatah, Aya Ashour Abd El-Naby Mahmoud Aref, Hala A. Abdulhamid, Suresh Ghotekar, and Doha H. Abou Baker

Abstract

In recent years, nanotechnology has developed more and more in the scientific field, which has met with great interest due to the large number of applications in almost all fields. The particular properties of nanoscale materials have prompted extensive research into nanoparticle (NP) development, characterization, and

H. S. Abbas (✉)
Microbiology Department, National Organization of Drug Control and Research (NODCAR), recently, Egyptian Drug Authority (EDA), Giza, Egypt

Microbiology Department, College of Pharmacy and Drug Manufacturing, Misr University for Science and Technology, Giza, Egypt

M. M. Nagy
Phytochemistry Department, National Organization of Drug Control and Research (NODCAR), recently, Egyptian Drug Authority (EDA), Giza, Egypt

W. E. Hammam
Medicinal Plants and Natural Products Department, National Organization of Drug Control and Research (NODCAR), recently, Egyptian Drug Authority (EDA), Giza, Egypt

A. A. Abd El Fatah
Faculty of Science, Benisuef University, Benisuef, Egypt

M. S. Abd-Elafatah
Faculty of Science, Port Said University, Port Said, Egypt

A. A. A. E.-N. M. Aref
Laboratories Department, Mansheyet El-Bakry Hospital, Cairo, Egypt

H. A. Abdulhamid
Department of Chemistry, Smt. Devkiba Mohansinhji Chauhan College of Commerce and Science, University of Mumbai, Silvassa, Dadra and Nagar Haveli (UT), India

S. Ghotekar · D. H. Abou Baker
Medicinal Plants Department, National Research Center, Giza, Egypt

S. Hameed, S. Rehman (eds.), *Nanotechnology for Infectious Diseases*,
https://doi.org/10.1007/978-981-16-9190-4_9

application. In order to find the increasingly preferred NP functionality, some researchers are looking into the realm of methods for obtaining NPs with new properties or enhanced functions. Nanoparticles can be synthesized by various chemical or physical techniques; however green nanotechnology is an alternative, an eco-friendly option. Reducing and stabilizing agents from plants and other natural resources were used for the biosynthesis of various shapes, sizes, and bioactivity NPs.

Selenium (Se) nanoparticles (SeNPs) have the prospective to function as a novel nutritional supplement with lower toxicity, higher breakdown, and the ability to be regularly removed from the body. In terms of therapeutic load and anticancer activity, selenium nanoparticles have a dual synergistic impact. Recent studies have looked at new applications for the nanoscale shape of selenium in biological activities including antifungal and antibacterial agents. Here we offer an intensive report on the latest developments in several biomedical practices of SeNPs, with a focus on chemical, and many purposes in the life.

Therefore, this overview summarizes the most recent information on the synthesis routes of SeNPs, and their surface decoration with functional groups, and natural polysaccharides to design functional selenium nanomaterials for medical purposes, such as anticancer and antimicrobial activities.

Keywords

Nanoselenium · Synthesis routes · Natural products · Polysaccharide · Surface decoration · Antimicrobial · Anticancer

9.1 Introduction

The scientific community originally considered Selenium (Se) to be poisonous, but Se was found to be an important element for the health of the living organism in the 1950s; it was not synthesized by our bodies and must be derived from the diet. It is only required in insignificant quantities, but it plays an essential role within our bodies, as in metabolism and thyroid function. Several literatures revealed that Se has a potential antioxidant and anticancer characteristics. In addition, organic Se compounds are absorbed more quickly than inorganic Se compounds, nevertheless SeNPs could provide a novel supplement to replace Se in the diet (Schwarz and Foltz 1957; Pham-Huy et al. 2008).

Selenium nanoparticles (SeNPs) sparked substantial attention a decade ago for a wide range of purposes due to their outstanding characteristics (Eswarapriya and Jegatheesan 2015). Nevertheless, nanoformulation of selenium (Se) is not entirely original. Se element, which is generated from biological origin, is usually found in nanoform (Buchs et al. 2013; Mal et al. 2016). Further, treatment of waters contaminated with Se comprises the release of colloidal SeNPs (Nancharaiah and Lens 2015a). Also, SeNPs propose a broad range of possibilities for human diet and disease treatment applications (Schwarz and Foltz 1957).

Several diseases have been related to the oxidative stress, such as heart diseases, cancer, and Alzheimer's disease and Se reduces the oxidative stress by keeping free radical numbers under control (Elahi et al. 2009; Markesbery and Lovell 2006; Cui et al. 2012; Shirley et al. 2014; Schnabel et al. 2008). Moreover, Se can reduce the risk of certain cancers by reducing DNA damage and oxidative stress and destroying cancer cells (Puspitasari et al. 2014). Sixty-nine studies displayed that a high blood level of Se was related to a lower risk of some cancers, including prostate, lung, breast, and colon cancers (Cai et al. 2016). Se may also protect against heart diseases, where the hazard of heart diseases was related to the rise of blood Se levels, and assist in the prevention of Alzheimer's disease, which causes memory loss and has a detrimental effect on thought and behavior (Flores-Mateo et al. 2006; González-Domínguez et al. 2014). Consumption of Se supplements raises the levels of the powerful antioxidant glutathione peroxidase and reduces the levels of the inflammatory marker C-reactive protein (Ju et al. 2017). Se is required for the thyroid gland to function perfectly. Thyroid tissue, in reality, has more selenium than any other organ in the body (Ventura et al. 2017). This powerful mineral helps protect the thyroid against oxidative damage and plays an essential role in the production of thyroid hormones, which regulate metabolism process and control growth and development (InformedHealth.org 2006). Studies have shown that high blood levels of Se are associated with enhanced immune response, but its deficiency would harm immunity (Hoffmann and Berry 2008). Additionally, Se supplements may help strengthen the immunity of people with influenza and hepatitis C (Steinbrenner et al. 2015). The relation of asthma with the increase of the oxidative stress and inflammation in the body have been reported. So, Se may help reduce asthma symptoms (Norton and Hoffmann 2012). However, Se toxicity is rare, it is important to keep the recommended amount of 55 μg per day and avoid the upper limit of 400 μg per day (Sunde 2010). Dizziness, hair loss, nausea, vomiting, muscle soreness, tremors, and facial flushing are all signs of Se toxicity. Se toxicity can cause severe neurological and intestinal symptoms, as well as a heart attack (Spiller and Pfiefer 2007).

Nanomaterials have distinctive physical and chemical structures due to their "nano-size." In many fields of science, nanotechnology is a hopeful process. There has been a giant advance of varied products with valuable functions in the field of nanomaterials, because of their distinctive properties, such as scale, shape, chemical compatibility, aggregation state, solubility, structure, and surface properties. Many benefits, such as chemical stability, biocompatibility, and low toxicity, drew attention to the synthesis and application of SeNPs (Zhang et al. 2001; Wang et al. 2007).

SeNPs were recommended as a dietary supplement, as well as having possible antimicrobial and anticancer treatment effects. Both of simple inorganic forms of Se compounds such as selenides and complex biogenic compounds like (Se nucleic acids and selenoenzymes) were in the environments and in living organisms (Kieliszek and Błażejak 2013).

SeNPs have recently attracted attention due to their lower surface area per unit volume, which lead to slow release and low interaction. And SeNPs are also a good choice for replacing other types of Se in clinical application since the toxicity of

elemental Se at nanoscale is smaller than that of selenate, selenite, or selenate ions (Zhang et al. 2008; Wadhwani et al. 2016).

Biological methods were used for the synthesis of metallic nanoparticles such as zinc oxide, silver, gold, copper oxide, and selenium. High-energy sustainable materials were used in the production of non-toxic, pure nanoparticles (Raja et al. 2017).

Plant-mediated nanoparticle construction is advantageous since it does not necessitate particular conditions in the media and culture as other biological species. Furthermore, plant extracts consist of cofactors such as proteins, enzymes, terpenoids, and flavonoids which act as capping and reducing agents (Kaliannan et al. 2019).

SeNPs are used in a variety of areas, including catalysis, solar energy conversion, water treatment, medicine, and environmental issues (Rayman 2005).

Mammals and higher animals require Se as one of the essential trace elements responsible for living cells to work ordinarily (Salem and Fouda 2020; Anu et al. 2020a; Gunti et al. 2019). Because of their low toxicity and high stability, SeNPs are now approved and recommended for use in a variety of scientific disciplines (Zhang et al. 2018). Physical, biological, and chemical methods can all be used to make it. Simultaneously, reports on a biological approach to producing SeNPs through plant parts are available (Alam et al. 2019; Surveswaran et al. 2009).

9.2 Chemical Synthesis of SeNPs

9.2.1 Chemical Reduction Methods

SeNPs were produced chemically by the reduction of selenite salt using glutathione as reductant and bovine serum albumin as stabilizer (Verma and Maheshwari 2018). Using the same precursor, Barnaby et al. (2011) synthesized Se nanospheres using dithiothreitol and gallic acid as a reducing agent. Ascorbic acid has been used as a reductant in many trials with polyvinyl alcohol or chitosan as stabilizers and to produce stable and spherical narrowly size distributed SeNPs from Se tetrachloride (Barnaby et al. 2011; Boroumand et al. 2019). Another precursor (selenious acid) was used in presence freshly prepared ice-cold $NaBH_4$ or ascorbic acid or hydroquinone in the presence of Daxad 11G as reducing agents (Gangadoo et al. 2017; Nath et al. 2004). In addition, polymers were used as stabilizing agents in many studies, e.g., stabilization with arabinogalactans, and beta-lactoglobulin (Nath et al. 2004; Zeng et al. 2018) (Fig. 9.1).

9.2.2 Wet Chemical Method

Using selenosulfate as a precursor and different organic acids (acetic, oxalic, gallic acids) in aqueous medium with polyvinyl alcohol as a stabilizer, SeNPs were formed in less than 1 min. On the other hand, Langi et al. used the same precursor with ionic

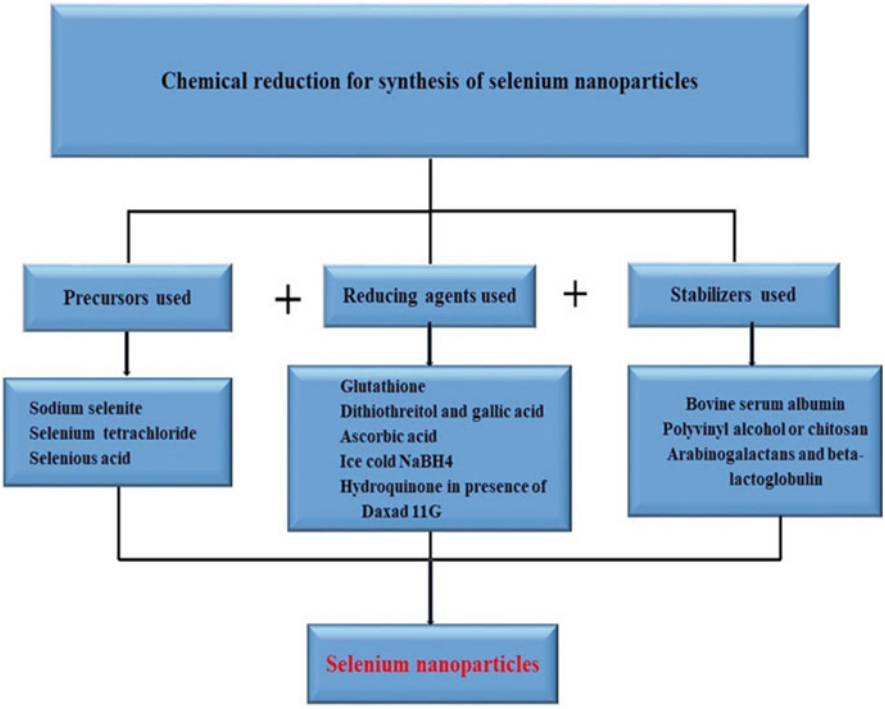

Fig. 9.1 Chemical reduction method used for SeNPs synthesis

liquid (3-methylimidazolium methanesulfonate) (Dwivedi et al. 2011; Langi et al. 2010), while Gao et al. (2003) prepared Se nanowire at room temperature by decomposition of selenodiglutathiones. Another way wet chemical method used the acetone and Se dioxide as a redox system to produce SeNPs (Gao et al. 2003; Shah et al. 2010).

9.2.3 Hydrothermal Methods

Hydrothermal method depends on mixing of an aqueous solution vapor with solid substance at elevated temperature and pressure, which leads to formation of small particles (Jamkhande et al. 2019). The trials of this method included the use of sodium selenite as a precursor with ascorbic acid dissolved in deionized water, stable colloids of cellulose nanocrystals as a reductant and a structure directing agent (Shar et al. 2019; Shin et al. 2007).

9.2.4 Solvothermal Method

It is used for the preparation of nanoparticles in presence of water or other organic chemicals like methanol or ethanol as a solvent. Reaction takes place in pressure vessel allowing heating of solvents above their boiling point temperature (Yang et al. 2007). Zeng et al. used a solvothermal process to produce trigonal butterfly-shaped Se nanoparticle by dissolving Se into ethylenediamine in Teflon-lined autoclave sealed and maintained at 160 °C for 2 h, then cooled at room temperature followed by adding acetone at −18 °C, after 24 h at −18 °C the precipitates were centrifuged (Zeng et al. 2013). Similarly, Liao et al. (2018) used solvothermal scheme for production of trigonal Se nanowires. The SeO_2 was reduced by glucose at elevated temperature (150 °C) for 12 h in Teflon-lined autoclave (Liao et al. 2018).

9.2.5 Sol–Gel Method

The appropriate amounts of Se and ascorbic acid were mixed and diluted forming elemental SeNPs-ascorbic acid (sol). This (sol) was stirred with selenocystine and allowed to stand for 48 h at 25 °C and the nano sol–gel compounds were formed (Bai et al. 2011).

9.3 Physical Synthesis of SeNPs

9.3.1 Laser Ablation

Amorphous Se (precursor) was pressed into 5 mm thick targets, different substrates were used as gold films, silicon wafers, or glass slides. Target and substrate were placed in vacuum chamber with a pump. Using pulsed laser at 532 nm resulted nanoparticles were deposited on substrates (Quintana et al. 2002).

9.3.2 Microwave Irradiation Method

A microwave-assisted approach was used for rapid synthesis of Se nanostructures, such as nanoball, nanotube, and multi-armed nanorod. This way is made by reduction of H_2SeO_3 with L-asparagine in polyethylene glycol for 30 min, and the temperature was maintained by automatic adjustment of microwave power (Yu et al. 2016a; Jadhav and Khanna 2015). In addition, SeNPs with hexagonal shape were synthesized using microwave irradiation on Se tetrachloride in distilled water at various conditions (Panahi-Kalamuei et al. 2014).

9.3.3 Sonochemical (Ultrasonic)

Se nanoparticle was formed by a sonochemical (ultrasonic) method using $SeCl_4$ as starting reagent and hydrazine, potassium borohydride, and thioglycolic acid as reducing agents (Panahi-Kalamuei et al. 2015).

9.3.4 Gamma Radiation

This is based on water radiolysis by applied radiation and the concomitant generation of free radicals. These free radicals form oxidizing and reducing species allowing formation of Se nanostructure. Sodium selenite was mixed with yeast growth media in culture bottles and placed it at 7 cm away from radiation source to produce SeNPs. It has the advantages of being efficient, allows the synthesis of spherical, dispersed nanoparticles, less toxic than chemical methods which uses aggressive chemical agents (Ribeiro et al. 2018).

9.3.5 Low Temperature Reactive Aerosol Processing

Reactive aerosol processing is perfect for the large-scale production of SeNPs (up to hundreds of kilograms). Organic precursor (such as glycine, citric acid, or betaine), phosphoric acid, sodium selenite, and water were used for aerosol with an inlet temperature of 250 °C (Viacava et al. 2020).

9.3.6 Heterogeneous Condensation Method

Se with Tungsten was used as the original starting material for producing the SeNPs. Se and tungsten at high temperature possess different levels of volatility. So, fabrication of SeNPs was done owing to the various condensation in the gaseous volume. Following, the SeNPs were placed onto a quartz substrate (Suslov et al. 2017).

9.3.7 Ball Milling Method

Ball milling depends on wet grinding of Se powder in distilled water at 800 rpm for 4 h using bidirection ball milling. This is followed by ultrasonic radiation to reach the nanoscale (Khubulava et al. 2018) (Table 9.1).

Table 9.1 Advantages and disadvantages of different methods used for Se nanoparticles synthesis (Panahi-Kalamuei et al. 2015; Ribeiro et al. 2018; Viacava et al. 2020; Suslov et al. 2017; Khubulava et al. 2018; Chhabria and Desai 2016a; Sharma et al. 2014; Shoeibi et al. 2017; Pouri et al. 2016)

Method	Advantage	Disadvantage
Chemical methods		
1. Chemical reduction (Gangadoo et al. 2017)	• Ability to control the size of the produced particles. • Short time, simple method, low cost.	Limitations associated with the use of reducing agents, e.g., cost, impurities, weak reducing ability.
2. Wet chemical (Langi et al. 2010)	• Simple method.	Cannot produce the needed quantity with the desired quality.
3. Hydrothermal (Jamkhande et al. 2019)	• Formation of high quality, monodispersed nanocrystals. • Size and shape control of particles.	Difficult to control the process.
4. Solvothermal (Zeng et al. 2013)	• Formation of high quality, monodispersed nanocrystals. • Large scale production. • Size control of particles.	High cost of equipment used.
5. Sol–gel (Bai et al. 2011)	• Simplest method. • Possibility of control of particle size and morphology.	
Physical methods (Quintana et al. 2002; Yu et al. 2016a; Jadhav and Khanna 2015; Panahi-Kalamuei et al. 2014; Panahi-Kalamuei et al. 2015; Ribeiro et al. 2018; Viacava et al. 2020)		
1. Laser ablation	• Clean technique. • Simplest method that can produce large amounts of nanostructure, low cost. • No need for final calcination step as in chemical methods.	• Particle size is not always uniform. • Splashing is a problem.
2. Microwave assisted	• Highly effective-fast method. • Rapid and homogenous heating can control and speed up the process in a better way than conventional heating methods.	• Homogenous nucleation leading to crystal growth.
3. Sonochemical	• Forms of nanostructures are easily controlled by controlling the reaction conditions. • Simple-green-rapid method.	• The rate of reduction depends totally on ultrasonic frequency.
4. Gamma radiation	• Efficient, allows the synthesis of spherical, dispersed nanoparticles, less toxic than chemical methods which uses aggressive chemical agents.	
5. Low temperature reactive aerosol	Large-scale production of SeNPs producing up to hundreds of kilograms.	

9.4 Biological Synthesis of SeNPs

Many of the chemical methods that are used in the synthesis of SeNPs represent a danger to the ecosystem and are costly as well. Instead, they use the biological synthesis methods that are reliable, eco-friendly, non-toxic, most of them are done at room temperature and pressure and inexpensive, unlike the chemical methods which are high cost (Chhabria and Desai 2016a). Plants and microbes like bacteria, fungi, and protozoa are used in the synthesis of SeNPs (Sharma et al. 2014).

The SeNPs that are synthesized by microbes are of complex arrangement in their nanostructure and unique. This means the diversity of reducing enzymes that exist in various microorganisms. Some of the microorganisms could remove or decrease the toxicity which exists in metal ions by modifying the redox state of the metal ions, which leads to the synthesis of described SeNPs (Shoeibi et al. 2017). Different microorganisms are used for biosynthesis of SeNPs as in Table 9.2.

9.5 Antimicrobial Activity and Mechanism of SeNPs

The increasing numbers of pathogens appearing with antibiotic resistance have become a critical health problem and thus, several researches have been focused to increase the recent antimicrobial therapies. About 70% of bacterial infections are considered to be resistant to one or more of the antibiotics commonly used to kill the infection (Allahverdiyev et al. 2011). Therefore, the development of new and more effective antimicrobial agents is very necessary. Metals such as silver (Ag), copper (Cu), gold (Au), zinc (Zn), and also Se (Se) have antimicrobial activity with various properties, potencies, and spectra of activity (Malarkodi et al. 2014). Recently, nanotechnology is well known as one of the most technology in the scientific research, as it offers great possibilities in various fields of science and technology. Many types of nanoparticles have received massive attention because of their potential antimicrobial effects. Specially, metal nanoparticles such as silver, zinc, and Se have been commonly used in several fields due to their antimicrobial activity (Adibkia et al. 2007). Also, biogenic metal nanoparticles have recently gained more interest compared to chemically synthesized metal nanoparticles because of their low toxicity, economically feasible, and eco-friendly (Adibkia et al. 2011; Sabzevari et al. 2013).

Se (Se) is an essential trace element and one of the dietary supplements in the human diet, Se has attracted a lot of attention due to its significant role in medicine as it is incorporated into many antioxidant enzymes, such as glutathione, peroxidase, deiodinase, and thioredoxin reductase (TRx). In several processes, these enzymes play an important role as antioxidant activity to raise muscle function and prevent cancer. Therefore, Se has become antagonistic to several diseases such as cancer, brain, and heart sickness (Xu et al. 2018; Mehdi et al. 2013). SeNPs have some unique physicochemical features which make them noticeable in the field of bio-medicine, photoelectrochemistry, and catalysis for the technological applications. Different microbes have been considered as a possible biocatalyst compared to the

Table 9.2 Biosynthesis of SeNPs

Biological method	Nanoparticles-morphology	Adv	Reference
Bacillus cereus	110 nm, spherical shape	Do not have environmental contamination	Pouri et al. (2016)
Pantoea agglomerans	Smaller than 100 nm, spherical	Eco-friendly, high antioxidant activity	Torres et al. (2012)
Vitis vinifera (raisin)	3–18 nm, spherical	Eco-friendly, non-toxic nature	Sharma et al. (2014)
Klebsiella pneumoniae	100–550 nm	Non-toxic, eco-friendly	Fesharaki et al. (2009)
Diospyros montana leaf extract	4–16 nm, spherical, crystalline	Stable	Kokila et al. (2017)
Withania somnifera leaf extract	45–90 nm, spherical, crystalline	Low cost	Alagesan and Venugopal (2018)
Bacterium *Zooglea ramigera*	30–150 nm, spherical	Stable, eco-friendly, uniform nanostructure	Srivastava and Mukhopadhyay (2013)
Fenugreek seed extract	50–150 nm, oval with smooth surface	Inexpensive, eco-friendly	Ramamurthy et al. (2013)
Terminalia arjuna leaf extract	10–80 nm, crystalline in shape	Minimizing toxic hazards	Prasad and Selvaraj (2014)
Archaea-haloarchaeon *Halococcus salifodinae*	28 nm, rod shaped, hexagonal crystal lattice	Eco-friendly	Rajasree and Gayathri (2014)
Lactic acid bacteria-species of lactobacillus	20–150 nm, spherical	Non-toxic, eco-friendly	Srivastava et al. (2014)
Sargassum latifolium	22.31–95.16 nm, spherical shape	Eco-friendly	El-Khateeb et al. (2019)
Cassia auriculata leaf extract	Amorphous, crystalline surface	Stable	Anu et al. (2020b)
Broccoli	50–150 nm	Eco-friendly	Kapur et al. (2017)

conventional methods to reduce selenite salts to non-toxic SeNPs, which are more eco-friendly (Sweety et al. 2016; Nancharaiah and Lens 2015b). In medical applications, SeNPs have been used as antimicrobial and anticancer agents (Chhabria and Desai 2016b). Bacteria have been shown to demonstrate excellent selenium tolerance and to be able to bio-SeNPs under anaerobic or aerobic conditions via the detoxification (Li et al. 2014; Deshpande et al. 1993).

So, bacteria are generally regarded as a biological source to synthesize SeNPs such as *Sulfurospirillum barnesii*, *Bacillus* sp., *Klebsiella pneumoniae*, *Selenihalanaerobacter shriftii*, etc. (Oremland et al. 2004; Fesharaki et al. 2010; Shakibaie et al. 2015). During the synthesis and purification of biogenic SeNPs from microbes, it was discovered that biosynthesized SeNPs could be used in anticancer drug (Chen et al. 2008; Luo et al. 2012), antimicrobial compounds (Li et al. 2017a), targeted drug deliveries (Maiyo and Singh 2017), and antioxidant agents (Yu et al.

2016b). As for the special antibacterial activity of bio-SeNPs, there are about 99% inhibition effects have been observed with relatively low concentration of bio-SeNPs for many pathogens such as *P. aeruginosa*, *S. aureus*, *E. coli*, and *Enterococcus faecalis* (Srivastava and Mukhopadhyay 2015; Webster and Tran 2011; Hariharan et al. 2012). However, the antibacterial activity of SeNPs has been documented to rely on the concentration, size, and method of synthesis. Scientists proved that SeNPs' antibacterial properties reduced with the increase in their particles size in the range of 81–205 nm (Huang et al. 2019).

Although SeNPs attracted many scientists as a possible future antimicrobial agent, there is restricted number of researches on the antibacterial activity and possible mechanisms of bio-SeNPs. there are few references reported about the antimicrobial mechanism of bio-SeNPs. Beheshti et al. have confirmed that *Bacillus* sp. synthesized bio-SeNPs could help apoptosis of protozoa leishmania major via DNA breaking up (Beheshti et al. 2013). In addition, Emanuele et al. noted that the adding of bio-SeNPs could efficiently enhance the generation of reactive oxygen species (ROS), but their intensity did not depend on either size or shape (Emanuele et al. 2015). However, the antimicrobial activity of other nanoparticles has generally been achieved through mitochondrial destruction, cell membrane disruption, and even interruption of transmembrane electron transport (Hajipour et al. 2012). The antibacterial properties of SeNPs have been documented in prior studies.

Also, the biosynthesized SeNPs decreases the growth of some foodborne bacteria, such as *B. Cereus*, *E. faecalis*, and *S. aureus*. Furthermore, the growth of *E. coli* and *S. aureus* is completely inhibited by SeNPs (average size of 115 nm) (Guisbiers et al. 2016; Zhang et al. 2019).

They found that the bio-SeNPs displayed strong inhibition activity against pathogens' growth. Most of the studied bacteria were killed within 12 h when treated with 500 mg/L SeNPs, of which the Gram-negative bacteria mortality rates were much higher. The leakage tests showed that after interacting with bio-SeNPs, there were more proteins and polysaccharides outside the cells. It has been shown that protein and polysaccharide leaks are caused by membrane permeability changes and cell wall disruption. And the change in the strength of reactive oxygen species (ROS) has shown the oxidative damage, which is important part in antibacterial manners. The results showed the ability to use the biosynthesized SeNPs as antibacterial agents for infectious bacterial diseases (Zhang et al. 2020).

Also, the antiviral activity of SeNPs gained the attention of the scientists. As an example, the mortality of Se deficient mice with the H1N1 influenza virus was three times higher compared to those which getting Na_2SeO_3 at a dose of 0.5 mg Se/kg, and mice with low serum selenium concentrations reported a decrease in body weight (BW) and lower TNF-α and IFN γ concentrations (Yu et al. 2011).

The administration of SeNPs may also be an important and feasible approach to enhance the immune response in the body. Li et al synthesized surface modified SeNPs (Se@OTV) of oseltamivir (OTV) with a better antiviral property and drug resistance restriction (Li et al. 2017b).

Though the clinical use of OTV itself as a real antiviral agent is typically restricted by the existence of drug resistant viruses, SeNPs' OTV decoration clearly

prevented H1N1 influenza virus infection and showed low toxicity. By inhibiting the activity of influenza virus glycoproteins-hemagglutinin and neuraminidase, Se@OTV interfered withH1N1's entry into host cells. Modified NPs have been able to prevent Madin-Darby canine kidney cells from getting infected by H1N1 and block chromatin and DNA breakdown (Yang et al. 2017).

Moreover, they suppressed the production of both ROS and of phosphorylation of cellular tumor antigen p53 and Akt. Thus, ROS play a vital part in the antiviral action. The H1N1 virus improved the production of intracellular ROS from 100% (control) to 380%. The OTV and SeNPs marginally reduced the generation of ROS to 270% and 210%, respectively. However, Se@OTV significantly reduced the production of ROS (120%). Therefore, this indicates the efficiency of Se@OTV as antiviral agents against H1N1 (Yang et al. 2017).

9.6 Surface Decoration of SeNPs

Generally, the surface of nanoparticles (NPs) has to be very hydrophilic and able to inhibit the adsorption of proteins (Nie et al. 2007). Hydroxyl, carboxyl, and amino are hydrophilic groups, most natural polysaccharides can adhere to biological tissues (primarily epithelial and mucous membranes) via non-covalent bonds. Although nanoparticles treat cancer cells through the passive targeting process, they still have limitations due to their nonspecific delivery mode. However, when nanoparticles are functionalized with polysaccharides derived from microalgae, macroalgae, micro seaweed, or bacteria, they can interact with biological targets specifically (Lotan and Raz 1988). Therefore, the surface modification of nanomaterials by polysaccharides could improve cell-permeating and cancer-targeting capabilities (Nie et al. 2007). There are different mechanisms of surface of SeNPs on normal and cancer cells (Fig. 9.2).

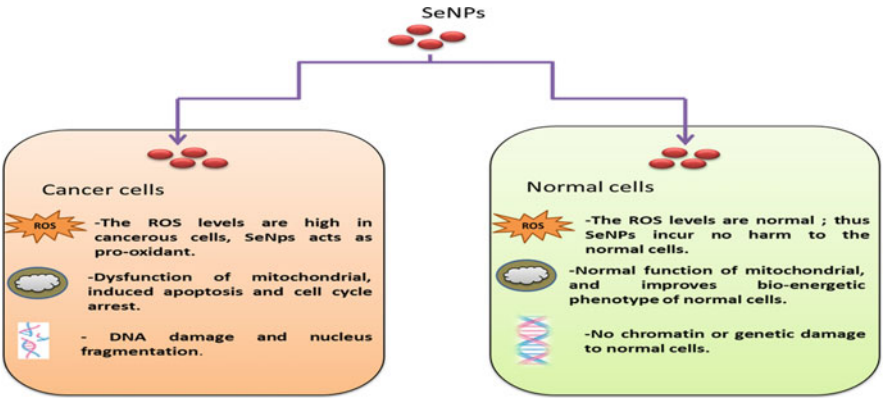

Fig. 9.2 Different mechanisms of surface of SeNPs against normal and cancer cell

SeNPs show high ROS levels cancer cells, and act as pro-oxidant. Dysfunction of mitochondrial induced apoptosis and cell cycle arrest, DNA damage, and nucleus fragmentation. On the other hand, SeNPs show that the ROS levels are normal, thus SeNPs incur no harm to the normal cells and improve the bio-energetic phenotype of normal cells. No genetic damage for the normal cells.

Also, SeNPs improved genetic actions and have superior adsorptive capacity due to the interaction between the SeNP and NH, C=O, COO− and C-N groups of proteins. Recently, the use of SeNPs in gene delivery studies has gained momentum. Most studies have focused on the development of multifunctional vehicles to deliver therapeutic nucleic acid. The modification of the particles is essential in order to confer reactivity to the nucleic acid and also to allow drug attachment. Se using as gene nanocarriers remains largely unexplored. The chemistry plays an essential role in multidisciplinary branch of science including different areas of electronics, biomedical applications, environmental concerns, therapeutics, and assures to play a central role in upcoming research areas (Guo et al. 2010; Elingarami et al. 2014). The well tunable and multivalent surface structures of SeNPs provide a better platform to integrate several therapeutics or bio-macromolecules by covalent or non-covalent combinations of the SeNPs' surface. Furthermore, these bio-conjugations over SeNPs are usually believed as the "worthy" product that can do wonders in joint combination in comparison to each individual component. In this segment, the central attention is on the functionalization of the SeNPs' surface with different kind of biological macromolecules including different types of proteins, peptides, nucleic acids, lipids, fatty acids, carbohydrates, etc.

9.6.1 5-Fluorouracil (5-FU)

The surface fabrication of SeNPs with 5-fluorouracil (5-FU) enhances the anticancer activity of formed particles (Liu et al. 2012). The 5-FU functionalized SeNPs possess higher stability of around 200 h under physiological conditions of pH = 7.4. 5-FU and have the ability to work as a weak acid in aqueous media and it is easily engulfed by the cancer cells and easily transported via uracil.

9.6.2 *Polyporus rhinoceros*

Water-soluble polysaccharides–protein complexes (PRW) of *Polyporus rhinoceros* has a small molecular weight (4×10^5 96 kDa) and has high protein content (41.3%) and mannose (12.1%) (Lai et al. 2008). In special, the apoptosis-promoting signaling pathway and the PRW-SeNPs were examined on the lung adenocarcinoma A549 cells. Wu et al. use the PRW of *Polyporus rhinoceros* for the surface functionalization of SeNPs, which has higher anti-proliferative activity for lung and breast cancer (Wu et al. 2013). The surface decoration of SeNPs with PRW can enhance the cellar uptake via endocytosis with greater stability. The developed monodisperse, spherical PRW-SeNPs particles offered suitable size distribution and

stability in the solution. The conjugated PRW-SeNPs considerably prevented the growth of A549 cells through initiation of apoptosis and G2/M phase capture (Wu et al. 2013).

9.6.3 Chitosan

On the other hand, Yu et al. have carried out the surface functionalization of SeNPs with chitosan (CS). The positive charge of the NH_3^+ group of CS on the nanoparticle surface consolidates the water solubility of the respective system, furthermore averts the plasma protein adsorption and ameliorates the cell penetration ability of the nanoparticle. The internalization of CS functionalized SeNPs further augment via the interaction of NH_3^+ group with the phosphoryl groups of phospholipid components in the cell membrane (Yu et al. 2012a).

9.6.4 Other Amino Acids

Feng et al. showed the capability of amino acids for the surface functionalization of SeNPs (Feng et al. 2014). Being an active component of proteins and intermediate product of various metabolic activities, these amino acids provide better stability to SeNPs, and enhance the anticancer efficacy. Comparison of different amino acids such as neutral (valine), acidic (aspartic acid), and basic (lysine) has shown a spacious spectrum of surface modification. The pH difference has paid the greatest attention in drug targeting and release. The pH standards in the human body differ between organs and tissues, which make the pH as important factor for drug delivery (Wang et al. 2010). In addition, encapsulation conjugated with targeting groups such as folic acid (FA), and Arginine-Glycine-Aspartic acid (RGD) can specifically be directed to the uptake by cancer cells that overexpress their receptors by endocytosis and provide drugs into the cytosol (Zhou et al. 2009; Hu et al. 2012). However, the contact between the conjugated proteins and the nanoparticles remains unknown. Some scientists have examined the contact between amino acids and nanoparticles (Nel et al. 2009; Blair et al. 2012). For instance, Yu et al. explored that the peptide binding manner in the construction of gold nanostructures should be reflected a three-step sequence, which is greatly dependent on amino acid structure and sequence. The SeNPs prepared under a simple redox system, which was modified by amino acids on its surface (Yu et al. 2012b).

9.6.5 Proteins or Polypeptide

There are several researches about the surface NPs engineered by protein or polypeptide (Mooney et al. 2010). For instance, researchers were studied the modification of SeNPs surface by bacterial protein and bovine serum albumin (BSA) (Yu et al. 2013; Rouse et al. 2007). Kong et al. found that BSA-functionalized

SeNPs could reduced the growth of LNCaP prostate cancer cell by the stimulation of cell apoptosis via Akt/Mdm2/AR pathways (Kong et al. 2011). Additionally, SeNPs reduce the transcriptional action of the androgen receptor (AR) through down-regulating the mRNA and protein expression. SeNPs trigger Akt kinase promoting Akt-dependent AR phosphorylation and Mdm2 regulated degradation via the proteasome pathway. So, SeNPs conquer prostate cancer cells growth by androgen receptor disruption, associating a prospective use in the cancer therapy (Kong et al. 2011).

9.6.6 Natural Products

Wang et al. demonstrated the effectiveness of SeNPs to overcome intraperitoneally injected H22 hepatic cancer cells. Remarkably, the NPs were initiated to be localized inside the cancer cells and reduced the generation of reactive oxygen species (ROS) (Wang et al. 2014). In vivo efficacy of SeNPs in hepatocarcinoma was verified by Ahmed et al., and the SeNPs were lessening the hepatocarcinoma. Also, SeNPs can induce ROS, which facilitate cancer cell death, reduce the anti-oxidation, and prevent the cancer progress in the healthy tissues at low doses (Ahmed et al. 2014). Though they are hopeful anticancer agents with rarer side effects, their application is restricted by their comparatively low toxicity to cancer cells (Zhuang et al. 2020). Such mitochondrion-targeted SeNPs could professionally raise ROS assembly and mitochondrion destruction in cancer cells. Lettering by Guo et al. the curcumin plays a significant part in the antitumor agent, whereas its clinical use is imperfect by its poor solubility. The anticancer activity of an aqueous formulations of Se, curcumin, and Se@ curcumin were considered (Fig. 9.3) (Guo et al. 2017). Curcumin, a hydrophobic polyphenol derived from turmeric *Curcuma longa*, has anticancer, antioxidant, antibacterial, antifungal, and antiviral activities (García-Niño and Pedraza-Chaverrí 2014; Kim et al. 2014; Abdel-Wahhab et al. 2016; Barbara et al. 2017).

Curcumin has a vital part in anticancer activity by adjusting the expression of numerous cellular targets like controlling the cancer cell proliferation, growth, invasion, and prompting apoptosis (Wang et al. 2017). Moreover, no sign of toxicity of curcumin in humans or animals, it was verified to be a natural and harmless agent (Kumari et al. 2017). So, an aqueous preparation of curcumin is vital for the clinical use.

In another report by Wu et al. mushroom polysaccharides–protein complexes (PSP) surface modification considerably improved the cellular uptake of SeNPs via endocytosis and repressed the growth of MCF-7 human breast carcinoma cells via inhibition of apoptosis with the association of PARP cleavage and caspase initiation (Wu et al. 2012). Mushroom polysaccharide has a numerous of hydroxyl groups, this distinctive chemical structure gives its strong physical adsorption onto SeNPs, eluding their aggregation and precipitation (Zhang et al. 2010). Not only the decoration of mushroom polysaccharides improved the cancer cell's uptake in the

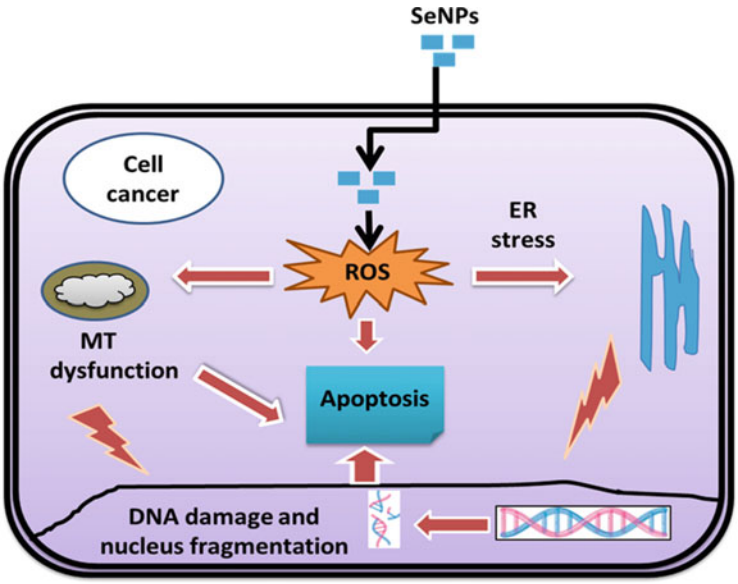

Fig. 9.3 Mode of SeNPs anticancer activity. SeNPs are thought to enter the body through receptor-mediated endocytosis. This malignant cell microenvironment causes pro-oxidant variation of SeNPs, resulting in the generation of more free radicals, which on the one hand destroys mitochondrial membrane, resulting in the leakage of mitochondrial (MT) proteins, and on the other hand causes endoplasmic reticulum stress. Damage to the MT membrane causes protein leakage and apoptosis. Furthermore, SeNPs have been demonstrated to inhibit angiogenic signaling in cancer cells, slowing their growth and proliferation. DNA damage is caused when these disruptive biological processes come together

treatment of cancer but also it displayed strong elevation of bone formation in vitro and in vivo (Wu et al. 2013; Zhang et al. 2010; Yu et al. 2018).

The surface of SeNPs was decorated with a monosaccharide sialic acid (SA) displayed a well-known cell internalization and discrimination between the cancer and normal cells. SAs are classically originated as terminal monosaccharides adhered to the cell surface glycoconjugates, which has an essential role in several biological processes, and unusual sialylation is related with various diseases, mainly cancers. For instance, SAs are overexpressed in tumor-associated glycoproteins, the recognition and definite binding of SA are vital for monitoring, examining, and adjusting cancer cells, which would have influence on the analytic and therapeutic purposes. However, the recognition of SA on the cancer cell surface is still interesting, but the instability problem could restrict its further uses (Xiong et al. 2017). Spirulina polysaccharide (SPS) from the blue-green *microalga Spirulina platensis* is supposed to have several biological purposes, immune-stimulators, free radical scavenging activity, and antiviral effect (Pugh et al. 2001). So, the SPS could be used as a surface modifier of nanomaterial to inhibit the adsorption plasma protein and improve their cell-penetrating capabilities. For example, SPS is used for

synthesis of SeNPs, which show antimicrobial activities against multidrug resistant *Klebsiella pneumoniae* (Abbas et al. 2021).

9.7 Conclusion

Recent studies on SeNPs have shown that SeNPs provide a unique form of Se with the same or higher antioxidant and biocompatibility as conventional organo-selenium compounds, but with minor toxicity. SeNPs are widely used as chemoprophylactic, therapeutic, and micronutrient alone or by limiting target molecules. In addition, some SeNPs combined with chemotherapy have shown synergistic effects acting as drug carriers. SeNPs have confirmed superior antioxidant characteristics due to their prophylactic effects linked to oxidative stress for use as a new generation of drugs to treat cancer, arthritis, diabetic, and Alzheimer's disease. SeNPs' ability to fight cancer development, which has been studied by many researchers, is due to its ability to induce apoptosis selectively only in malignant tissue. In general, SeNPs will play an important part in oncology and as the production of antibiotics. So, the increasing attention in the design and progress of an advanced nanoscale materials made from Se will increase in the years to come.

This overview focuses on depth of the green synthesis of SeNPs. This green synthetic method has turned out to be one of the most hopeful and eco-friendly methods for NPs synthesis. SeNPs unique applications are used in areas such as biosensors, the cosmetics industry, biomedicine, and agriculture. Therefore, SeNPs can be a promising candidate for future progress in the medical sector.

References

Abbas HS, Abou Baker DH, Ahmed EA (2021) Cytotoxicity and antimicrobial efficiency of selenium nanoparticles biosynthesized by Spirulina platensis. Arch Microbiol 203:523–532. https://doi.org/10.1007/s00203-020-02042-3

Abdel-Wahhab MA, Salman AS, Ibrahim MI, El-Kady AA, Abdel-Aziem SH, Hassan NS, Waly AI (2016) Curcumin nanoparticles loaded hydrogels protects against aflatoxin B1-induced genotoxicity in rat liver. Food Chem Toxicol 94:159–171

Adibkia K, Omidi Y, Siahi MR, Javadzadeh AR, Barzegar-Jalali M, Barar J, Maleki N, Mohammadi G, Nokhodchi A (2007) Inhibition of endotoxin-induced uveitis by methylprednisolone acetate nanosuspension in rabbits. J Ocul Pharmacol Ther 23:421–432

Adibkia K, Javadzadeh Y, Dastmalchi S, Mohammadi G, Niri FK, Alaei-Beirami M (2011) Naproxen–eudragit RS100 nanoparticles: preparation and physicochemical characterization. Colloids Surf B Biointerfaces 83:155–159

Ahmed HH, Khalil WK, Hamza AH (2014) Molecular mechanisms of Nano-Se in mitigating hepatocellular carcinoma induced by N-nitrosodiethylamine (NDEA) in rats. Toxicol Mech Methods 24(8):593–602

Alagesan V, Venugopal S (2018) Green synthesis of Se nanoparticle using leaves extract of Withania somnifera and its biological applications and photocatalytic activities. BioNanoScience 105. https://doi.org/10.1007/s12668-018-0566-8

Alam H, Khatoon N, Raza M, Ghosh PC, Sardar M (2019) Synthesis and characterization of nano Se using plant biomolecules and their potential applications. BioNanoScience 9(1):96–104

Allahverdiyev AM, Abamor ES, Bagirova M, Rafailovich M (2011) Antimicrobial effects of TiO_2 and Ag_2O nanoparticles against drug-resistant bacteria and leishmania parasites. Future Microbiol 6:933–940

Anu K, Devanesan S, Prasanth R, AlSalhi MS, Ajithkumar S, Singaravelu G (2020a) Biogenesis of SeNPs and their antileukemia activity. J King Saud Univ Sci 32(4):2520–2526

Anu K, Devanesan S, Prasanth R, AlSalhi M, Ajithkumar S, Singaravelu G (2020b) Biogenesis of SeNPs and their anti-leukemia activity. J King Saud Univ Sci 32:2520. https://doi.org/10.1016/j.jksus.2020.04.018

Bai Y, Qin B, Zhou Y, Wang Y, Wang Z, Zheng W (2011) Preparation and antioxidant capacity of element SeNPs sol–gel compounds. J Nanosci Nanotechnol 11(6):5012–5017

Barbara R, Belletti D, Pederzoli F, Masoni M, Keller J, Ballestrazzi A, Grabrucker AM (2017) Novel Curcumin loaded nanoparticles engineered for Blood-Brain Barrier crossing and able to disrupt Abeta aggregates. Int J Pharm 526(1–2):413–424

Barnaby S, Sarker N, Dowdell A, Banerjee I (2011) The spontaneous formation of SeNPs on gallic acid assemblies and their antioxidant properties. Fordham Undergraduate Res J 1:41–46

Beheshti N, Soflaei S, Shakibaie M, Yazdi MH, Ghaffarifar F, Dalimi A, Reza Shahverdi A (2013) Efficacy of biogenic SeNPs against Leishmania major: in vitro and in vivo studies. J Trace Elem Med Biol 27(3):203–207. https://doi.org/10.1016/j.jtemb.2012.11.002

Blair AC, Fisher ER, Rickey D (2012) Discovering nanoscience. Science 337(6098):1056–1057

Boroumand S, Safari M, Shaabani E, Shirzad M, Faridi-Majidi R (2019) SeNPs: synthesis, characterization and study of their cytotoxicity, antioxidant and antibacterial activity. Mater Res Exp 6(8):0850d8

Buchs B, Evangelou MW, Winkel LH, Lenz M (2013) Colloidal properties of nanoparticular biogenic selenium govern environmental fate and bioremediation effectiveness. Environ Sci Technol 47(5):2401–2407

Cai X, Wang C, Yu W, Fan W, Wang S, Shen N, Wu P, Li X, Wang F (2016) Se exposure and cancer risk: an updated meta analysis and meta-regression. Sci Rep 6:19213. https://doi.org/10.1038/srep19213

Chen T, Wong YS, Zheng W, Bai Y, Huang L (2008) SeNPs fabricated in Undaria pinnatifida polysaccharide solutions induce mitochondria-mediated apoptosis in A375 human melanoma cells. Colloids Surf B Biointerfaces 67(1):26–31. https://doi.org/10.1016/j.colsurfb.2008.07.010

Chhabria S, Desai K (2016a) SeNPs and their applications. In: Encyclopedia of nanoscience and nanotechnology, vol 4. American Scientific Publishers, USA

Chhabria S, Desai K (2016b) SeNPs and their applications. In: Nalwa HS (ed) Encyclopedia of nanoscience and nanotechnology. American Scientific Publishers, USA

Cui H, Kong Y, Zhang H (2012) Oxidative stress, mitochondrial dysfunction, and aging. J Signal Transduct 2012:646354. https://doi.org/10.1155/2012/646354

Deshpande LM, Kapadnis BP, Chopade BA (1993) Metal resistance in Acinetobacter and its relation to β-lactamase production. Biometals 6(1):55–59. https://doi.org/10.1007/BF00154233

Dwivedi C, Shah P, Singh K, Kumar M, Bajaj PN (2011) An organic acid-induced synthesis and characterization of SeNPs. J Nanotechnol 2011:651971

Elahi MM, Kong YX, Matata BM (2009) Oxidative stress as a mediator of cardiovascular disease. Oxidative Med Cell Longev 2(5):259–269. https://doi.org/10.4161/oxim.2.5.9441

Elingarami S, Deng Y, Fan J, Zhang Y, He N (2014) NEIL-2 single nucleotide polymorphism genotyping using single base extension on core–shell Fe_3O_4@ SiO_2@ Au magnetic nanoparticles and association of the genotypes with gastric cancer risk in Northern Jiangsu (China). Sci Adv Mater 6(5):899–907

El-Khateeb A, Hamed E, Ibrahim F, Hamed S (2019) Eco-friendly synthesis of Se and zinc nanoparticles with biocompatible Sargassum latifolium algae extract in preservation of edible oils. J Food Dairy Sci 10(5):141

Emanuele Z, Silvia L, Raymond JT, Junaid SSQ, Giovanni V (2015) Biogenic Se and tellurium nanoparticles synthesized by environmental microbial isolates efficaciously inhibit bacterial

planktonic cultures and biofilms. Front Microbiol 6:584. https://doi.org/10.3389/fmicb.2015.00584
Eswarapriya B, Jegatheesan K (2015) Antifungal activity of biogenic selenium nanoparticles. Synthesized from electronic waste. Int J PharmTech Res 8(3):383–386
Feng Y, Su J, Zhao Z, Zheng W, Wu H, Zhang Y, Chen T (2014) Differential effects of amino acid surface decoration on the anticancer efficacy of SeNPs. Dalton Trans 43(4):1854–1861
Fesharaki P, Nazari P, Shakibaie M, Rezaie S, Banoee M, Abdollahi M, Shahverdi A (2009) Biosynthesis of SeNPs using Klebsiella pneumoniae and their recovery by a simple sterilization process. Braz Arch Biol Technol 6:1. https://doi.org/10.1590/S1517-83822010000200028
Fesharaki PJ, Nazari P, Shakibaie M, Rezaie S, Banoee M, Abdollahi M, Shahverdi AR (2010) Biosynthesis of SeNPs using Klebsiella pneumoniae and their recovery by a simple sterilization process. Braz J Microbiol 41:461–466. https://doi.org/10.1590/S151783822010000200028
Flores-Mateo G, Navas-Acien A, Pastor-Barriuso R, Guallar E (2006) Se and coronary heart disease: a meta-analysis. Am J Clin Nutr 84(4):762–773. https://doi.org/10.1093/ajcn/84.4.762
Gangadoo S, Stanley D, Hughes RJ, Moore RJ, Chapman J (2017) The synthesis and characterisation of highly stable and reproducible SeNPs. Inorg Nano-Metal Chem 47(11):1568–1576
Gao X, Gao T, Zhang L (2003) Solution–solid growth of α-monoclinic Se nanowires at room temperature. J Mater Chem 13(1):6–8
García-Niño WR, Pedraza-Chaverrí J (2014) Protective effect of curcumin against heavy metals-induced liver damage. Food Chem Toxicol 69:182–201
González-Domínguez R, García-Barrera T, Gómez-Ariza JL (2014) Homeostasis of metals in the progression of Alzheimer's disease. Biometals 27(3):539–549. https://doi.org/10.1007/s10534-014-9728-5
Guisbiers G, Wang Q, Khachatryan E, Mimun LC, Mendoza-Cruz R, Larese-Casanova P, Webster TJ, Nash KL (2016) Inhibition of E. coli and S. aureus with SeNPs synthesized by pulsed laser ablation in deionized water. Int J Nanomedicine 11:3731–3736. https://doi.org/10.2147/IJN.S106289
Gunti L, Dass RS, Kalagatur NK (2019) Phytofabrication of SeNPs from Emblica officinalis fruit extract and exploring its biopotential applications: antioxidant, antimicrobial, and biocompatibility. Front Microbiol 10:931
Guo P, Coban O, Snead NM, Trebley J, Hoeprich S, Guo S, Shu Y (2010) Engineering RNA for targeted siRNA delivery and medical application. Adv Drug Deliv Rev 62(6):650–666
Guo M, Li Y, Lin Z, Zhao M, Xiao M, Wang C, Zhu B (2017) Surface decoration of SeNPs with curcumin induced HepG2 cell apoptosis through ROS mediated p53 and AKT signaling pathways. RSC Adv 7(83):52456–52464
Hajipour MJ, Fromm KM, Ashkarran AA, de Aberasturi DJ, de Larramendi IR, Rojo T, Mahmoudi M (2012) Antibacterial properties of nanoparticles. Trends Biotechnol 30(10):499–511. https://doi.org/10.1016/j.tibtech.2012.06.004
Hariharan H, Al-harbi N, Karuppiah P, Rajaram S (2012) Microbial synthesis of Se nanocomposite using Saccharomyces cerevisiae and its antimicrobial activity against pathogens causing nosocomial infection. Chalcogenide Lett 9:509–515. https://doi.org/10.1063/1.4757928
Hoffmann PR, Berry MJ (2008) The influence of Se on immune responses. Mol Nutr Food Res 52(11):1273–1280. https://doi.org/10.1002/mnfr.200700330
Hu C, Song G, Zhang B, Liu Z, Chen R, Zhang H, Hu T (2012) Intestinal metabolite compound K of panaxoside inhibits the growth of gastric carcinoma by augmenting apoptosis via Bid-mediated mitochondrial pathway. J Cell Mol Med 16(1):96–106
Huang T, Holden JA, Heath DE, O'Brien-Simpson NM, O'Connor AJ (2019) Engineering highly effective antimicrobial SeNPs through control of particle size. Nanoscale 11(31):14937–14951. https://doi.org/10.1039/C9NR04424H
InformedHealth.org (2006) How does the thyroid gland work? [Internet]. Institute for Quality and Efficiency in Health Care (IQWiG), Cologne, Germany. 2010 Nov 17 [Updated 2018 Apr 19]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK279388/

Jadhav A, Khanna PK (2015) Impact of microwave irradiation on cyclo-octeno-1,2,3-selenadiazole: formation of SeNPs and their polymorphs. RSC Adv 5(56):44756–44763
Jamkhande G, Ghule NW, Bamer AH, Kalaskar MG (2019) Metal nanoparticles synthesis: an overview on methods of preparation, advantages and disadvantages, and applications. J Drug Deliv Sci Technol 53:101174
Ju W, Li X, Li Z, Wu GR, Fu XF, Yang XM, Zhang XQ, Gao XB (2017) The effect of Se supplementation on coronary heart disease: a systematic review and meta-analysis of randomized controlled trials. J Trace Elem Med Biol 44:8–16. https://doi.org/10.1016/j.jtemb.2017.04.009
Kaliannan D, Palaninaicker S, Palanivel V, Mahadeo MA, Ravindra BN, Jae-Jin S (2019) A novel approach to preparation of nano-adsorbent from agricultural wastes (Saccharum officinarum leaves) and its environmental application. Environ Sci Pollut Res 26(6):5305–5314
Kapur M, Soni K, Kohli K (2017) Green synthesis of SeNPs from broccoli, characterization, application and toxicity. Adv Tech Biol Med 5(1):198. https://doi.org/10.4172/2379-1764.1000198
Khubulava S et al (2018) Preparation of SeNPs with mechano-sonochemical methods. Asian J Pharm 12:02
Kieliszek M, Błażejak S (2013) Selenium: significance, and outlook for supplementation. Nutrition 29(5):713–718
Kim S, Stébé MJ, Blin JL, Pasc A (2014) pH-controlled delivery of curcumin from a compartmentalized solid lipid nanoparticle@ mesostructured silica matrix. J Mater Chem B 2(45):7910–7917
Kokila K, Elavarasan N, Sujatha V (2017) Diospyros montana leaf extract-mediated synthesis of SeNPs and their biological applications. New J Chem 41. https://doi.org/10.1039/C7NJ01124E
Kong L, Yuan Q, Zhu H, Li Y, Guo Q, Wang Q, Gao X (2011) The suppression of prostate LNCaP cancer cells growth by SeNPs through Akt/Mdm2/AR controlled apoptosis. Biomaterials 32(27):6515–6522
Kumari M, Ray L, Purohit MP, Patnaik S, Pant AB, Shukla Y, Gupta KC (2017) Curcumin loading potentiates the chemotherapeutic efficacy of SeNPs in HCT116 cells and Ehrlich's ascites carcinoma bearing mice. Eur J Pharm Biopharm 117:346–362
Lai CK, Wong KH, Cheung PCK (2008) Antiproliferative effects of sclerotial polysaccharides from Polyporusrhinocerus Cooke (Aphyllophoromycetideae) on different kinds of leukemic cells. Int J Med Mushrooms 10(3)
Langi B, Shah C, Singh K, Chaskar A, Kumar M, Bajaj PN (2010) Ionic liquid-induced synthesis of SeNPs. Mater Res Bull 45(6):668–671
Li DB, Cheng YY, Wu C, Li WW, Li N, Yang ZC, Tong ZH, Yu HQ (2014) Selenite reduction by Shewanella oneidensis MR-1 is mediated by fumarate reductase in periplasm. Sci Rep 4:3735. https://doi.org/10.1038/srep03735
Li Y, Lin Z, Guo M, Xia Y, Zhao M, Wang C, Zhu B (2017a) Inhibitory activity of SeNPs functionalized with oseltamivir on H1N1 influenza virus. Int J Nanomedicine 12:5733–5743. https://doi.org/10.2147/IJN.S140939
Li Y, Lin Z, Guo M et al (2017b) Inhibitory activity of SeNPs functionalized with oseltamivir on H1N1 influenza virus. Int J Nanomedicine 12:5733–5743
Liao F, Han X, Zhang Y, Chen H, Xu C (2018) CTAB-assisted solvothermal synthesis of ultralong t-Se nanowires and bundles using glucose as green reducing agent. Mater Lett 214:41–44
Liu W, Li X, Wong YS, Zheng W, Zhang Y, Cao W, Chen T (2012) SeNPs as a carrier of 5-fluorouracil to achieve anticancer synergism. ACS Nano 6(8):6578–6591
Lotan R, Raz A (1988) Lectins in cancer cells. Ann N Y Acad Sci 551:385–396
Luo H, Wang F, Bai Y, Chen T, Zheng W (2012) SeNPs inhibit the growth of HeLa and MDA-MB-231 cells through induction of S phase arrest. Colloids Surf B Biointerfaces 94:304–308. https://doi.org/10.1016/j.colsurfb.2012.02.006
Maiyo F, Singh M (2017) SeNPs: potential in cancer gene and drug delivery. Nanomedicine 12(9):1075–1089. https://doi.org/10.2217/nnm-2017-0024

Mal J, Veneman WJ, Nancharaiah YV, van Hullebusch ED, Peijnenburg WJ, Vijver MG, Lens PN (2016) A comparison of fate and toxicity of selenite, biogenically and chemically synthesized selenium nanoparticles to zebrafish (Danio rerio) embryogenesis. Nanotoxicology 11(1):87–97

Malarkodi C, Rajeshkumar S, Paulkumar K, Vanaja M, Gnanajobitha G, Annadurai G (2014) Biosynthesis and antimicrobial activity of semiconductor nanoparticles against oral pathogens. Bioinorg Chem Appl 2014:1–10

Markesbery WR, Lovell MA (2006) DNA oxidation in Alzheimer's disease. Antioxid Redox Signal 8(11–12):2039–2045. https://doi.org/10.1089/ars.2006.8.2039

Mehdi Y, Hornick JL, Istasse L, Dufrasne I (2013) Se in the environment, metabolism and involvement in body functions. Molecules 18(3):3292–3311

Mooney A, Corry AJ, Ruairc CN, Mahgoub T, O'Sullivan D, O'Donovan N, Kenny PT (2010) Synthesis, characterisation and biological evaluation of N-(ferrocenyl) naphthoyl amino acid esters as anticancer agents. Dalton Trans 39(35):8228–8239

Nancharaiah YV, Lens PNL (2015a) Selenium biomineralization for biotechnological applications. Trends Biotechnol 33:323–330

Nancharaiah YV, Lens PNL (2015b) Se biomineralization for biotechnological applications. Trends Biotechnol 33(6):323–330. https://doi.org/10.1016/j.tibtech.2015.03.004

Nath S, Ghosh SK, Panigahi S, Thundat T, Pal T (2004) Synthesis of Se nanoparticle and its photocatalytic application for decolorization of methylene blue under UV irradiation. Langmuir 20(18):7880–7883

Nel AE, Mädler L, Velegol D, Xia T, Hoek EM, Somasundaran P, Thompson M (2009) Understanding biophysicochemical interactions at the nano–bio interface. Nat Mater 8(7):543–557

Nie S, Xing Y, Kim GJ, Simons JW (2007) Nanotechnology applications in cancer. AnnuRev Biomed Eng 9:257–288

Norton RL, Hoffmann PR (2012) Se and asthma. Mol Asp Med 33(1):98–106. https://doi.org/10.1016/j.mam.2011.10.003

Oremland RS, Herbel MJ, Blum JS, Langley S, Curran S (2004) Structural and spectral features of Se nanospheres produced by Se-respiring bacteria. Appl Environ Microbiol 70(1):52–60. https://doi.org/10.1128/aem.70.1.52-60.2004

Panahi-Kalamuei M, Salavati-Niasari M, Hosseinpour-Mashkani SM (2014) Facile microwave synthesis, characterization, and solar cell application of SeNPs. J Alloys Compounds 617: 627–632

Panahi-Kalamuei M, Mousavi-Kamazani M, Salavati-Niasari M, Hosseinpour-Mashkani SM (2015) A simple sonochemical approach for synthesis of Se nanostructures and investigation of its light harvesting application. J Alloys Ultrason Sonochem 23:246–256

Pham-Huy LA, He H, Pham-Huy C (2008) Free radicals, antioxidants in disease and health. Int J Biomed Sci 4(2):89–96

Pouri S, Motamedi H, Honary S, Kazeminezhad I (2016) Biological synthesis of SeNPs and evaluation of their bioavailability. Braz Arch Biol Technol 6:1. https://doi.org/10.1590/1678-4324-2017160452

Prasad K, Selvaraj K (2014) Biogenic synthesis of SeNPs and their effect on As(III)-induced toxicity on human lymphocytes. Biol Trace Elem Res 157:275. https://doi.org/10.1007/s12011-014-9891-0

Pugh N, Ross SA, ElSohly HN, ElSohly MA, Pasco DS (2001) Isolation of three high molecular weight polysaccharide preparations with potent immunostimulatory activity from Spirulinaplatensis, Aphanizomenonflos-aquae and Chlorella pyrenoidosa. Plantamedica 67(08):737–742

Puspitasari IM, Abdulah R, Yamazaki C, Kameo S, Nakano T, Koyama H (2014) Updates on clinical studies of Se supplementation in radiotherapy. Radiat Oncol (London, England) 9:125. https://doi.org/10.1186/1748-717X-9-125

Quintana M, Haro-Poniatowski E, Morales J, Batina N (2002) Synthesis of SeNPs by pulsed laser ablation. Appl Surf Sci 195(1–4):175–186

Raja S, Ramesh V, Thivaharan V (2017) Green biosynthesis of silver nanoparticles using Calliandra haematocephala leaf extract, their antibacterial activity and hydrogen peroxide sensing capability. Arab J Chem 10(2):253–261

Rajasree S, Gayathri S (2014) Extracellular biosynthesis of SeNPs using some species of lactobacillus. Indian J Geo-Marine Sci 43(5):766

Ramamurthy C, Sampath K, Arunkumar P, Kumar S, Sujatha V, Premkumar K, Thirunavukkarasu C (2013) Green synthesis and characterization of SeNPs and its augmented cytotoxicity with doxorubicin on cancer cells. Bioprocess Biosyst Eng 36(8):1131–1139. https://doi.org/10.1007/s00449-012-0867-1

Rayman MP (2005) Se in cancer prevention: a review of the evidence and mechanism of action. Proc Nutr Soc 64(4):527–542

Ribeiro Filho JN, Oliveira A, Neves M (2018) SeNPs synthesized by gamma radiation. Quarta Semana de Engenharia Nuclear e Ciências das Radiações 4:286–290

Rouse JG, Yang J, Ryman-Rasmussen JP, Barron AR, Monteiro-Riviere NA (2007) Effects of mechanical flexion on the penetration of fullerene amino acid-derivatized peptide nanoparticles through skin. Nano Lett 7(1):155–160

Sabzevari A, Adibkia K, Hashemi H, Hedayatfar A, Mohsenzadeh N, Atyabi F, Ghahremani MH, Dinarvand R (2013) Polymeric triamcinolone acetonide nanoparticles as a new alternative in the treatment of uveitis: in vitro and in vivo studies. Eur J Pharm Biopharm 84:63–71

Salem SS, Fouda A (2020) Green synthesis of metallic nanoparticles and their prospective biotechnological applications: an overview. Biol Trace Elem Res 199:344–370

Schnabel R, Lubos E, Messow CM, Sinning CR, Zeller T, Wild PS, Peetz D, Handy DE, Munzel T, Loscalzo J, Lackner KJ, Blankenberg S (2008) Se supplementation improves antioxidant capacity in vitro and in vivo in patients with coronary artery disease: the Se Therapy in Coronary Artery disease Patients (SETCAP) Study. Am Heart J 156(6):1201.e1–1201.e12011. https://doi.org/10.1016/j.ahj.2008.09.004

Schwarz K, Foltz CM (1957) Se as an integral part of factor 3 against dietary necrotic liver degeneration. J Am Chem Soc 79(12):3292–3293

Shah P, Dwivedi C, Singh KK, Kumar M, Bajaj PN (2010) Riley oxidation: a forgotten name reaction for synthesis of SeNPs. Mater Res Bull 45(9):1213–1217

Shakibaie M, Mohazab NS, Ayatollahi Mousavi SA (2015) Antifungal activity of SeNPs synthesized by Bacillus species Msh-1 against Aspergillus fumigatus and Candida albicans. Jundishapur J Microbiol 8:e26381. https://doi.org/10.5812/jjm.26381

Shar AH, Lakhan M, Wang J, Ahmed M, Alali K, Ahmed R, Ali I, Dayo A (2019) Facile synthesis and characterization of SeNPs by the hydrothermal approach. Digest J Nanomater Biostruct 14:867–872

Sharma G, Sharma A, Bhavesh R, Park J, Ganbold B, Nam J, Lee S (2014) Biomolecule-mediated synthesis of SeNPs using dried Vitis vinifera (raisin) extract. www.mdpi.com/journal/molecules, 2761. https://doi.org/10.3390/molecules19032761

Shin Y, Blackwood JM, Bae I-T, Arey BW, Exarhos GJ (2007) Synthesis and stabilization of SeNPs on cellulose nanocrystal. Mater Lett 61(21):4297–4300

Shirley R, Ord EN, Work LM (2014) Oxidative stress and the use of antioxidants in stroke. Antioxidants (Basel, Switzerland) 3(3):472–501. https://doi.org/10.3390/antiox3030472

Shoeibi S, Mozdziak P, Narenji A (2017) Biogenesis of SeNPs using green chemistry. Springer International Publishing, Cham, pp 2–3. https://doi.org/10.1007/s41061-017-0176-x

Spiller H, Pfiefer E (2007) Two fatal cases of Se toxicity. Forensic Sci Int 171:67–72. https://doi.org/10.1016/j.forsciint.2006.06.077

Srivastava N, Mukhopadhyay M (2013) Biosynthesis and structural characterization of SeNPs mediated by Zooglea ramigera. Powder Technol 244:26–29. https://doi.org/10.1016/j.powtec.2013.03.050

Srivastava N, Mukhopadhyay M (2015) Green synthesis and structural characterization of SeNPs and assessment of their antimicrobial property. Bioprocess Biosyst Eng 38(9):1723–1730. https://doi.org/10.1007/s00449-015-1413-8

Srivastava P, Braganca J, Kowshik M (2014) In vivo synthesis of SeNPs by Halococcus salifodinae BK18 and their anti-proliferative properties against HeLa cell line. Biotechnol Prog 30:1480–1487. https://doi.org/10.1002/btpr.1992

Steinbrenner H, Al-Quraishy S, Dkhil MA, Wunderlich F, Sies H (2015) Dietary Se in adjuvant therapy of viral and bacterial infections. Adv Nutr (Bethesda, Md) 6(1):73–82. https://doi.org/10.3945/an.114.007575

Sunde RA (2010) Selenium. In: Coates PM, Betz JM, Blackman MR et al (eds) Encyclopedia of dietary supplements, 2nd edn. London, Informa Healthcare, pp 711–718

Surveswaran S, Kamble MY, Yadav SR, Sun M (2009) Molecular phylogeny of Ceropegia (Asclepiadoideae, Apocynaceae) from Indian Western Ghats. Plant Syst Evol 281:51–63. https://doi.org/10.1007/s00606-009-0182-8

Suslov A, Bikorimana S, Lama P, Sukharenko V, Walser A, Dorsinville R (2017) Synthesis of SeNPs and their nonlinear optical properties. J Nonlinear Opt Phys Mater 26(02):1750016

Sweety AW, Utkarsha US, Richa S, Balu AC (2016) Biogenic SeNPs: current status and future prospects. Appl Microbiol Biotechnol 100(6):2555–2566. https://doi.org/10.1007/s00253-016-7300-7

Torres S, Campos V, León C, Rodríguez-Llamazares S, Rojas S, González M, Smith M, Mondaca M (2012) Biosynthesis of SeNPs by Pantoea agglomerans and their antioxidant activity. J Nanopart Res 14:1236. https://doi.org/10.1007/s11051-012-1236-3

Ventura M, Melo M, Carrilho F (2017) Se and thyroid disease: from pathophysiology to treatment. Int J Endocrinol 2017:1297658. https://doi.org/10.1155/2017/1297658

Verma P, Maheshwari SK (2018) Preparation of sliver and SeNPs and its characterization by dynamic light scattering and scanning electron microscopy. J Microsc Ultrastruct 6(4):182

Viacava K, Ammann E, Bravo D, Lenz M (2020) Low-temperature reactive aerosol processing for large-scale synthesis of SeNPs. Ind Eng Chem Res 59(36):16088–16094

Wadhwani SA, Shedbalkar UU, Singh R, Chopade BA (2016) Biogenic SeNPs: current status and future prospects. Appl Microbiol Biotechnol 100(6):2555–2566

Wang H, Zhang J, Yu H (2007) Elemental Se at nano size possesses lower toxicity without compromising the fundamental effect on selenoenzymes: comparison with selenomethionine in mice. Free Radic Biol Med 42(10):1524–1533

Wang Y, Yan Y, Cui J, Hosta-Rigau L, Heath JK, Nice EC, Caruso F (2010) Encapsulation of water-insoluble drugs in polymer capsules prepared using mesoporous silica templates for intracellular drug delivery. Adv Mater 22(38):4293–4297

Wang X, Sun K, Tan Y, Wu S, Zhang J (2014) Efficacy and safety of SeNPs administered intraperitoneally for the prevention of growth of cancer cells in the peritoneal cavity. Free Radic Biol Med 72:1–10

Wang L, Chen X, Du Z, Li G, Chen M, Chen X, Chen T (2017) Curcumin suppresses gastric tumor cell growth via ROS-mediated DNA polymerase γ depletion disrupting cellular bioenergetics. J Exp Clin Cancer Res 36(1):1–14

Webster TJ, Tran (2011) SeNPs inhibit Staphylococcus aureus growth. Int J Nanomedicine 6:1553–1558. https://doi.org/10.2147/IJN.S21729

Wu H, Li X, Liu W, Chen T, Li Y, Zheng W, Wong KH (2012) Surface decoration of SeNPs by mushroom polysaccharides–protein complexes to achieve enhanced cellular uptake and antiproliferative activity. J Mater Chem 22(19):9602–9610

Wu H, Zhu H, Li X, Liu Z, Zheng W, Chen T, Wong KH (2013) Induction of apoptosis and cell cycle arrest in A549 human lung adenocarcinoma cells by surface-capping SeNPs: an effect enhanced by polysaccharide–protein complexes from Polyporusrhinocerus. J Agric Food Chem 61(41):9859–9866

Xiong Y, Li M, Lu Q, Qing G, Sun T (2017) Sialic acid-targeted biointerface materials and bio-applications. Polymers 9(7):249

Xu C, Qiao L, Guo Y, Ma L, Cheng Y (2018) Preparation, characteristics and antioxidant activity of polysaccharides and proteins capped SeNPs synthesized by Lactobacillus casei ATCC 393. Carbhydr Polym 195:576–585

Yang Y, Matsubara S, Xiong L, Hayakawa T, Nogami M (2007) Solvothermal synthesis of multiple shapes of silver nanoparticles and their SERS properties. J Phys Chem C 111(26):9095–9104
Yang J, Shim SM, Nguyen TQ et al (2017) Poly-γ-glutamic acid/chitosan nanogel greatly enhances the efficacy and heterosubtypic cross-reactivity of H1N1 pandemic influenza vaccine. Sci Rep 7: 44839
Yu L, Sun L, Nan Y, Zhu LY (2011) Protection from H1N1 influenza virus infections in mice by supplementation with selenium: a comparison with selenium-deficient mice. Biol Trace Elem Res 141(1–3):254–261
Yu B, Zhang Y, Zheng W, Fan C, Chen T (2012a) Positive surface charge enhances selective cellular uptake and anticancer efficacy of SeNPs. Inorg Chem 51(16):8956–8963
Yu J, Becker ML, Carri GA (2012b) The influence of amino acid sequence and functionality on the binding process of peptides onto gold surfaces. Langmuir 28(2):1408–1417
Yu C, Wo F, Shao Y, Dai X, Chu M (2013) Bovine serum albumin nanospheres synchronously encapsulating "gold selenium/gold" nanoparticles and photosensitizer for high-efficiency cancer phototherapy. Appl Biochem Biotechnol 169(5):1566–1578
Yu B, You P, Song M, Zhou Y, Yu F, Zheng W (2016a) A facile and fast synthetic approach to create SeNPs with diverse shapes and their antioxidation ability. New J Chem 40(2):1118–1123
Yu B, You P, Song M, Zhou Y, Yua F, Zheng W (2016b) A facile and fast synthetic approach to create SeNPs with diverse shapes and their antioxidation ability. New J Chem 40:1118–1123. https://doi.org/10.1039/c5nj02519b
Yu S, Luk KH, Cheung ST, Kwok KWH, Wong KH, Chen T (2018) Polysaccharide-protein complex-decorated Se nanosystem as an efficient bone-formation therapeutic. J Mater Chem B 6(32):5215–5219
Zeng K, Chen S, Song Y, Li H, Li F, Liu P (2013) Solvothermal synthesis of trigonal Se with butterfly-like microstructure. Particuology 11(5):614–617
Zeng S, Ke Y, Liu Y, Shen Y, Zhang L, Li C, Liu A, Shen L, Hu X, Wu H (2018) Synthesis and antidiabetic properties of chitosan-stabilized SeNPs. Colloids Surf B Biointerfaces 170:115–121
Zhang JS, Gao XY, Zhang LD, Bao YP (2001) Biological effects of a nano red elemental selenium. Biofactors 15(1):27–38
Zhang J, Wang X, Xu T (2008) Elemental Se at nano size (Nano-Se) as a potential chemopreventive agent with reduced risk of Se toxicity: comparison with se-methylselenocysteine in mice. Toxicol Sci 101(1):22–31
Zhang Y, Wang J, Zhang L (2010) Creation of highly stable SeNPs capped with hyperbranched polysaccharide in water. Langmuir 26(22):17617–17623
Zhang W, Zhang J, Ding D, Zhang L, Muehlmann LA, Deng SE et al (2018) Synthesis and antioxidant properties of Lycium barbarum polysaccharides capped SeNPs using tea extract. Artif Cells Nanomed Biotechnol 46(7):1463–1470
Zhang HL, Zhou H, Bai JD, Li Y, Yang J, Ma Q, Qu YY (2019) Biosynthesis of SeNPs mediated by fungus Mariannaea sp. HJ and their characterization. Colloids Surf A 571:9–16. https://doi.org/10.1016/j.colsurfa.2019.02.070
Zhang H, Li Z, Dai C, Wang P, Fan S, Yu B, Qu Y (2020) Antibacterial properties and mechanism of SeNPs synthesized by Providencia sp. DCX. Environ Res. https://doi.org/10.1016/j.envres.2020.110630
Zhou Z, Shen Y, Tang J, Fan M, Van Kirk EA, Murdoch WJ, Radosz M (2009) Charge-reversal drug conjugate for targeted cancer cell nuclear drug delivery. Adv Funct Mater 19(22): 3580–3589
Zhuang Y, Li L, Feng L, Wang S, Su H, Liu H, Wu Y (2020) Mitochondrion-targeted SeNPs enhance reactive oxygen species-mediated cell death. Nanoscale 12(3):1389–1396

Part III

Nanotechnology and Drug Carriers

Nanotechnology in Drug Delivery Systems: Ways to Boost Bioavailability of Drugs

10

Touseef Amna, M. Shamshi Hassan, Fatehia Nasser Gharsan, Suriya Rehman, and Faheem A. Sheikh

Abstract

Medicinal plants and their products have been utilized ever since prehistoric era for health and medicinal assessment and are exploited thus far. Undoubtedly, robust utilization of medicinal wealth has resulted progressive exhaustion. Additionally, these plant by-products face tribulations such as inadequate absorption at specific sites. To overcome proper accumulation problems, nanotechnology is playing a significant role. This chapter aims to highlight novel drug delivery systems, their challenges and potential in innovative nanomedicines. In present times, it has been well recognized that nanocarrier systems encompass multifaceted relevances than conservative formulations. Currently, imperative thoughtfulness is being given to synthesis of therapeutic formations comprising biocompatible nanocomposites, e.g., micellar, liposomes, nanocapsules as well nanofibers, etc. These aforesaid delivery arrangements usually are polymeric in nature and possess nanostructured morphology and consequently are used to offer targeted delivery of drugs and to improve bioavailability. Certainly, nanomedicine and nano-delivery systems are moderately new, however, swiftly emerging discipline possessing supplies in nanoscale dimensions are engaged to deliver purposes such as diagnostic apparatus or to carry therapeutic drugs to

T. Amna (✉) · F. N. Gharsan
Department of Biology, Faculty of Science, Albaha University, Albaha, Saudi Arabia

M. S. Hassan
Department of Chemistry, Faculty of Science, Albaha University, Albaha, Saudi Arabia

S. Rehman
Department of Epidemic Diseases Research, Institute for Research and Medical Consultations (IRMC), Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia

F. A. Sheikh
Department of Nanotechnology, University of Kashmir, Srinagar, Jammu and Kashmir, India

S. Hameed, S. Rehman (eds.), *Nanotechnology for Infectious Diseases*,
https://doi.org/10.1007/978-981-16-9190-4_10

desired locations in an organized mode. Nanotechnology proffers manifold applications in curing persistent sicknesses in human beings by targeted delivery of accurate medicines. Currently, a plethora of uses of nanomedicine are there; for instance, chemotherapeutic drugs, biological materials, immunotherapeutic agents, etc. for cure of a range of ailments. Accordingly, present chapter portrays rationalized synopsis of current progress in area of nano-oriented drug carriers and nanomedicines. These nano-based drug delivery systems have improved effectiveness of old as well as new drugs, eventually, accurate diagnosis via novel indicators. In addition, the limitations and benefits of nanomedicine have also been discussed in brief.

Keywords

Delivery systems · Nanofibers · Quantum dots · Nanoparticles · Drugs · Nanohydrogels · Nanoemulsions · Nanomedicine

10.1 Introduction

Plant oriented natural products are being utilized as medicines for a variety of ailments since prehistoric era. Admittedly, the natural products demonstrate extraordinary properties, viz. astonishing chemical variety, chemical and biological characteristics with macromolecular explicitly as well as negligible toxicity (Rehman 2016; Rehman et al. 2019; Aldakheel et al. 2020). The aforementioned properties craft their approval for unearthing of new drugs. Additionally, computational research has facilitated to visualize molecular communications of drugs and formulate future drug discovery such as targeted drug discovery and drug delivery. Nevertheless, compound derived from natural products is currently being validated for curing various most important sicknesses such as cancer, diabetes, cardiovascular, inflammatory, and microbial infections. It is primarily due to unique characteristics and advantages of natural drugs, for instance, negligible toxicity and side effects, economic cost, and excellent therapeutic prospective. Conversely, the unease connected with the biocompatibility, and toxicity of natural compounds displays a bigger dispute for utilization as medicine.

Keeping in contemplation the restrictions attached with output and vulnerability of flora as sources of preferred pharmaceuticals. Accordingly, loads of natural compounds are not passing clinical trial stages due to these tribulations. The utilization of bulky materials in drug delivery pretense big challenges, such as in vivo unsteadiness, meager bioavailability, and less solubility, negligible absorption in body, complications with targeted delivery, and stimulant efficacy, as well as possible unpleasant effects of drugs. As a result, utilization of novel drug delivery carriers for targeting drugs to definite body parts might be an alternative which can resolve these critical problems. To augment their therapeutic outcomes and diminish connected aftereffects, active drug molecules ought to be discriminatorily mount up on infection region for an expanded time with towering control (Amna et al. 2020).

In this direction nanotechnology depicts noteworthy responsibility in sophisticated medicine/drug formulations, targeting dome and their restricted drug liberation and release with enormous victory (Patra et al. 2018; Rehman et al. 2021a). The flow in nanomedicine study all through the previous several years is currently decoding into substantial commercialization works all over the world, with numerous goods in the marketplace and a rising numeral under manufacture (Nahvi et al. 2021; Qureshi et al. 2021). At present, nanomedicine is subjugated by drug delivery systems, claiming approximately 75% of collective sales (Bamrungsap et al. 2012). Drug delivery is procedure of managing a pharmaceutical component to attain remedial effects in human beings as well as animals. In other words the drug delivery implies to methods, formulations, machinery, and schemes for carrying therapeutics in body to desired locations as safe and sound as well as competently accomplish their much preferred therapeutic outcomes (Liu et al. 2016). Generally speaking, for the healing of human sicknesses, nasal and pulmonary passages of drug delivery are attracting escalating significance. As a result, delivering drugs at proscribed flow as well as, on purpose delivery, are the other superior ways which have dynamically been in practice.

The present chapter provides an overview about the various methods tailored for targeted delivery of novel and potent drugs. Nevertheless, our focus is only on various commonly used drug delivery systems/or carriers such as nanoparticles (NPs), quantum dots (QDs), electrospun nanofibers, nanoemulsions, coreshell nanoparticles (CSNs), and nanohydrogels in this chapter.

10.2 Nanotechnology Oriented Drug Delivery Systems

Nanotechnology oriented therapeutics are tremendously being utilized to enhance drug solubility, steadiness, and lessening broad spectrum confrontation and to improve protection and effectiveness of treatment (Rehman et al. 2020a). Here are several competent carriers in nanotechnology-oriented drug delivery systems such as nanoparticles, dendrimers, polymeric micelles, liposomes, polymeric drug conjugates, exosomes, polymersomes (Fig. 10.1), etc.

It is not easy to cover all these delivery methods in this chapter but we have discussed some commonly used competent carriers in nanotechnology-based drug delivery systems (Fig. 10.2).

10.3 Nanoparticles as Drug Carrier

Nanoparticles (NPs) are quickly budding nanocarriers which can be characterized as ultra-dispersed solid supramolecular moieties generally having dimension in the range of 10–1000 nm (Rehman et al. 2020b). In recent times, impending attention in the area of nanopharmaceuticals has produced quite a lot progression. NPs have a prospective influence on the cure of illnesses and infections, in which drugs could either be encapsulated, entrapped, dissolved, or adhered to NP matrix behaving as a

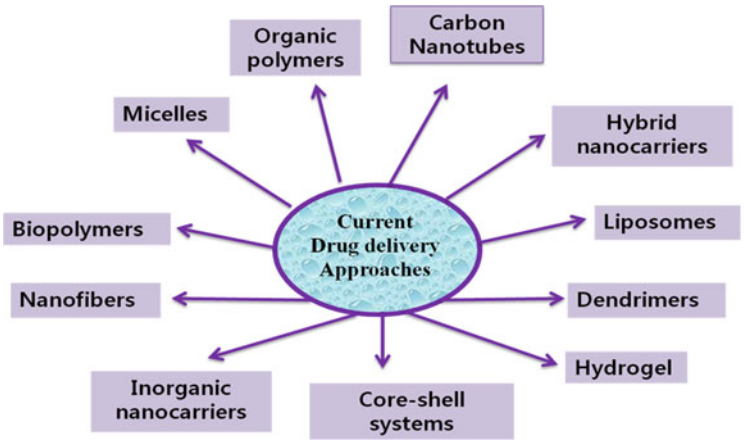

Fig. 10.1 Schematic illustration of encapsulation vehicles for novel drugs as drug delivery approaches

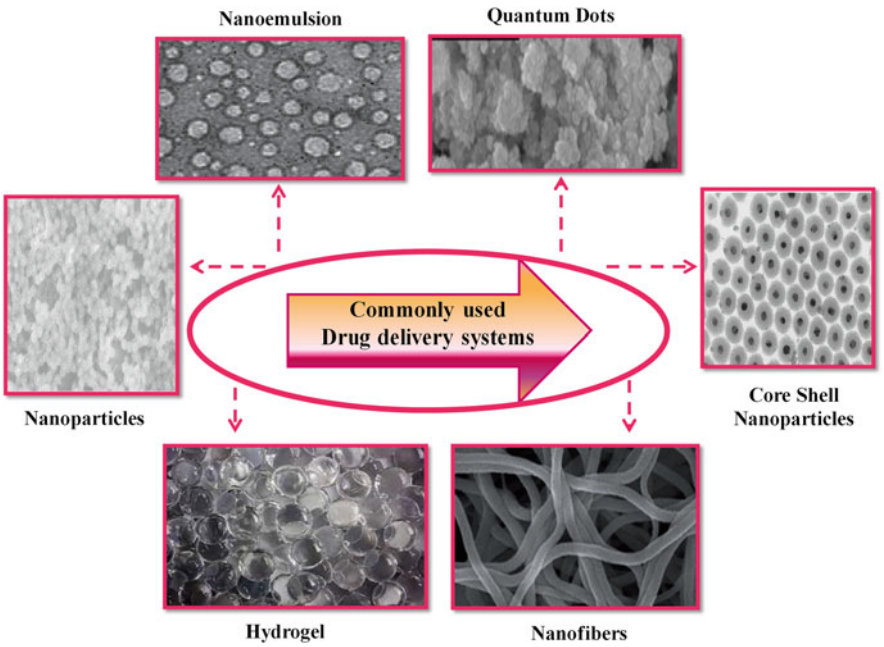

Fig. 10.2 Diagrammatic representation of commonly used drug delivery systems which has been focused in this chapter

basin for required drug (Bawarski et al. 2008; Hani et al. 2014). In this regard, a number of polymers and biopolymers are frequently utilized for the synthesis of NPs. The most commonly applied polymers include Poly(lactic-co-glycolic acid)

(PLGA), polyethylene glycol (PEG), and modified PLGA, etc. Chiefly, the NPs can as well assist in augmentation of solubility of scantily water-soluble drugs, for instance, curcumin. Thereupon, the encapsulation of curcumin in PLGA NPs appreciably shields curcumin from surroundings and enhances its bioavailability and thus, its liberation in cytoplasm ensuing in G2 receptor blockage act on MCF-7 cancer cells as reported elsewhere (Verderio et al. 2013).

Undoubtedly the NPs are the drug transporter system which is prepared from a wide range of materials, for example, poly(alkylcyanoacrylates) (pacas), polyacetates, polysaccharides, copolymers, etc. The accurate method of nanoparticle transportation into head is not implied, however, it is considered to rely on the particles dimension, material constitution, and configuration. In a few cases, it is accounted to imitate molecules that would usually be carried to brain. Furthermore, NPs offer enormous benefits concerning drug targeting, release, and discharge, and with their added prospective to merge diagnostics and treatment; therefore, come out as one of the key tools in nanomedicine (Rehman et al. 2020c, 2021a, b). Conclusively, the chief aims are to progress their stability in the natural atmosphere, to arbitrate the bio-distribution of active components, advance drug loading, targeting, transportation, liberation, and communication with biological barricades.

The cytotoxicity of nanoparticles or their disintegration by-products is the major concern, evidently enhancement in biocompatibility are apprehensions of future investigations (Bawarski et al. 2008).

Additionally, the majority of nanostructured molecules exist at the particle facade, which enhances the loading and release of loaded compounds, for instance, curative medicines, proteins, and polynucleotides, to desired cells and tissues. Interestingly, the exceedingly competent drug release consisting of nanomaterials can greatly reduce price as well as lessen the side effects connected with specific drugs. Moreover, NP dimension and exterior properties can effortlessly maneuver to attain both reflexive and active drug targeting. Likewise, the NPs are also able to manage and retain delivery of a drug for the period of transportation or/at the site of localization, changing drug circulation and ensuing authorization of the drug to enhance therapeutic efficiency coupled with very less aftereffects. Earlier, reports are on the encapsulation of vitamin C and E encapsulated NPs as well as NPs were synthesized from *Hibiscus rosa-sinensis* petal extracts and chitosan. The purpose of using chitosan is targeted delivery as well as biocompatibility. These nanoformulations depicted excellent hemocompatible behavior with approximately 76% encapsulation efficiency. Additionally, superior anticancer behavior was experienced towards BC cells (MCF-7) (Nayak et al. 2016).

Furthermore, the mesoporous silica nanoparticles (MSNs) are acknowledged as outstanding nanoparticles for anticancer drug delivery. These MSNs were observed to be unwavering by means of their exciting characteristics for biomedical uses, for instance, elevated chemical stability, huge surface area, and adjustable hole size, thus, allocating the integration of high quantity of drugs (Sábio et al. 2019). Identically, the magnetic nanoparticles (MNPs) also display very essential part in the detection of cancer abrasions because these MNPs possess unique magnetic properties and biological communications at cellular rank. Assuredly, these MNPs

have revolutionized diagnostic techniques as well as clinical cure for a lot of illnesses (Attari et al. 2019). Conclusively, the improvement in human health conditions at present experiences an outburst of consideration directed by utilization of NPs to distribute drugs to cells. These NPs are wheedled in organized manner that they liberate drugs at specific sites of unhealthy cells and therefore, permit for the straight management besides increasing the efficiency and simultaneously reducing side effects, an overall enhancement of human health.

10.4 Quantum Dots Nanocarriers

Quantum dots (QDs), often acknowledged as nanoscale semiconductor crystals, are nanoparticles with exceptional optical and electronic characteristics such as intense and rigorous fluorescence. They have properties, for instance, fine chemical and photo-stability, excellent quantum produce, and dimension adjustable brightness discharge. Therefore, these QDs hold immense prospective for such uses. Subsequently, novel outcomes are available in these areas (sensors, drug delivery, and biomedical imaging). Undoubtedly, QDs are excellent contenders as theranostic platforms, as they can be capable of key nanocarrier or be a fraction of multifaceted design as the fluorescent tags. Subsequently, fluorescence emission of QDs is constant and tuned by changing dimensions or sonata of particles. Interestingly, their plane region is elevated, and this formulates them outstanding materials for the composite nanosystem. Considering their unique characteristics, QDs catch ample applications in numerous fields (chemistry, physics, biology, material science, pharmaceutics as well as biomedicals, etc.). In current times, they are effectively applied in the fields of cell labeling, intracellular tagging, in vivo bio-imaging, gene/drug delivery and marking, and cancer detection.

In this regard, a novel drug paclitaxel, an extensive used drug for the cure of a variety of human cancers, along with CdTe@CdS@ZnS QDs have been co-loaded in nanostructure lipid carriers to possess theranostic mode regarding cancer treatment (Olerile et al. 2017). In another study, an encapsulation efficacy of ~80% and drug loading of 4.68%, having tumor reticence tempo about 77.85% (Olerile et al. 2017) been reported. Identically, in other investigation, crossbreed silica nanocapsules which have been loaded with ZnSe:Mn/ZnS core–shell as well as paclitaxel have been anticipated as theranostic stage for chemotherapy and fluorescence imaging (Zhao et al. 2017).

In contrast to semiconductor QDs, the carbon quantum dots (CQDs) possess squat toxicity, superior biocompatibility, and wide excitation spectra. Similarly, in other study the CQDs from sodium alginate, prepared by a facile one step method for gene delivery purpose and the as-prepared CQDs, showed excellent strong ability to condense plasmid DNA and therefore, soaring competence for transfection. These CQDs possibly will take part in a twin role as gene vectors and as bio-imaging probes (Zhou et al. 2016). An additional exciting application for graphene quantum dots (GQDs) was documented to battle against Alzheimer's syndrome. These GQDs were found to have inhibitory influence on agglomeration of amyloid-β fibrils,

however, count of freshly developed neuronal precursors and neurons augmented (Xiao et al. 2016). Furthermore, the encapsulated QDs in polymers are in actual fact nontoxic to cell lines as well as animal models. Even though, additional detailed investigations are required to investigate in vivo metabolic pathways, cellular imbibition method, bio-disintegration, and finally extended in vivo toxic reactions.

Admittedly, it has been now undoubtedly recognized that QDs possess excellent potential for utilization in fields, for instance, drug release, sensors as well bio-imaging. However, to facilitate QDs practically in clinical functions, numerous problems exit which are to be resolved, for example, toxic reactions in general, body cleansing, preparation procedure scalability, ecological crash, synthesis expenditure, etc. In spite of the research investigations conducted on in vivo toxicity of QDs, still extensive research is considered necessary in this field. Likewise, the reproducibility of synthesis of QDs too is an imperative challenge.

10.5 Electrospun Nanofibers as Carriers

Nanofibers are fibers having diameter ranging from only some nanometers to 1000 nm showing exceptional functions and essential nanoscale distinctiveness (El-Aswar et al. 2022). Nanofibers have gained huge consideration in nanomedicine owing to their likeness with extracellular matrix (ECM). Akin to nanoparticles, their distinctive individuality of high surface area and polymers tuning using nanofiber has improved efficacy in encapsulation and drug loading capacities. The drug delivery systems made up of electrospin nanofibers that have displayed incredible progresses in controlled and continual release supplemented by their elevated surface area, adjustable porosity, mechanical stamina, present well-suited atmosphere for drug encapsulation, biocompatibility, efficient drug loading, and sustained liberation (Kamsani et al. 2021). On the basis of the characteristics of drug to be distributed, an appropriate approach of drug loading has to be devised to attain most wanted drug-release kinetics. With a suitable blend of procedure, solution, and atmospheric conditions, nanofiber characters, for instance, fiber diameter, porosity, and thickness of nanofiber mat can be regulated (Patel and Yadav 2018). Polymeric nanofiber due to its distinctive practical designer may be applied for both systemic and localized drug delivery. In this regard, Joshi and co-workers applied nanofiber oriented systems for controlled drug delivery in cure of periodontitis (Joshi et al. 2015).

Electrospinning prepares composite and multi-layered nanofiber employing various polymers to regulate cellular reaction. In these two distinct types of polymers are managed to create two different coating, inner core and external case displayed restricted discharge of entrapped drug assists to regulate therapeutic efficiency. Huang et al., applied co-axial electrospinning method to trap resveratrol and gentamycin sulfate in bioabsorbable polymer such as polycaprolactone (Huang et al. 2006).

Drug loading capability is main factor responsible for the impending of drug delivery systems. Earlier research determines elevated drug loading efficacy of

electrospun nanofibers. The drug loading efficacy of nanofibers is associated with physiochemical characters of drug and polymers. Commonly, lofty drug loading will be possible if both drug and polymers possess comparable solubility prototype. Recently, nanofibers display significant loading efficiency of around 89% of peclitaxel in chosen solvent system of cremophor (Ma and Mumper 2013). Furthermore, electrospinning produces a cheap substitute to yield continuous nanofibers. Nanofibers owing to its multi-layered structure aid in masking bitter taste of drugs, create electrospun nanofibers appropriate for buccal and sublingual application. Clogged cycle electrospinning procedure keeping a sterilized atmosphere during production procedure formulates it conducive for ocular drug delivery. Additional nanofibers can be synthesized from a range of polymers to attain required physiochemical and pharmaceutical properties appropriate for transmucosal drug delivery. Similarly, the electrospun polymeric nanofibers were found very useful for encapsulation of novel anticancer pro-drug camptothecin to progress discharge and to amend steadiness and anticancer action (Amna et al. 2012, 2013).

10.6 Nanoemulsions for Delivery of Drugs

Nanoemulsions are emulsions having nano-dimension, prepared for enhancing the delivery of drugs. In nanoemulsions, two non-miscible liquids are assorted to structure a solitary segment using emulsifying mediator, i.e., surfactant and co-surfactant (Choradiya and Patil 2021). The basic dissimilarity between emulsion and nanoemulsion is shape and size of particles diffused in continuous juncture. Nanoemulsions particle size differs from 10 to 1000 nm. These nanocarriers are solid spheres having amorphous surface and lipophilic with a negative charge. Magnetic nanoparticles can be utilized to augment site specificity. They improve medicinal effectiveness of drug and lessen harmful impact and noxious response. The word nanoemulsion also called miniemulsion is oil-water or vice versa diffusion stabilized by surfactant film having droplet size range between 20 and 600 nm. As the sizes of nanoemulsions are small, they are transparent. Three different types of nanoemulsion have been reported as (a) oil in water nanoemulsion where oil is discrete in liquid stage, (b) water in oil nanoemulsion where water droplets are scattered in oil phase, and (c) bi-continuous nanoemulsions. The diverse uses of nanoemulsions are as follows: It can be functional as replacement for liposomes and vesicles, get better bioavailability of drug, safe and does not create skin problems, possess superior physical stability, have advanced surface area for better absorption, may be developed in an assortment of forms such as foams, creams, liquids, sprays, etc. It proffers enhanced uptake of oil-soluble appendages in culture, make possible to dissoluble lipophilic drugs, supportive in flavor masking, lesser quantity of vigor is prerequisite.

Designing nanoemulsion comprises active drug, additive, and emulsifier. Two different techniques for synthesis of nanoemulsion are: (a) high-energy emulsification and (b) low-energy emulsification. The high-energy emulsification scheme contains high-energy stirring, ultrasonic emulsification, high-pressure

homogenization, microfluidization, as well as membrane emulsification (Banker et al. 1996; Tiwari and Amiji 2006; Valdivia et al. 1997). The low-energy emulsification method involves phase inversion temperature, emulsion inversion point, and spontaneous emulsification (Shakeel et al. 2008). Combined method consists of high and low-energy emulsification; it is likely to formulate overturn nanoemulsion in a highly viscous system. Nanoemulsion can also shield drugs, which are prone to hydrolysis and oxidation. Currently, nanoemulsions are utilized for targeted drug delivery of a range of anticancer drugs, photosensitizers, or therapeutic compound. Nanoemulsion may supply with prolonged accomplishment of drugs as well.

Emulsion preparations are usually applied to control drugs as they improve solubility of hydrophobic composite, increase pharmacokinetic outline, and lower harmful outcome suffered by patients. Locoid Crelo® is an emulsion consisting of hydrocortisone butyrate stabilized by non-ionic surfactant cetostearyl alcohol that is managed by topical use to skin (Otto et al. 2009; Pierard et al. 1996). The characteristics of emulsion such as emulsion kind, drop dimension, surfactant, emollients have an influence on dermal delivery of drug (Otto et al. 2009). Diprivan® is a common anesthetic emulsion enclosing propofol stabilized by egg lecithin that is intravenously given to calm patients. Drug-emulsion uptake by means of intraperitoneal and intramuscular procedure is under considerable investigation for use in insulin delivery and as vaccine adjuvants, correspondingly (Haggag et al. 2018).

Emulsion nanomedicine possesses benefits of free drug involving enhanced bioavailability, stability against disintegration, less clearance, and distinct biological exchanges. Nevertheless, nanomedicines tried to fight back in order to enhance effectiveness of cure in clinical sites. Cancer is a varied sickness and it has been recognized that particular cancers need distinctive nanomedicine approaches to considerably enhance healing (Garg et al. 2019) effectiveness. Recently, approaches are being made to formulate novel emulsion nanomedicine through superior efficiency and protection for an assortment of cancers.

Specific drug delivery employing emulsion nanomedicine is advanced to non-targeted procedures as it enhances bio-distribution and diminishes non-specific imbibitions in hale and hearty tissues. Nanoemulsion is an appealing approach to design efficient nanomedicines for drug delivery. An easy synthesis and clear characteristics of emulsions and surfactants offer pliant frame to design a variety of nanomedicines. Its tiny size permits them to infiltrate deeply inside cells, extend their circulation, and enclose exceptional bio-nano communications.

10.7 Nanohydrogel Delivery Systems

Hydrogels are defined as well hydrated materials formed by means of chemical and/or physical crosslinking of molecules outlining a three-dimensional arrangement capturing the solvent. In other words, merging elastic properties of solids with microviscous behavior of fluids able to generate a pool of drug and make possible the limited and continued drug delivery that can result an enhanced therapeutic efficiency and diminution of unpleasant side impacts of a systemic drug

management. *Nanohydrogels* (NGs), like *hydrogels*, can be described as a 3D system of hydrophilic polymers comprising size in the range of less than 100 nm. These NGs have come out as most useful drug delivery system owing to some unique properties such as possess massive drug loading, excellent stability, bio-reliability, targeted delivery as well as response to various environmental stimuli. In other words, in the area of medicine these hydrogels have emerged as elegant materials for multiplicity of functions and have established colossal clinical applications (Mahinroosta et al. 2018; Narayanaswamy and Torchilin 2019). Likewise, the supramolecular hydrogels, created by reversible, non-covalent intermolecular connections, are incredibly eye-catching for biomedical uses (Webber and Dankers 2019). Additionally these supramolecular hydrogels are easy to prepare and possess hierarchical arrangement as well as excellent stimuli receptiveness, ultimately improve the therapeutic result of drug delivery (Dalwadi and Patel 2015). Supramolecular nanohydrogels fashioned by a gel medium enclosing drug loaded into nanoparticles (Gao et al. 2016), symbolize an improvement as drug liberation strategy, as they join mechanical possessions of a hydrogel along compensation of a nanoparticle with the intention of a nanocontainer and a nanocarrier capable of enhancing drug delivery (Granata et al. 2020).

In general the NGs are fashioned by drug-loaded nanoparticles combined with gel template (Mahanta et al. 2018), however, there is a rising demand in NGs produced by molecules self-assembling in nanoparticles capable of ensnaring a drug and outline a gel devoid of adding up of additives (Altunbas et al. 2011). Hydrogels own an exceptional 3D, cross-linked system of polymers competent of gripping hefty amount of water and biological solution devoid of liquefying. NGs or nanogels are collected of miscellaneous kind of polymers, either of synthetic or normal foundation. Their amalgamation is bound by a chemical covalent bond or is physically cross-linked with non-covalent linkage similar to electrostatic connections, hydrophobic interactions, and hydrogen linkage. Its amazing capacity to take up water or additional liquids is mostly credited to hydrophilic clusters akin to hydroxyl, amide, sulfate, etc.

Furthermore, the usual biomolecules such as protein- or peptide oriented nanohydrogels are significant group of hydrogels which own elevated biocompatibility and metabolic degradation. Synthesis of protein NGs plus successive encapsulation procedure usually engrosses utilization of eco-friendly solvents and can be made up by means of diverse proteins, for example, fibroins, albumin, collagen, elastin, gelatin, lipoprotein, etc. connecting emulsion, electrospray, desolvation methods, etc. Additionally, these NGs are outstanding biomaterials with wide purposes in fields of regenerative medicine, tissue engineering, and drug delivery owing to various rewards such as biodegradability, biocompatibility, amendable mechanical potency, molecular linking capability, and tunable responses to some stimuli such as ionic concentration, pH, and temperature.

Similarly, biopolymeric hydrogels also are being employed as a carrier for contemporary and local drug delivery owing to their adjustable viscoelasticity, exceptional water absorption capability plus biocompatibility. Generally, polymeric materials utilized in hydrogels are attained from usual as well as artificial

possessions. However, the synthetic hydrogels relate to problems allied to their biocompatibility and biodegradability. Nevertheless, such hydrogels own excellent quality mechanical power and water incorporation competence (Chyzy et al. 2020; Sosnik and Seremeta 2017).

10.8 Conclusion

This chapter presents an outline about the novel drug delivery carriers which are nowadays in practice in medical sciences. The prospects and confront of nanomedicines in drug delivery from synthetic/or natural resources up to their clinical uses have been argued in present chapter. Additionally, this chapter incorporated information concerning about viewpoint and development in nanomedicine vicinity. Above all, the nanotechnology has initiated many delivery systems for delivery of drugs at exact spots. Nonetheless, diverse sorts of nanovehicles have been prepared for various important drugs. However, drug delivery methods which have been talked about in this chapter include NPs, QDs, electrospun nanofibers, nanoemulsions, CSNs, and nanohydrogels. The nanohydrogel which coalesces mechanical characters of a hydrogel as well as uses of a nanoscale micelle in drug delivery comes into view as gifted matter for drug delivery. Likewise, nanofibers possess high surface area and polymers, tuning using nanofibers hold enhanced efficacy in encapsulation and drug-loading. On the other hand, the nanoemulsions facilitate to dissoluble lipophilic drug and NP matrix helps to encapsulate, entrap drugs, whereas QDs are practiced for labeling purposes. In summary nanomaterials hold versatile multi-uses (Fig. 10.3) due to their exceptional configuration and makeup.

Despite the pledge of nanomedicine for multiple disorders, there are some drawbacks for utilizing these nanodrug delivery vehicles, the drugs delivered from

Fig. 10.3 Pictorial representation of key advantages of the encapsulated drug and drug delivery systems

nanoscale entities operate in a different way from standard or predictable form. We expect that this chapter will make available understanding about drug delivery systems which will assist in expansion of effectual ways for therapy.

References

Aldakheel RK, Rehman S, Almessiere MA, Khan FA, Gondal MA, Mostafa A, Baykal A (2020) Bactericidal and in vitro cytotoxicity of Moringa oleifera seed extract and its elemental analysis using laser-induced breakdown spectroscopy. Pharmaceuticals 13(8):193

Altunbas A, Lee SJ, Rajasekaran SA, Schneider JP, Pochan DJ (2011) Encapsulation of curcumin in self-assembling peptide hydrogels as injectable drug delivery vehicles. Biomaterials 32(25): 5906–5914

Amna T, Hassan MS, Nam K-T, Bing YY, Barakat NA, Khil M-S, Kim HY (2012) Preparation, characterization, and cytotoxicity of CPT/Fe_2O_3-embedded PLGA ultrafine composite fibers: a synergistic approach to develop promising anticancer material. Int J Nanomedicine 7:1659

Amna T, Barakat NA, Hassan MS, Khil M-S, Kim HY (2013) Camptothecin loaded poly (-ε-caprolactone) nanofibers via one-step electrospinning and their cytotoxicity impact. Colloids Surf A Physicochem Eng Asp 431:1–8

Amna T, Hassan MS, Sheikh FA (2020) Nanocamptothecins as new generation pharmaceuticals for the treatment of diverse cancers: overview on a natural product to nanomedicine. In: Application of nanotechnology in biomedical sciences. Springer, Cham, pp 39–49

Attari E, Nosrati H, Danafar H, Kheiri Manjili H (2019) Methotrexate anticancer drug delivery to breast cancer cell lines by iron oxide magnetic based nanocarrier. J Biomed Mater Res A 107(11):2492–2500

Bamrungsap S, Zhao Z, Chen T, Wang L, Li C, Fu T, Tan W (2012) Nanotechnology in therapeutics: a focus on nanoparticles as a drug delivery system. Nanomedicine 7(8):1253–1271

Banker GS, Lieberman HA, Rieger MM (1996) Pharmaceutical dosage forms: disperse systems. M. Dekker, New York

Bawarski WE, Chidlowsky E, Bharali DJ, Mousa SA (2008) Emerging nanopharmaceuticals. Nanomedicine 4(4):273–282

Choradiya BR, Patil SB (2021) A comprehensive review on nanoemulsion as an ophthalmic drug delivery system. J Mol Liq 339(116751.10):1016

Chyzy A, Tomczykowa M, Plonska-Brzezinska ME (2020) Hydrogels as potential nano-, micro- and macro-scale systems for controlled drug delivery. Materials 13(1):188

Dalwadi C, Patel G (2015) Application of nanohydrogels in drug delivery systems: recent patents review. Recent Pat Nanotechnol 9(1):17–25

El-Aswar EI, Ramadan H, Elkik H, Taha AG (2022) A comprehensive review on preparation, functionalization and recent applications of nanofiber membranes in wastewater treatment. J Environ Manag 301:113908

Gao W, Zhang Y, Zhang Q, Zhang L (2016) Nanoparticle-hydrogel: a hybrid biomaterial system for localized drug delivery. Ann Biomed Eng 44(6):2049–2061

Garg U, Chauhan S, Nagaich U, Jain N (2019) Current advances in chitosan nanoparticles based drug delivery and targeting. Adv Pharm Bull 9(2):195

Granata G, Petralia S, Forte G, Conoci S, Consoli GML (2020) Injectable supramolecular nanohydrogel from a micellar self-assembling calix[4]arene derivative and curcumin for a sustained drug release. Mater Sci Eng C 111:110842

Haggag YA, Faheem AM, Tambuwala MM, Osman MA, El-Gizawy SA, O'Hagan B, Irwin N, McCarron PA (2018) Effect of poly (ethylene glycol) content and formulation parameters on particulate properties and intraperitoneal delivery of insulin from PLGA nanoparticles prepared using the double-emulsion evaporation procedure. Pharm Dev Technol 23(4):370–381

Hani U, Shivakumar H, Anjum H, Pasha MY (2014) Preparation and optimization of curcumin-hydroxy propyl cyclodextrin bioadhesive vaginal films for human papilloma virus-induced cervical cancer. J Biomater Tissue Eng 4(10):796–803

Huang ZM, He CL, Yang A, Zhang Y, Han XJ, Yin J, Wu Q (2006) Encapsulating drugs in biodegradable ultrafine fibers through co-axial electrospinning. J Biomed Mater Res A 77(1): 169–179

Joshi D, Garg T, Goyal AK, Rath G (2015) Development and characterization of novel medicated nanofibers against periodontitis. Curr Drug Deliv 12(5):564–577

Kamsani NH, Haris MS, Pandey M, Taher M, Rullah K (2021) Biomedical application of responsive 'smart' electrospun nanofibers in drug delivery system: a minireview. Arab J Chem 2021:103199

Liu D, Yang F, Xiong F, Gu N (2016) The smart drug delivery system and its clinical potential. Theranostics 6(9):1306

Ma P, Mumper RJ (2013) Paclitaxel nano-delivery systems: a comprehensive review. J Nanomed Nanotechnol 4(2):1000164

Mahanta AK, Senapati S, Paliwal P, Krishnamurthy S, Hemalatha S, Maiti P (2018) Nanoparticle-induced controlled drug delivery using chitosan-based hydrogel and scaffold: application to bone regeneration. Mol Pharm 16(1):327–338

Mahinroosta M, Farsangi ZJ, Allahverdi A, Shakoori Z (2018) Hydrogels as intelligent materials: a brief review of synthesis, properties and applications. Mater Today Chem 8:42–55

Nahvi I, Belkahla S, Asiri SM, Rehman S (2021) Overview and prospectus of algal biogenesis of nanoparticles. In: Microbial nanotechnology: green synthesis and applications. Springer, Singapore, pp 121–134

Narayanaswamy R, Torchilin VP (2019) Hydrogels and their applications in targeted drug delivery. Molecules 24(3):603

Nayak D, Minz AP, Ashe S, Rauta PR, Kumari M, Chopra P, Nayak B (2016) Synergistic combination of antioxidants, silver nanoparticles and chitosan in a nanoparticle based formulation: characterization and cytotoxic effect on MCF-7 breast cancer cell lines. J Colloid Interface Sci 470:142–152

Olerile LD, Liu Y, Zhang B, Wang T, Mu S, Zhang J, Selotlegeng L, Zhang N (2017) Near-infrared mediated quantum dots and paclitaxel co-loaded nanostructured lipid carriers for cancer theragnostic. Colloids Surf B Biointerfaces 150:121–130

Otto A, Du Plessis J, Wiechers J (2009) Formulation effects of topical emulsions on transdermal and dermal delivery. Int J Cosmet Sci 31(1):1–19

Patel GC, Yadav BK (2018) Polymeric nanofibers for controlled drug delivery applications. In: Organic materials as smart nanocarriers for drug delivery. Elsevier, Amsterdam, pp 147–175

Patra JK, Das G, Fraceto LF, Campos EVR, del Pilar Rodriguez-Torres M, Acosta-Torres LS, Diaz-Torres LA, Grillo R, Swamy MK, Sharma S (2018) Nano based drug delivery systems: recent developments and future prospects. J Nanobiotechnol 16(1):1–33

Pierard G, Lachapelle J, Frentz G, Schopf E, Stolz E (1996) Hydrocortisone 17-butyrate topical emulsion (Locoid Crelo®) in psoriasis. J Eur Acad Dermatol Venereol 6(1):11–14

Qureshi A, Blaisi NI, Abbas AA, Khan NA, Rehman S (2021) Prospectus and development of microbes mediated synthesis of nanoparticles. In: Microbial nanotechnology: green synthesis and applications. Springer, Singapore, pp 1–15

Rehman S (2016) Endophytes: the producers of important functional metabolites. Int J Curr Microbiol App Sci 5(5):377–391

Rehman S, Al Salem Z, Al Jindan R, Hameed S (2019) Microbial natural products: exploiting microbes against drug-resistant bugs. In: Pathogenicity and drug resistance of human pathogens. Springer, Singapore, pp 393–404

Rehman S, Asiri SM, Khan FA, Jermy BR, Ravinayagam V, Alsalem Z, Al Jindan R, Qurashi A (2020a) Anticandidal and in vitro anti-proliferative activity of sonochemically synthesized indium tin oxide nanoparticles. Sci Rep 10(1):1–9

Rehman S, Farooq R, Jermy R, Mousa Asiri S, Ravinayagam V, Al Jindan R, Alsalem Z, Shah MA, Reshi Z, Sabit H, Alam Khan F (2020b) A wild fomes fomentarius for biomediation of one pot synthesis of titanium oxide and silver nanoparticles for antibacterial and anticancer application. Biomol Ther 10(4):622

Rehman S, Jermy R, Asiri SM, Shah MA, Farooq R, Ravinayagam V, Ansari MA, Alsalem Z, Al Jindan R, Reshi Z, Khan FA (2020c) Using Fomitopsis pinicola for bioinspired synthesis of titanium dioxide and silver nanoparticles, targeting biomedical applications. RSC Adv 10(53): 32137–32147

Rehman S, Almessiere MA, Al-Suhaimi AE, Hussain M, Yousuf Bari M, Mehmood Ali S, Al-Jameel SS, Slimani Y, Khan FA, Baykal A (2021a) Ultrasonic synthesis and biomedical application of Mn0.5Zn0.5ErxYxFe2− 2xO4 nanoparticles. Biomolecules 11(5):703

Rehman S, Almessiere MA, Al-Jameel SS, Ali U, Slimani Y, Tashkandi N, Al-Saleh NS, Manikandan A, Khan FA, Al-Suhaimi EA, Baykal A (2021b) Designing of Co0.5Ni0.5GaxFe2− xO4 ($0.0 \leq x \leq 1.0$) microspheres via hydrothermal approach and their selective inhibition on the growth of cancerous and fungal cells. Pharmaceutics 13(7):962

Sábio RM, Meneguin AB, Ribeiro TC, Silva RR, Chorilli M (2019) New insights towards mesoporous silica nanoparticles as a technological platform for chemotherapeutic drugs delivery. Int J Pharm 564:379–409

Shakeel F, Baboota S, Ahuja A, Ali J, Faisal M, Shafiq S (2008) Stability evaluation of celecoxib nanoemulsion containing Tween 80. Thai J Pharm Sci 32:4–9

Sosnik A, Seremeta KP (2017) Polymeric hydrogels as technology platform for drug delivery applications. Gels 3(3):25

Tiwari S, Amiji M (2006) Nanoemulsion formulations for tumor-targeted delivery. In: Nanotechnology for cancer therapy. CRC Press, Boca Raton, FL

Valdivia FJG, Dachs AC, Perdiguer NC (1997) Nanoemulsion of the oil water type, useful as an ophthalmic vehicle and process for the preparation thereof. Google Patents

Verderio P, Bonetti P, Colombo M, Pandolfi L, Prosperi D (2013) Intracellular drug release from curcumin-loaded PLGA nanoparticles induces G2/M block in breast cancer cells. Biomacromolecules 14(3):672–682

Webber MJ, Dankers PY (2019) Supramolecular hydrogels for biomedical applications. Macromol Biosci 19:1800452

Xiao S, Zhou D, Luan P, Gu B, Feng L, Fan S, Liao W, Fang W, Yang L, Tao E (2016) Graphene quantum dots conjugated neuroprotective peptide improve learning and memory capability. Biomaterials 106:98–110

Zhao T, Liu X, Li Y, Zhang M, He J, Zhang X, Liu H, Wang X, Gu H (2017) Fluorescence and drug loading properties of ZnSe:Mn/ZnS-Paclitaxel/SiO_2 nanocapsules templated by F127 micelles. J Colloid Interface Sci 490:436–443

Zhou J, Deng W, Wang Y, Cao X, Chen J, Wang Q, Xu W, Du P, Yu Q, Chen J (2016) Cationic carbon quantum dots derived from alginate for gene delivery: one-step synthesis and cellular uptake. Acta Biomater 42:209–219

Recent Developments in Silica Nanoparticle Based Drug Delivery System

11

Monika Sohlot, Sumistha Das, and Nitai Debnath

Abstract

Infectious diseases are one of the leading causes of death worldwide. Though there are many standard medicines available in the market to combat all these diseases, gradually the pathogens are becoming resistant against these medications. Moreover, many of these drugs may show severe side effects in patients as well. Researchers are trying to find alternative strategies to overcome these problems. Because of having many novel properties, nanoparticles are now being exploited in various disease management. Among different nanoparticles, silica nanoparticles are considered to be one of the most biocompatible one. Its surface silanol groups can be modified with different molecules of choice and it makes it suitable for targeted drug therapy. As among different silica nanostructures, mesoporous silica nanoparticles carry pores, loading and release of drug from mesoporous silica nanoparticles are very easy. Hence mesoporous silica nanoparticles are being extensively used as a drug delivery platform to combat different diseases including infectious diseases.

Keywords

Biocompatibility · Mesoporous silica · Slow release · Surface functionalization · Targeted drug delivery

M. Sohlot · S. Das · N. Debnath (✉)
Amity Institute of Biotechnology, Amity University Haryana, Gurugram, India
e-mail: ndebnath@ggn.amity.edu

S. Hameed, S. Rehman (eds.), *Nanotechnology for Infectious Diseases*,
https://doi.org/10.1007/978-981-16-9190-4_11

11.1 Introduction

Infectious diseases are communicable diseases or transmissible diseases caused by the entry of pathogen into other organism's body, tissues, or cells followed by proliferation within the host's body (Hay et al. 2014). The pathogens responsible for causing the infection can be any bacteria, viruses, parasites, and fungi. Based on the causative agents, infectious diseases are classified as bacterial infection, viral infection, and parasitic infection. Process of infection is not a single step process, rather it is a multi-step process and this can become even lethal if it is not diagnosed early and a proper medication regime is followed. Infection may spread through droplets, direct contact, or through vector, etc. All the organisms have their own strategies to deal with infectious diseases like avoidance, resistance, and tolerance (Medzhitov et al. 2012). Though there are many ways to treat infectious diseases, the conventional methods include administration of antibiotics, antivirals, antiparasitic, or antifungal drugs. Each treatment is specific for the particular causative agents. Ciprofloxacin, benznidazole, nifurtimox, carvacrol, thymol, etc. are some of the agents which are being regularly used for the successful treatment of infectious diseases for years. But many a time these treatments may have severe side effects in the patients. Antibiotic treatment sometimes results in hypersensitive reactions, nephritis, nausea, vomiting, etc. (Heta and Robo 2018). Antiparasitic agents such as amodiaquine increase the chances of causing agranulocytosis and hepatitis when used for long term (Kuhlmann and Fleckenstein 2017). More importantly there is ever emerging problem of development of resistance in the pathogenic agents against antibiotics, antiparasitic, and antiviral drugs. To overcome these barriers and to make the infectious disease management more robust, development of new strategies is of paramount importance.

Nanotechnology can offer a new tool in the infectious disease management. Nanoparticles (NPs) offer many novel properties because of their enhanced surface area to volume ratio, presence of more number of electrons on the surface in comparison with the core, quantum confinement effect, etc. Already smart delivery system has been developed by exploiting the beneficial properties of NPs. Among various NPs, silica NPs (SNPs) are very popular in the field of medical science as these are comparatively non toxic and surface silanol groups can be modified with several ligands. It can be used for controlled drug release even at a specific tissue target and this is already being used in infectious disease management (Aderibigbe 2017). SNPs of different size and shape can be synthesized depending on the requirement of a particular delivery system. Among different SNPs mesoporous SNPs became most popular for combating infectious diseases. For example, some of the drugs have great phagocytosis activity but they are ineffective because of their low solubility and penetration effect. So, to overcome those issues, mesoporous SNPs are most widely used as a smart nanocarrier because they have porous structure which can increase the adsorbing effect and lead to improve solubility of drugs (Subramaniam et al. 2019).

In this chapter we have extensively discussed different types of SNPs and their synthesis process. Later we have emphasized on the role of mesoporous SNPs on infectious disease management.

11.2 Types of Silica Nanoparticles

There are different types of SNPs which may be classified into following categories:

(a) Based on structural organization
- Nonporous SNPs.
- Mesoporous silica nanoparticles (MSNs).
- Core shell SNPs.

(b) Based on shape
- Spherical SNPs.
- Short rod shaped SNPs.
- Long rod shaped SNPs.

11.3 Non-porous Silica Nanoparticle

Though non-porous SNPs are solid in nature, they can be utilized for the delivery of cargos through the encapsulation and conjugation in which release of payload can be controlled by means of chemical linkers and degradation of silica matrix (Tang and Cheng 2013).

Particle size—Size of SNPs depends on the type of silicon alkoxide and the chain length of the alcohol used for synthesis of the nanomaterial. Alcohol with shorter chain length normally produces SNP of smaller size (though concentration of the reactants, energy used for the synthesis process also control the particle size). Application of the SNPs is influenced by the size, shape, dispersion nature, etc. Monodisperse non-porous SNPs find application in drug delivery and molecular imaging, whereas micron sized silica particles are used in liquid chromatography (Tang and Cheng 2013). NPs of size around 100 nm circulate in vivo and reach target tumor efficiently (Shang et al. 2014) (Table 11.1 and Fig. 11.1).

Surface properties—Surface properties can also be modulated to achieve efficient targeting and improving therapy. The surface properties of NPs are known to play an important role in determining the interactions between NP and biological system which influence the cellular internalization and trafficking, bio-distribution, and tissue penetration of NPs. To facilitate their use in specific applications, the suitable design of surface ligands on these NPs is necessary. Physicochemical properties of the surface (as hydrophobicity/hydrophobicity and zeta potential as well as dispersibility in solution) are govern by surface ligands (Shang et al. 2014). SNPs with positive, negative, or zwitterionic surface charge could be prepared with the addition of different functional group such as addition of

Table 11.1 Average particle size (±S.E.) of non-porous SNPs under different reaction conditions

Sample	Experimental conditions				Average particle size (nm)
	Silicon alkoxide [M]	Alcohol [M]	Water [M]	NH_4OH [M]	
a.	TEOS [0.12]	Ethanol [8]	14	14	183 ± 19
b.	TEOS [0.12]	Ethanol [8]	3	14	268 ± 14
c.	TEOS [0.12]	Ethanol [4]	14	14	77 ± 8
d.	TEOS [0.12]	Ethanol [4]	3	14	371 ± 6
e.	TEOS [0.012]	Ethanol [8]	14	14	87 ± 6
f.	TEOS [0.012]	Ethanol [8]	3	14	139 ± 21
g.	TEOS [0.012]	Ethanol [4]	14	14	55 ± 9
h.	TEOS [0.045]	Ethanol [4]	14	14	59 ± 9
i.	TMOS [0.012]	Methanol [8]	14	14	59 ± 7
j.	TMOS [0.012]	Methanol [8]	3	14	91 ± 8
k.	TMOS [0.012]	Methanol [4]	3	14	70 ± 6
l.	TMOS [0.045]	Methanol [4]	14	14	29 ± 7

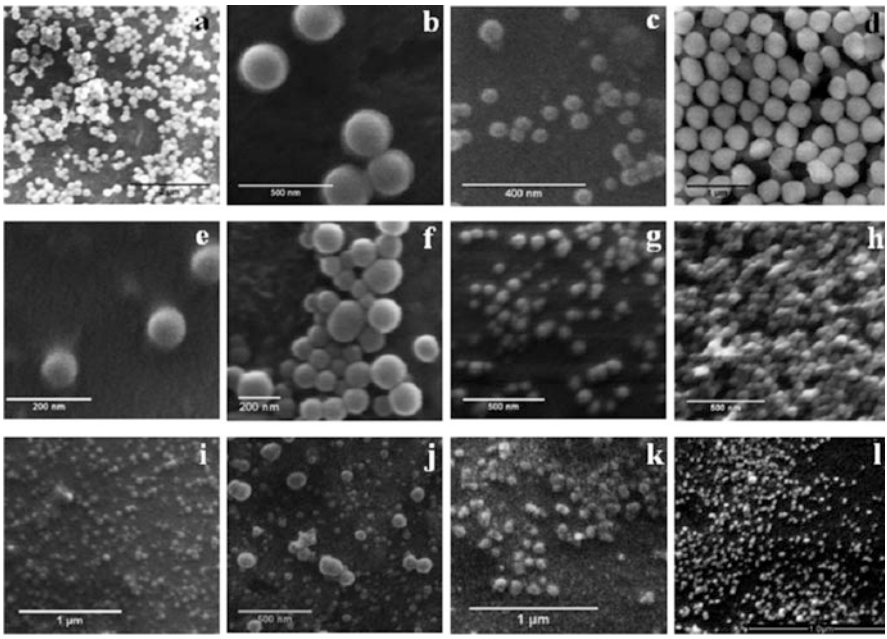

Fig. 11.1 FE SEM image of non-porous SNPs of different sizes synthesized by modifying the reactant concentrations (**a**–**l**)

3-aminopropyltriethoxysilane, 3- (trihydroxysilyl)-propyl methylphosphonate or carboxyethylsilanetriol, or zwitterion silanes following the formulation of silica NPs. SNP surface can be functionalized with polymers either chemically (through

covalent bonding) or physically (by physical adsorption) The former is favored due to the stable covalent bonding between the polymer and NP such as polyethylene glycol (PEG). The surface of SNPs can also be functionalized with various targeting ligands like antibodies or aptamers (Tang and Cheng 2013).

Shapes of non-porous nanoparticle—Shape of the non-porous silica NPs is controllable. Different templates and polymer adsorption methods are used for making different shapes of SNPs.

Control of SNP shape is important for fundamental studies of understanding the effect of shape in different application fields like nanomedicine, etc. Optimization of shape is required to get the best result in biological system for improved diagnosis and therapy. Shape control of NPs favors circulation or tissue penetration properties (Geng et al. 2007). Effect of different shape of silica NPs on circulation and penetration has shown below:

Worm and spherical micelles—Worm-like micelles have superior circulation time compared to spherical micelles likely due to the enhanced evasion of phagocytosis (Tang and Cheng 2013).

Nanorods and nanospheres—It is also noticeable that nanorods penetrate tumor tissues more rapidly than nanospheres likely because of improved transport through tumor vasculature pores (Wong et al. 2011; Tang and Cheng 2013).

Spherical and non-spherical non-porous silica nanoparticle—Spherical shaped non-porous SNPs can enter the cells more effectively as compared to non-spherical NPs. (Geng et al. 2007).

11.4 Mesoporous Silica Nanoparticles

Mesoporous silica nanoparticles (MSNs) have particle diameters in the range of 50–300 nm having narrow pore size distributions of the order 2–6 nm (Huang et al. 2014). Since MSNs were first reported in 1992, various kinds of MSNs, including different sizes, surface functionalities, pore properties, and shapes, have been explored (Hao et al. 2014). The most common type of mesoporous nanoparticles are MCM-41 and SBA-15. Their structure and morphology are controllable at both the nanometer and micrometer scale. The controllable structure of MSNs contributes towards high surface area and pore size of MSNs (Huang et al. 2014). Unlike conventional nano or micro-particles, MSNs provide super facial structure regulation for adapting different accommodations, convenient surface functionalization for ligating various targets, and pore size and pore type for encapsulating abundant bioactive substances (drug, protein, gene, or dye, etc.) (Hao et al. 2014).

Pore size—Though MSNs have different pore size, most commonly they have smaller pores. MSNs with larger pores show diffraction peaks in the lower-angle region, while MSNs with smaller pores show diffraction peaks in the higher-angle region. MSNs with different pore sizes result different levels of cellular immune responses. MSNs with larger pore size show stronger immune activation and antitumor efficacy in comparison with MSNs with smaller pore size (Hong et al. 2020).

Surface area—MSNs have high surface area which is responsible for different surface functionalities (Hao et al. 2014). Due to their high surface area, MSNs have been extensively studied as a delivery vehicle. In MSNs, the silica surface has a high density of negatively charged silanol groups (Si-OH) which can be modified with a wide range of organic functional groups. (Huang et al. 2014).

Particle size—The size of SNPs plays a vital role in their toxicity. Moreover, particle size also influences the biodistribution and pharmacokinetics of drugs. Different particle size shows different toxic response like smaller NPs which may have low toxic effects in some tissues but they can cross blood–brain barrier and can show toxicity, larger NPs may cause toxicity such as liver inflammation, etc. SNPs are comparatively non-toxic up to 100 mg/mL concentration if the size of the particles is 100 nm.

Shape of MSN—MSN shapes can be categorized based on two main categories: MSNs with different aspect ratios (ARs) and MSNs with different morphologies. AR has a great attention due to the possibility of various orientations in the bio-nano interface, whereas MSNs with morphologies have received more and more attention for their unique spatial architectures (Hao et al. 2014). MSNs with a smaller AR generally affect cells to a minor degree compared to MSNs with a larger AR (Huang et al. 2010). There are different shaped MSNs like spheres, and short and long rods which display great potential in different fields. Many more shapes like MSNs with hexagonal-symmetry (HMSNP), blackberry-like MSNPs (BMSNP), chrysanthemum-like MSNPs (CMSNP) are also being used in different fields. A few examples of the popular MSNs are MCM-41/SBA-15, SBA-16, FDU-2, KIT-5, MCM-50.

11.5 Core/Shell Silica Nanoparticles

Core/shell NPs (CSNs) are referred as biphasic type of NPs because their inner core and outer shell are made up of different components (Ghosh Chaudhuri and Paria, 2011). In case of core/shell SNPS (C/S SNPs) one of the phases comprises of silica. Core and shell components influence the properties of NPs so the materials are selected on the basis of purpose and function for which the resulted NPs are used (Khatami et al. 2018). The combination of core and shell material provides unique properties such as improved reactivity, stability, etc.

C/S SNPs have shown remarkable biocompatibility both in vitro and in vivo and have huge potential in diagnostics and drug delivery including cancer. The shell structure in C/S SNPs was found particularly suitable for imaging agent delivery. The shell structures can preserve imaging agents inside the NPs (Wu et al. 2014). There are several factors that affect the bio-distribution and efficiency (drug loading and targeting efficiency) of CSNs. Particle size is one of such factors.

Particle size—Uniform sized NPs are created using reverse microemulsion method. C/S SNPs with controllable size can be constructed with varying organic solvents used in reverse microemulsions (Zhao et al. 2019 & Wu et al. 2014). C/S SNPs with a tunable size range from 10 to 100 nm were developed by simply

Table 11.2 Core/shell SNPs with different pore size prepared by different methods

Pore size of C/S-SiNPs	Method
Up to 3 nm	Template method
Up to 20 nm	Multilayer by multilayer method
Up to 45 nm	Coacervation method
Up to 16 nm	Dual-template method

varying the types of organic solvents. Surface molecule occupied by the surfactant molecule is influenced by the different type of organic solvent (Wu et al. 2014).

Pores size—The pore size of the core-shell silica particles is mostly generated by the co-surfactant. The pore size can be enlarged from 7.1 to 22.3 nm while keeping the unique fibrous shell structure (Qu et al. 2017). C/S SNPs of different pore size may be prepared by using various method (Table 11.2).

11.6 Synthesis of Silica Nanoparticles

Various methods that have been used to obtain SNPs can be categorized into two main approaches: Top-down and bottom-up.

Top-down—This method is also known as physical approach. In this process the size of particle is reduced through some physical techniques like ball milling, etc.

Bottom-up—It is known as a chemical approach which involves a common route used to produce SNPs from atomic or molecular scale.

Some of the widely used methods to synthesize silica nanoparticles are as follows:

Sol–gel process—The term "sol-gel" was firstly introduced by Graham in 1864 during his work on silica sols in which sols refer to the liquid medium (Singh et al. 2014). This process is substantially more preferred because of its ability to produce refined and comparable products at mild conditions. This process is based on the hydrolysis and condensation reaction of organometallic compounds in alcoholic solutions. In this process, there is hydrolysis of metal alkoxide or inorganic salt in the presence of acid or base as a catalyst. There is formation of silanol groups after the hydrolysis of metal alkoxide such as tetraethyl orthosilicate (TEOS), etc. For the formation of entire silica structure, condensation reaction occurs between silanol groups and ethoxy groups (Stöber et al. 1968).

Synthesis of SNPs with TEOS has the following reaction steps:

Hydrolysis

$$\mathrm{Si(OC_2H_5)_4 + H_2O \overset{hydrolysis}{\rightarrow} Si(OC_2H_5)_3OH + C_2H_5OH}$$

Condensation

$$\equiv Si - O - H + H - O - Si \equiv \overset{\text{water condensation}}{\rightarrow} \equiv Si - O - Si \equiv + H_2O$$

$$\equiv Si - OC_2H_5 + H - O - Si \equiv \overset{\text{alcohol condensation}}{\rightarrow} \equiv Si - O - Si \equiv + C_2H_5OH$$

Formation of sol and gel can be divided into two stages:

Colloidal suspension of particles—particles are suspended into liquid medium which is referred as sol.

Conversion of sol into gel—The particles react with each other forming a cross-linked 3D polymeric chain which converts into gel (Singh et al. 2014).

Precipitation—Precipitation is the process behind the condensation reaction. It is based upon the mixing of ionic solution to form the precipitate. It is defined as a chemical event that occurs in an aqueous solution when two ionic bonds combine, forming an insoluble precipitate. So for the synthesis of SNPs, precipitate is silica. The properties of precipitated silica get influenced by many factors such as

- synthesis temperature
- time of precipitation
- pH
- the addition of surfactants
- Approaches on washing and drying.

*Steps for the synthesis***:**

1. *Mixing of solutions*—First of all, precursor is mixed with distilled water and then there is addition of anionic solution. It can be added by two different techniques depending upon the optimizing process such as for the optimization of pH at different temperature dropwise mixing is preferred and to obtain large scale production direct mixing is conducted (Joni et al. 2020).
2. *Stirring*—After addition, stirring is followed at room temperature in order to adjust the pH (Joni et al. 2020).
3. *Recovery of nanoparticle*—To recover the NPs, filtration is followed by cleaning and drying at specific temperature for 12 h. Cleaning is done with distilled water to neutralize the acid (Joni et al. 2020) (Fig. 11.2).

Pyrolysis—Pyrolysis is the thermolysis of materials at elevated temperatures in an inert atmosphere. Here pyro refers "fire" and lysis means "separating." This causes the change in chemical composition of the material. In general, pyrolysis of organic substances produces volatile products and leaves a solid residue. SNPs can be synthesized by using ultrasonic spray pyrolysis (USP). In this technique tetraethyl orthosilicate (TEOS) is used as a precursor at different pyrolysis temperatures. USP

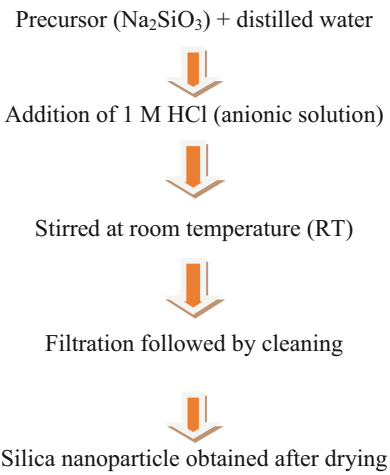

Fig. 11.2 Illustration of the synthesis of silica particles by precipitation method

can also be used to directly prepare silica nanoparticles through a continuous process (Watanabe et al. 2011; Das et al. 2019).

Reverse microemulsion method—In reverse microemulsion, there is formation of micelles by dissolving surfactant molecules into organic solvents. In the presence of water, the polar head groups organize themselves to form microcavities containing water, which is often called as reverse micelles. In synthesis of SNPs, the NPs can be grown inside the microcavities by carefully controlling the addition of silicon alkoxides and catalyst into the medium containing reverse micelles (Rahman and Padavettan 2012). This method enables a fine control of the silica shell thickness with nanometer precision. It enables straightforward surface functionalization via co-condensation between tetraethyl orthosilicate and another silane with desired functional group.

Vapor phase method—Synthesis of SNP by vapor phase method involves chemical supersaturation. Chemical supersaturation refers to the reaction between vapor molecules which results in condensed phase (Swihart 2003). Vapor phase synthesis involves the following process (Fig. 11.3):

Chemical vapor synthesis or chemical vapor condensation—It is the method which involves the conditions that support the nucleation of particles in vapor phase. This method does not involve any film deposition on the wall. The precursor in reactor could be solid, liquid, or gas but they should be delivered in reactor as vapor. Silicon tetrachloride ($SiCl_4$) and Tetraethoxysilane (TEOS) are used as precursor for the synthesis of silica nanoparticle (Yan et al. 2014). However, handling of TEOS is more facile than silicon tetrachloride but synthesis of particle by using silicon tetrachloride is beneficial from the environmental perspective because silicon tetrachloride is a potent environmental pollutant. NPs obtained by this method have the following characteristics:

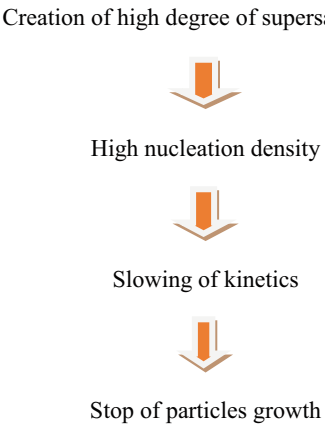

Fig. 11.3 Illustration of the steps in vapor phase synthesis process

- higher purity
- uniform particle size distribution
- larger surface area
- smoother surface

All these characteristics are influenced by the temperature and reaction time used during the synthesis of particle by this methodology. Surface area decreases with increase in temperature and reaction time while there is increase in particle size with increase in reaction time (Fig. 11.4).

11.7 Mesoporous Silica Nanoparticles as Drug Delivery System

MSNs are well studied by various research groups as an efficient drug delivery system (DDS). DDS is advantageous as not only the quantity of drug dosage can be controlled but also a sustainable release paradigm can also be achieved. MSNs are further used for the stimuli responsive drug release due to its loading efficiency, biocompatibility, ease of synthesis, controllable size, and pore diameter. NP surface area to volume ratio is also very significant to produce useful DDS by using NPs.

Different factors of MSNs such as pore size, shape, and structural arrangement affect the drug delivery.

Pore size—Pore size should be larger than the drug molecules to be loaded into MSNs. Larger pore size leads to increase in loading capacity but will have faster release rate (Xu et al. 2019; Vallet-Regi et al. 2008; Cirujano et al. 2017). It has been estimated that larger pore size would lead to increase in the loading capacity by almost two times as compared to small pores.

Pore structure—Pore geometry is also an important factor in context of drug delivery and release (Xu et al. 2019). Different pore structures show that different effect on the release rate and loading capacity such as MSNs with cone shaped pore has higher drug loading capacity (Meka et al. 2016; Xu et al. 2019), whereas MSNs

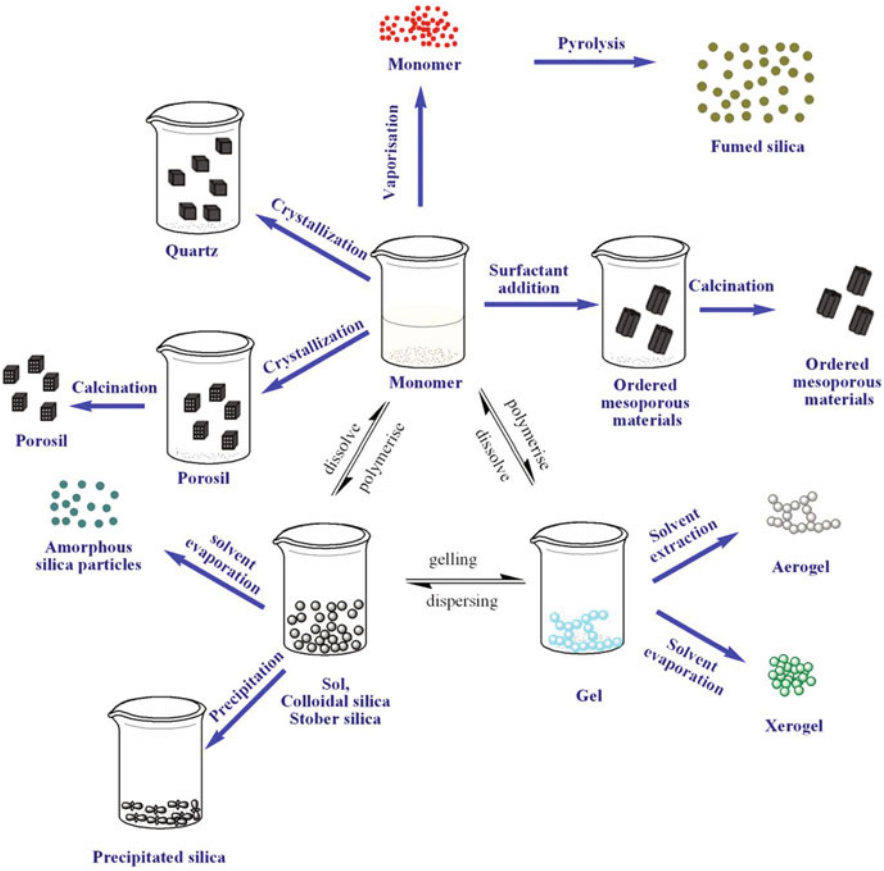

Fig. 11.4 General scheme for synthesis of silica (Adapted from Brinker CF, Schrerer GW: Sol-Gel Science. The Physics and Chemistry of Sol–Gel Processing. second edition. London: Academic Press; 1990)

with cylindrical pores have better loading capacity than other shape pore such as cylindrical pore shape, etc. (Andersson et al. 2004; Xu et al. 2019).

The drug release is subsequently influenced by endogenous and exogenous factors (Song et al. 2016a, b). These two factors are important for the synthesis of stimuli responsive MSNs based DDS (Raza et al. 2019).

Endogenous stimuli—Endogenous stimuli are the internal or biological stimuli that include native environment of tissues (Raza et al. 2019; Song et al. 2016a, b). MSN based nanocarriers are sensitive to the internal stimuli and provide controlled drug delivery to the targeted site efficiently. Stimuli that affect the drug delivery are pH, redox, enzyme, ATP, glucose, and H_2O_2, ionic microenvironment. In other words, endogenous stimuli are the combination of chemical as well as biochemical elements (Raza et al. 2019; Song et al. 2016a, b.

11.8 pH Responsive MSN Based Carrier System

Nanocarriers are optimized as a DDS by functionalizing their surfaces. pH responsive nanocarrier is sensitive to pH, so it will work by sensing the pH conditions within the target site. Diseased tissues and healthy tissues differ in the pH range as healthy tissues have pH of 7.4, whereas diseased tissues have pH range of 6–7 which is slightly acidic than healthier tissues (Song et al. 2016b). Drug is released by sensing the pH of tissues. Under acidic conditions, linkers are cleaved as their bonds are degraded in acidic pH. This stimulates the release of drug from nanocarrier. For example, Song et al. (2016b) synthesized a gold NP coated MSN and tested its efficacy as a delivery platform of dye. By the use of linkers such as acetal bond, hydrazine bond, hydrazone bond, and ester bond, gold NPs were coated on the surface of MSNs. After that dye ($[Ru(bipy)_3]Cl_2$) was loaded as a model drug in order to test the release of drug within the tissues. The release of the dye from the nanocarrier was found to be dependent on pH. When the conditions are normal, like in healthy tissues when there is pH of 7.0 then there is no release of drug because nanopores are blocked by the acetal linkers and gold NPs which are coated on the surface of MSNs (Song et al. 2016b; Raza et al. 2019).

11.9 Redox Responsive Nanocarrier

Just like the pH redox potential differences also exist between the normal and diseased cells. In redox responsive DDS, concentrations of reducing agent play an important role as its concentration varies in the normal and diseased cells. For example, reducing agents such as GSH are found to be higher in cancerous cells. GSH is capable of cleaving the disulfide bond, so this property is exploited for the generation of redox responsive nanocarrier system. MSNs may be coated with NPs such as CdS through the disulfide bond to block the pores of MSN. When DDS reaches the tumor site, disulfide bonds are degraded due to the presence of GSH in tumor cells and as a result the drugs are release at the target site. This is the basic principle that followed for the establishment of redox responsive nanocarrier (Song et al. 2016b; Raza et al. 2019).

11.10 Enzyme Responsive Nanocarrier

Enzyme responsive nanocarrier is the one in which enzymes act as stimuli to release the drug from the DDS (Ulijn 2006). In diseased tissue, particularly there is overexpression of specific enzymes in tumor tissues and this property is exploited for the enzyme responsive DDS. Enzymes such as proteases, hydrolases, oxidoreductases are overexpressed in abnormal tissue. Release of drugs from the nanocarrier is based upon the principle of enzyme aided degradation. So for the formulation of enzyme responsive nanocarrier, that carrier should be the one that can release the drug in the presence of particular enzyme.

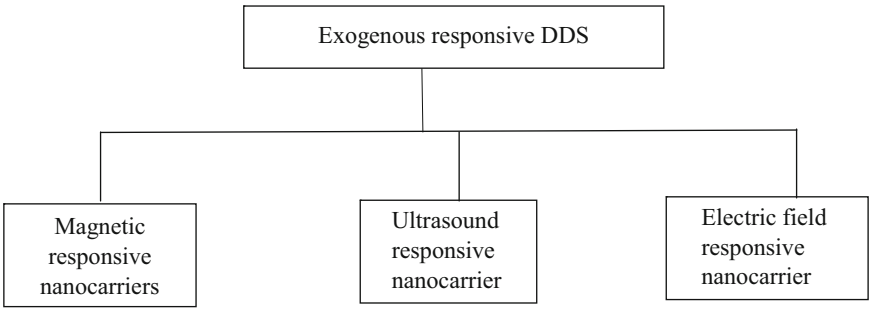

Fig. 11.5 Classification of enzyme responsive DDS

In an enzyme responsive DDS three components are required: (a) carrier, (b) stimuli, i.e., enzyme, (c) gatekeeper. For example, enzymes such as metalloproteinases may act as stimuli and is able to cleave the gatekeeper (-β-cyclodextrin) that is coated on the surface of the MSNs which act as carrier for the drug to be release at the target site.

Exogenous stimuli—Exo refers to "outside." So exogenous stimuli are the ones which come from the outside environment. In exogenous stimuli DDS, drug release depends upon the response to external factors such as temperature, light, electrical field, etc. (Yadav et al. 2018). Exogenous DDS is broadly classified as follows (Fig. 11.5):

Magnetic responsive nanocarriers—DDS releases the drug to tumor tissue in response to the magnetic field (Vinhas et al. 2020). Magnetic responsive nanocarrier shows high efficiency in delivering the drug to the target site. The basic requirements for the magnetic nanocarrier to become a DDS are biocompatibility, magnetic property of the NP, etc.

Ultrasound responsive nanocarrier—The application of ultrasound is combined with other factors (such as thermal, mechanical) to release the drug from the nanocarrier (Entzian and Aigner 2021). The ultrasound responsive nanocarrier is constructed in such a way that both ultrasound and drug carrier NP come into contact with each other and then the drug is released at the target site in response to mechanical or thermal stimuli. The sensing of stimuli is based on the type of NPs used as a carrier for the DDS. If the carrier is organic, the drug is released in response to thermal stimuli but if the carrier is of inorganic nature such as MSN, then mechanical factors act as a stimulus to release the drug at the target site. MSNs are the most preferred NP for the DDS as they offer high drug loading capacity due to the presence of pores on their surface. Ultrasound responsive MSNs nanocarriers have been developed by surface modification of MSN with sodium alginate and carboxyl-calcium coordination bonds that can act as a gatekeepers by blocking pores of MSNs (Entzian and Aigner 2021 & Li et al. 2019).

Electric field responsive nanocarrier—Electric field responsive nanocarrier refers to the smart drug delivery systems in which drug release is controlled by the application of an electric field. Electric pulses can provide many benefits in

biomedical field such as stimulation of cell fusion, increasing penetration of therapeutics agents by acting on drug carriers, cells, and tissues. This type of DDS is used for the treatment of cancer cells through the electro chemotherapy in which antitumor drugs are combined with the electric field pulses. Electro responsive DDS is available as implantable material that can be administered via injection and then upon the stimulation of electric field pulses release the drug to the tumor and leading to decrease the size of tumor (Kolosnjaj-Tabi et al. 2018).

Post-synthetic surface functionalization of silica nanoparticle—SNPs are used as carrier in DDS through surface modification of these NPs. This surface modification allows precise control over drug loading and their release at the target site (Liberman et al. 2014). Due to the presence of inner and outer surface MSNs act as a suitable scaffold for a variety of modifications and functionalization that provide efficient cargo delivery as well as maintain biocompatibility and stability (Rastegari et al. 2021). Functionalization with a specific group such as capping with particular hydrophobic group also helps reduce the hydrolysis of silica (Liberman et al. 2014). The surface of SNPs is usually modified via alkoxysilane or halosilane. In halosilanes first the hydrolyzation reaction takes place in which alcohol group is formed and then it undergoes a condensation reaction to form Si–O–Si linkage. There are two methods for modifying and functionalizing the MSN.

1. Co-condensation modifications.
2. Post-synthetic modifications.

In this chapter we are discussing about the post-synthetic modifications only. To achieve any kind of surface modifications, estimation of the surface available for modification is an important requirement which is resolved by taking into account two factors such as pore volume and surface of MSNs. In this type of modifications, modifying agents with special functional groups are attached to the pore surface selectively. Functionalization can be done with different groups depending upon the purpose such as 3-aminopropyl triethoxysilane (APTES), 3-mercaptopropyl trimethoxysilane (MPTS) is preferred for bonding between two parts and to achieve biocompatibility and improve stability in biological fluids. PEGylation of MSNs can also be done. Liberman et al. (2014) made a pH responsive MSN based DDS where the surface was functionalized with APTES and nitroveratryl carbamate. Nitroveratryl carbamate acted as a protecting group which controlled the drug release in low pH and resulted in electrostatic repulsion and triggered the release of doxorubicin (DOX) from the MSNs surface.

11.11 Silica Nanoparticle as a DDS for Infectious Diseases

Infectious diseases are those diseases that can spread from one organism to another. It is caused by the pathogens like bacteria, parasites, virus through the process of pathogenesis. Transmission can be through living (plants, humans, and other animals) or non-living things (air droplets or any other object). Infectious diseases

may be classified into two groups: emerging and re-emerging infectious diseases. This classification is based on the type of infection, e.g., if the disease is caused by a new type of infection it is called an emerging infectious disease and when the diseases are due to the reappearance of an old type of infection it is called as re-emerging infectious diseases (Aparna et al. 2019). Infectious diseases re-emergence is the main cause of morbidity and death due to lack of the treatment because the re-emergence of infection causes the causative agents (parasites, bacteria, etc.) to become resistant to specific drugs that make the drug ineffective against those microorganisms (Aparna et al. 2019 & Carvalho et al. 2020). Therefore, to address this problem a new approach has to be discovered. Combination of nanotechnology and medicine is continuously being explored as a new paradigm to combat the menace of infectious diseases. Already various nanomaterial based smart delivery systems have been developed for the effective treatment of infectious diseases. In this approach, the first step is the selection of NP in which the drug is incorporated and then delivered to target tissue (Torres-Sangiao et al. 2016). NPs are very useful as a carrier because they have an ability to fight with pathogens by many ways like damaging cell membrane, ROS production, etc. (Chen et al. 2018).

11.12 Beneficial Attributes of NPs for Treatment of Infectious Diseases

- Tunable surface property
- Enhanced drug loading due to increased surface area to volume ratio
- Selective antimicrobial effect
- Low toxicity against the host
- Selective targeting
- Increase pharmacokinetic profile

Among various NPs, SNPs are of great interest as a nanocarrier for drug delivery. Various SNPs like conventional non-porous SNPs, MSNs, core/shell SNPs can be used as a drug carrier to combat infectious diseases. Among these due to its flexibility in the surface groups, biocompatibility, and porous structure, MSNs have become popular as DDS (Selvarajan et al. 2020). Development of smart delivery system based on MSN provides the efficiency in drug release and minimizes the risk from direct application of drug (Carvalho et al. 2020). Even MSNs can be used for targeted drug delivery. Targeted drug delivery mechanism proves to be most efficient method for the treatment of infectious diseases specifically for the re-emerging infectious diseases (Aparna et al. 2019).

11.13 Specific Properties of MSN Due to Which they are Used as a Nanocarrier for Infectious Disease Treatment

MSNs are widely accepted nanocarriers for the treatment of infectious diseases for the following reasons:

- Adaptable pore size
- It provides sustained release of drugs due to covalently bonded one or more amine group at inner pore surface (Ghosh 2019).
- It has pores on inner and outer surface and there is special mechanism by which these pores may be blocked and then on the special stimulation these pores may also be opened, and drugs can be released. Thus, this whole mechanism improves the drug delivery at the target site.
- It has the potential of surface modification through which efficacy of drug can be improved and it is beneficial for toxicity reduction (Xu et al. 2019).
- Unique structural frame of MSNs provides protection of certain agents from denaturation and degradation (Kao et al. 2014; Xu et al. 2019).
- Some drugs can provide efficient treatment, but they fail to do so due to their low solubility. MSNs can conjugate drugs on its surface thereby improving solubility (Narayan et al. 2018).
- MSNs have different structural arrangement and pore size which may influence their properties (Narayan et al. 2018).

Parasitic infectious diseases—Parasites are defined as pathogens that live in or on another organism which is known as the host organism for the parasites. Parasites obtain nutrition from their host organisms for their growth and survival. Infections caused or transmitted by the parasites are known as parasitic infectious diseases or parasitosis. Parasites are primarily responsible for persuading the chronic diseases that are difficult to diagnose at first. Parasites can spread infections to both humans and animals. Parasites are classified on the basis of their presence inside or outside body. Endoparasites are those that reside in the host and they can be found intracellular or extracellular spaces, whereas ectoparasites are those that live on the surface of the host body such as skin and get their nutrition by sucking blood or body fluid. Most ectoparasites serve as vectors for certain pathogens. Lice, rat, flea, ticks, and itch mite are examples of human ectoparasites while endoparasites include *Trichinella*, *Schistosoma,* etc. Both parasitic organisms can maintain parasitism between the host and the parasites. Targeted delivery mechanisms are more effective for treating endoparasitic infections because they require the drug to be delivered to the infection site inside the cell. MSNs are also now being used for the production of vaccines for the treatment of certain types of parasitic infections as they have enormous amount of silanol groups which can be further functionalized favoring enhanced cell targeting and release of the drug at the target site (Torres-Sangiao et al. 2016).

All these properties make MSN an efficient nanoplatform for the treatment of parasitic infectious diseases (Carvalho et al. 2020). MSN is used to treat a number of parasitic infections such as Schistosomiasis, Chagas diseases, etc.

Schistosomiasis—Schistosomiasis is a chronic and acute phase disease caused by blood trematodes that belong to the genus *Schistosoma*. Transmission to humans occurs through contact with fresh water that contains the parasite. The three main species that infect humans are *S. haematobium*, *S. japonium,* and *S. mansoni*. Infection in humans is due to the larval stage of parasite that enters the skin. There are two main types of treatments available for the infection, chemotherapy and administration of vaccine. Praziquantel is the only medicine by which the infection can be treated but it is not completely efficient as *Schistosoma* does not multiply within the host instead, they deposit eggs resulting in worm burden. Hence there is a need for treatments which can reduce the parasite ratio and viability of eggs within the tissues (Thétiot-Laurent et al. 2013). MSN-based vaccines were produced by the incorporation of SWAP (Soluble Worm Antigenic Preparation) on the surface of MSNs, followed by the immunological tests. This new technology showed high immune response due to the presence of high surface area, non-toxic nature of the carrier system, high pore volume, and biocompatibility. Thus, this nanocarrier is used as a successful tool for the treatment of schistosomiasis against the *S. mansoni* infection (Carvalho et al. 2020).

Chagas diseases—Chagas disease is an infectious disease caused by the parasite *Trypanosoma cruzi*. Transmission can occur in a number of ways such as through fecal contamination of Reduviidae insects with insect bites or another skin injury, from blood transfusion, organ transplantation, congenital contamination, and consumption of contaminated food. It shows symptoms in two phases: acute phase and chronic phase. The first is asymptomatic while the second consists of digestive and cardiac lesions. Infection can be controlled to great extent by preventing insect bites (Quijia Quezada et al. 2019). Two treatment methods are possible, one is the introduction of antiparasitic treatment by administration of medications and second is symptomatic treatment by controlling the infection. The medications available for the treatment are benznidazole and nifurtimox. But they often show side effects, so these drugs are incorporated into NPs to improve their efficiency (Arrúa et al. 2019). For action against *Trypanosoma cruzi*, the surface of MSNs is modified by functionalization of MCM-41 with (3- glycidoxypropy) trimethoxysilane (GPTMS) and chitosan succinate (CS) with benznidazole (BZ). This results in the formation of MSN nanocarriers with uniform pore size of 3.3 nm and successful treatment of the *Trypanosoma* infection (Carvalho et al. 2020).

Bacterial diseases—Bacterial diseases are caused by pathogenic bacteria such as *Mycobacterium tuberculosis*, *Staphylococcus* sp., *Pseudomonas* sp., etc. These bacteria transmit diseases by invading the normal system of the body mechanism and entering in an area where no other bacteria are found such as the blood (Chan et al. 2013). Treatment of bacterial diseases using conventional mechanisms is not as efficient as required. The two main reasons behind the difficulties are antimicrobial resistance (AMR) and biofilm formation. AMR is the main cause for the difficulty of treatment in bacterial infection. To treat infections more effectively, large amount of

antibiotics is used that can treat the infection more effectively but also make the pathogen resistant to antibiotics which leads to mortality in many such bacterial infection. Resistance to antibiotics means that bacteria can survive even in the presence of the antibiotics, e.g., carbapenem resistant pneumoniae strains are able to survive in the presence of carbapenem. Controlling resistant bacterial strains is a major challenge for the pharmaceutical industries. Antibiotics resistance in bacteria occurs due to the evolution of antibiotics resistance genes (Friedman et al. 2016) and this may arise for more than one reasons such as usage of antibiotics at high dosage, selection pressure, genetic mutations etc. This is one of the main reasons of increasing in number of resistant bacterial pathogens which are causing higher death among the organisms (Selvarajan et al. 2020).

Biofilm refers to the protective coating of an extracellular matrix around bacteria. Bacterial pathogen has a unique feature of forming a self-coating (Biofilm) around its surface, so in order to disrupt those biofilm, long drug therapy is required. Long drug therapy employs use of large amount of antibiotics which again increase in resistance in bacteria against antibiotics and helps them to survive in the presence of antibiotics.

Therefore, to address this problem there is need for a system that improves the healing process and reduces the effect of antimicrobial resistance. The targeted delivery mechanism is advantageous as it can deliver the drug to the target site, increase solubility of the drug, reduce side effects. MSN based nanocarrier works against several bacterial infection such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*. Tetracycline loaded on MSN surface showed inhibitory effect against *E. coli*. MSNs functionalized with sulfonate and drug ciprofloxacin showed antibacterial effect against *Pseudomonas aeruginosa* (Ghosh 2019).

Fungal infections—Mycosis or fungal infections caused by fungal agents like *Aspergillus*, *Candida*, dermatophytic molds from the genera *Trichophyton*, *Microsporum*, *Epidermophyton,* etc. Based on the site of the infection it can be classified into three groups, namely superficial, cutaneous, and systemic infection (Kainz et al. 2020). Cutaneous infection is one that occurs only on the upper body surface. But when the infection spreads throughout the body it is called as systemic infection. Systemic fungal infections are more harmful than cutaneous infection. Superficial fungal infections include tinea of the finger and feet, aspergillosis and mucormycosis are examples of systemic infections. Fungal infections are usually transmitted through spores either by inhalation or by direct skin contact. To reduce the side effects and to improve the antifungal treatment efficiency, the nano carrier systems are designed where the appropriate NPs with antifungal properties and compatibility for the drug to be loaded are chosen. The nano carrier system overcomes the drawbacks associated with the conventional treatment procedures and this can even work against biofilm formation, multiple resistance problems. A few examples of silica nanocarrier mediated fungal disease control strategies are discussed below:

Aspergillosis—This is a fungal infection caused by the fungus *Aspergillus* sp. that affects the lungs. The infection is harmful to people who have weak immune systems or those who are already suffering from diseases like asthma, cancer, or any other

serious illness. It is transmitted by the inhalation of spores from *Aspergillus*. The pathogen responsible for the infection is *A. niger*, *A. flavus*, *A. fumigatus* (Denning et al. 2012). Carvacrol, cinnamaldehyde, eugenol, and thymol are some of the agents that show stimulation of antifungal activity. Carvacrol and thymol are natural preservatives. These oils with antifungal properties are loaded onto the MSN and used for the targeted delivery of these agents to the infection site. The MSN-based nanodevice was found to reduce the adverse effects associated with the direct delivery of antifungal agents such as irritation and toxicity.

Candidiasis—Infection caused by any type of yeast that includes *Candida* species. It can affect people who have weakened immune systems such as older people and children. Infection usually occurs with the pathogenic *Candida albicans* (Walker 2008). The pH responsive MSN Smart Delivery System was formulated for treatment that released antifungal agents by sensing a low pH. In this system the surface of the MSN was functionalized with an amine group and loaded with fluorescein (S1-FL). At pH 5 the electrostatic interaction between the amine groups was weakened which stimulated the release of fluorescein at transition site. Thus, this type of nano delivery system stimulated the efficiency of drug action and also reduced the side effects associated with oral or topical treatments (Ghosh 2019). Smart delivery systems can also be combined with photodynamic therapy to inhibit biofilm formation. MSN was functionalized with a photosensitizer RB (Rose Bengal) resulting in the formation of reactive oxygen species (ROS) that could kill microbial growth (Carvalho et al. 2020).

Application of SNP based drug delivery system to combat different infectious diseases is tabulated in Table 11.3.

11.14 Biocompatibility, Biodistribution, and Clearance of Silica Nanoparticles

Biocompatibility refers to the ability of a material to contact with host and show the desired effect without making any damage within the host. The term biocompatibility is used differently in different contexts such as the biocompatibility of MSNs refers to the behavior of SNPs within the biological host. The biocompatibility of SNPs is determined by its successful response with respect to biomedical applications such that when it is used as a drug carrier it should not produce any toxic effects within the body. In other words, MSNs must maintain continuity with the tissue without any detrimental effects (Williams 2008). Biodistribution is the diffusion of a substance up to the target site or it is the potential of drug to reach into the cell or tissue. It is the major factor of pharmacokinetics, which is determined by the concentration of substance and change with time. Smart nanocarriers for targeted drug delivery use MSN –like NPs as nanocarriers that employ employment of enhanced permeability and retention effect (EPR) effect for internalization of drug within tissues followed by passive diffusion of drug molecules. Thus the bio-distribution of nanoparticles is a very important factor in the functionality of the nanocarrier.

Table 11.3 MSN based nano delivery system for treatment of different infectious diseases

Diseases	Causative agent	Conventional treatment	Treatment by SNP based nanocarrier	References
Schistosomiasis	*Schistosoma*	Chemotherapy-Praziquantel is the only medication used	MSN based vaccine—Antigenic preparation of soluble worms are incorporated on MSN surface	Carvalho et al. (2020)
Chagas diseases	*Trypanosoma cruzi*	Antibiotics administration like benznidazole and nifurtimox	MSN based nanocarrier—MCM-41 surface is functionalized with GPTMS and CS	Arrúa et al. (2019) Carvalho et al. (2020)
Bacteremia, pneumonia	*Bacillus subtilis*	Antibiotic treatment—Linezolid and daptomycin	Copper shell is loaded on MSN surface which will increase antibacterial efficiency	Ghosh (2019)
Aspergillosis	*Aspergillus* spp.	Antifungal agents—Thymol, carvacrol	Natural oil with antifungal agents loaded on MSN	Bernardos et al. (2014)
Candidiasis	*Candida* spp.	Azole drugs	pH responsive MSN nanocarrier by surface functionalization with amine group and loading of fluorescein	Ghosh (2019)
Small intestines infection	*E. coli*	Hydrolysis of bacterial wall by the protein factor such as lysozyme	MSN-41with larger pore size is loaded with lysozyme to enhance the treatment	Xu et al. 2019; Song et al. (2016a, b)
Bacterial bone infection	*Pseudomonas aeruginosa*, *Staphylococcus epidermis*	Antibiotics treatment	pH responsive MSNs—SBA-15 with amino and carboxylic acid modification on surface to enhance the antibacterial efficiency and low protein adsorption	Gisbert-Garzarán et al. (2020); Khatoon et al. (2016)
Intracellular infection	*S. aureus*	Antimicrobial peptides, phage therapy	HMM type MSNs loaded with rifampicin antibiotic	Subramaniam et al. (2019)

(continued)

Table 11.3 (continued)

Diseases	Causative agent	Conventional treatment	Treatment by SNP based nanocarrier	References
Tuberculosis	*Mycobacterium tuberculosis*	Drug therapy	Chaperon associated MSNs—MSNs loaded with clofazimine to enhance to solubility	Chen et al. (2018)
Tularemia	*Francisella tularensis*	Antibiotic treatment	MSN loaded with moxifloxacin	Lee et al. (2016)
Necrotic Enteritis (NE)	*Clostridium perfringens*	Antibiotic growth promoters; Vaccination-Netvax vaccine	MSN based nanovaccine—MSN loaded with chimeric antigen through conjugation and adsorption methods	Hoseini et al. (2021)
Vibriosis	*Vibrio alginolyticus*	Antibiotics treatment	MSN nanovaccine—dihydrolipoamide dehydrogenase (DLDH) antigens loaded on MSN	Pang et al. (2016)
Foot and mouth diseases	FMD virus (FMDV)	Vaccination	Nanovaccines—FMDV VLPs adsorbed on the surface of MSNs which enhance the immunity and overcome the drawbacks of traditional vaccines	Bai et al. (2019)

The behaviors of NPs such as biocompatibility, biodistribution, and clearance of NPs are influenced by some physicochemical properties:

- Size
- Shape
- Surface chemistry or modification

Shape of SNPs influences the cellular uptake and drug delivery such that rod like SNPs enhance the compatibility within the host tissue (Huang et al. 2011). The ability of surface modification of MSN is extensively harnessed for building smart drug distribution system. The functionalization with different groups has different effects on the behavior of silica NPs. Modifications with hydroxyl groups, carboxylic group, and PEG favors the clearance from systemic blood circulation (Huang

et al. 2011). Surface modified with particular PEG reduced the interaction between positively charged lipid bilayer of RBC and negatively charged silica NP and thus improve the compatibility of MSN.

References

Aderibigbe B (2017) Metal-based nanoparticles for the treatment of infectious diseases. Molecules 22(8):1370. https://doi.org/10.3390/molecules22081370

Andersson J, Rosenholm J, Areva S, Linden M (2004) Influences of material characteristics on ibuprofen drug loading and release profiles from ordered micro- and mesoporous silica matrices. Chem Mater 16:4160–4167. https://doi.org/10.1021/cm0401490

Aparna VA, Biswas R, Jayakumar R (2019) Targeted nanoparticles for treating infectious diseases. In: Biomimetic nanoengineered materials for advanced drug delivery, pp 169–185. https://doi.org/10.1016/b978-0-12-814944-7.00012-6

Arrúa EC, Seremeta KP, Bedogni GR, Okulik NB, Salomon CJ (2019) Nanocarriers for effective delivery of benznidazole and nifurtimox in the treatment of Chagas disease: a review. Acta Trop 198:105080. https://doi.org/10.1016/j.actatropica.2019.10

Bai M, Dong H, Su X, Jin Y, Sun S, Zhang Y, Guo H (2019) Hollow mesoporous silica nanoparticles as delivery vehicle of foot-and-mouth disease virus-like particles induce persistent immune responses in guinea pigs. J Med Virol 91(6):941–948. https://doi.org/10.1002/jmv.25417

Bernardos A, Marina T, Zacek P, Pérez-Esteve É, Martínez-Máñez R, Lhotka M, Kouřimská L, Pulkrábek J, Kloucek P (2014) Antifungal effect of essential oil components against Aspergillus niger when loaded into silica mesoporous supports. J Sci Food Agric 95(14):2824–2831. https://doi.org/10.1002/jsfa.7022

Carvalho GC, Sábio RM, de Cássia Ribeiro T, Monteiro AS, Pereira DV, Ribeiro SJL, Chorilli M (2020) Highlights in mesoporous silica nanoparticles as a multifunctional controlled drug delivery nanoplatform for infectious diseases treatment. Pharm Res 37(10):191. https://doi.org/10.1007/s11095-020-02917-6

Chan GJ, Lee AC, Baqui AH, Tan J, Black RE (2013) Risk of early-onset neonatal infection with maternal infection or colonization: a global systematic review and meta-analysis. PLoS Med 10(8):e1001502. https://doi.org/10.1371/journal.pmed.1001502

Chen W, Cheng C-A, Lee B-Y, Clemens DL, Huang W-Y, Horwitz MA, Zink JI (2018) A facile strategy enabling both high loading and high release amounts of the water-insoluble drug clofazimine using mesoporous silica nanoparticles. ACS Appl Mater Interfaces 10(38):31870–31881. https://doi.org/10.1021/acsami.8b09069

Cirujano FG, Luz I, Soukri M, Van Goethem C, Vankelecom IFJ, Lail M et al (2017) Boosting the catalytic performance of metal-organic frameworks for steroid transformations by confinement within a mesoporous scaffold. Angew Chem Int Ed 56:13302–13306. https://doi.org/10.1002/anie.201706721

Das H, Debnath N, Arai T, Kawaguchi T, Sakamoto N, Shinozaki K et al (2019) Superparamagnetic magnesium ferrite/silica core-shell nanospheres: a controllable SiO_2 coating process for potential magnetic hyperthermia application. Adv Powder Technol 30:3171–3181. https://doi.org/10.1016/j.apt.2019.09.026

Denning DW, Pleuvry A, Cole DC (2012) Global burden of chronic pulmonary aspergillosis complicating sarcoidosis. Eur Respir J 41(3):621–626. https://doi.org/10.1183/09031936.00226911

Entzian K, Aigner A (2021) Drug delivery by ultrasound-responsive nanocarriers for cancer treatment. Pharmaceutics 13(8):1135. https://doi.org/10.3390/pharmaceutics13081135

Friedman ND, Temkin E, Carmeli Y (2016) The negative impact of antibiotic resistance. Clin Microbiol Infect 22(5):416–422. https://doi.org/10.1016/j.cmi.2015.12.002

Geng Y, Dalhaimer P, Cai S, Tsai R, Tewari M, Minko T, Discher DE (2007) Shape effects of filaments versus spherical particles in flow and drug delivery. Nat Nanotechnol 2(4):249–255. https://doi.org/10.1038/nnano.2007.70

Ghosh S (2019) Mesoporous silica-based nano drug-delivery system synthesis, characterization, and applications. In: Nanocarriers for drug delivery, pp 285–317. https://doi.org/10.1016/b978-0-12-814033-8.00009-6

Ghosh Chaudhuri R, Paria S (2011) Core/shell nanoparticles: classes, properties, synthesis mechanisms, characterization, and applications. Chem Rev 112(4):2373–2433. https://doi.org/10.1021/cr100449n

Gisbert-Garzarán M, Manzano M, Vallet-Regí M (2020) Mesoporous silica nanoparticles for the treatment of complex bone diseases: bone cancer, bone infection and osteoporosis. Pharmaceutics 12(1):83. https://doi.org/10.3390/pharmaceutics12010083

Hao N, Li L, Tang F (2014) Shape-mediated biological effects of mesoporous silica nanoparticles. J Biomed Nanotechnol 10(10):2508–2538. https://doi.org/10.1166/jbn.2014.1940

Hay RJ, Johns NE, Williams HC, Bolliger IW, Dellavalle RP, Margolis DJ et al (*2014*) The global burden of skin disease in 2010: an analysis of the prevalence and impact of skin conditions. J Investig Dermatol 134(6):1527–1534. https://doi.org/10.1038/jid.2013.446

Heta S, Robo I (2018) The side effects of the most commonly used group of antibiotics in periodontal treatments. Med Sci 6(1):6. https://doi.org/10.3390/medsci6010006

Hong X, Zhong X, Du G, Hou Y, Zhang Y, Zhang Z, Sun X (2020) The pore size of mesoporous silica nanoparticles regulates their antigen delivery efficiency. Sci Adv 6(25):eaaz4462. https://doi.org/10.1126/sciadv.aaz4462

Hoseini ZS, Hajizade A, Razmyar J, Ahmadian G, Arpanaei A (2021) Mesoporous silica nanoparticles-based formulations of a chimeric proteinous vaccine candidate against necrotic enteritis disease. Mater Sci Eng C 128:112316. https://doi.org/10.1016/j.msec.2021.112316

Huang X, Teng X, Chen D, Tang F, He J (2010) The effect of the shape of mesoporous silica nanoparticles on cellular uptake and cell function. Biomaterials 31(3):438–448. https://doi.org/10.1016/j.biomaterials.2009.09.060

Huang X, Li L, Liu T, Hao N, Liu H, Chen D, Tang F (2011) The shape effect of mesoporous silica nanoparticles on biodistribution, clearance, and biocompatibility in vivo. ACS Nano 5(7):5390–5399. https://doi.org/10.1021/nn200365a

Huang X, Young NP, Townley HE (2014) Characterization and comparison of mesoporous silica particles for optimized drug delivery. Nanomater Nanotechnol 4:2. https://doi.org/10.5772/58290

Joni IM, Rukiah, & Panatarani, C. (2020). Synthesis of silica particles by precipitation method of sodium silicate: effect of temperature, pH and mixing technique. In: Third international conference 00

Kainz K, Bauer MA, Madeo F, Carmona-Gutierrez D (2020) Fungal infections in humans: the silent crisis. Microbial Cell 7(6):143–145. https://doi.org/10.15698/mic2020.06.718. ISSN 2311-2638. PMC 7278517. PMID 32548176

Kao K-C, Lin T-S, Mou C-Y (2014) Enhanced activity and stability of lysozyme by immobilization in the matching nanochannels of mesoporous silica nanoparticles. J Phys Chem C 118:6734–6743. https://doi.org/10.1021/jp4112684

Khatami M, Alijani HQ, Sharifi I (2018) Biosynthesis of bimetallic and core–shell nanoparticles: their biomedical applications—a review. IET Nanobiotechnol 12(7):879–887. https://doi.org/10.1049/iet-nbt.2017.0308

Khatoon S, Han HS, Lee M, Lee H, Jung D-W, Thambi T et al (2016) Zwitterionic mesoporous nanoparticles with a bioresponsive gatekeeper for cancer therapy. Acta Biomater 40:282–292. https://doi.org/10.1016/j.actbio.2016.04.011

Kolosnjaj-Tabi J, Gibot L, Fourquaux I, Golzio M, Rols M-P (2018) Electric field-responsive nanoparticles and electric fields: physical, chemical, biological mechanisms and therapeutic prospects. Adv Drug Deliv Rev 138:56–67. https://doi.org/10.1016/j.addr.2018.10.017

Kuhlmann FM, Fleckenstein JM (2017) Antiparasitic agents. In: Infectious diseases, pp 1345–1372.e2. https://doi.org/10.1016/b978-0-7020-6285-8.00157-x

Lee B-Y, Li Z, Clemens DL, Dillon BJ, Hwang AA, Zink JI, Horwitz MA (2016) Redox-triggered release of moxifloxacin from mesoporous silica nanoparticles functionalized with disulfide snap-tops enhances efficacy against pneumonic tularemia in mice. Small 12(27):3690–3702. https://doi.org/10.1002/smll.201600892

Li X, Wang Z, Xia H (2019) Ultrasound reversible response nanocarrier based on sodium alginate modified mesoporous silica nanoparticles. Front Chem 7:59. https://doi.org/10.3389/fchem.2019.00059

Liberman A, Mendez N, Trogler WC, Kummel AC (2014) Synthesis and surface functionalization of silica nanoparticles for nanomedicine. Surf Sci Rep 69(2–3):132–158. https://doi.org/10.1016/j.surfrep.2014.07.001

Medzhitov R, Schneider DS, Soares MP (2012) Disease tolerance as a defense strategy. Science 335(6071):936–941. https://doi.org/10.1126/science.1214935

Meka AK, Abbaraju PL, Song H, Xu C, Zhang J, Zhang HW et al (2016) A vesicle supra-assembly approach to synthesize amine-functionalized hollow dendritic mesoporous silica nanospheres for protein delivery. Small 12:5169–5177. https://doi.org/10.1002/smll.201602052

Narayan R, Nayak U, Raichur A, Garg S (2018) Mesoporous silica nanoparticles: a comprehensive review on synthesis and recent advances. Pharmaceutics 10(3):118. https://doi.org/10.3390/pharmaceutics10030118

Pang H, Chen L, Hoare R, Huang Y, ZaoheWu, Jian J (2016) Identification of DLD, by immunoproteomic analysis and evaluation as a potential vaccine antigen against three Vibrio species in Epinephelus coioides. Vaccine 34(9):1225–1231. https://doi.org/10.1016/j.vaccine.2015.11.001

Qu Q, Si Y, Xuan H, Zhang K, Chen X, Ding Y, Feng S, Yu HQ (2017) Synthesis of core-shell silica spheres with tunable pore diameters for HPLC. Mater Lett 211, S0167577X17314337. https://doi.org/10.1016/j.matlet.2017.09.087

Quijia Quezada C, Azevedo CS, Charneau S, Santana JM, Chorilli M, Carneiro MB, Bastos IMD (2019)

Rahman IA, Padavettan V (2012) Synthesis of silica nanoparticles by sol-gel: size-dependent properties, surface modification, and applications in silica-polymer nanocomposites—a review. J Nanomater 2012, 1–15. https://doi.org/10.1155/2012/132424

Rastegari. E., Hsiao, Y., Lai, W., Lai, Y., Yang, T., Chen, S., Huang, P., Chiou S., Mou C. Y., Chien Y. (2021) An Update on Mesoporous Silica Nanoparticle Applications in Nanomedicine. Pharmaceutics 2021, 13, 1067. https://doi.org/10.3390/pharmaceutics13071067.

Raza A, Rasheed T, Nabeel F, Hayat U, Bilal M, Iqbal H (2019) Endogenous and exogenous stimuli-responsive drug delivery systems for programmed site-specific release. Molecules 24(6):1117. https://doi.org/10.3390/molecules24061117

Selvarajan V, Obuobi S, Ee PLR (2020) Silica nanoparticles—a versatile tool for the treatment of bacterial infections. Front Chem 8:602. https://doi.org/10.3389/fchem.2020.00602

Shang L, Nienhaus K, Nienhaus G (2014) Engineered nanoparticles interacting with cells: size matters. J Nanobiotechnol 12(1):5. https://doi.org/10.1186/1477-3155-12-5

Singh LP, Bhattacharyya SK, Kumar R, Mishra G, Sharma U, Singh G, Ahalawat S (2014) Sol-Gel processing of silica nanoparticles and their applications. Adv Colloid Interf Sci 214 (2014):17–37. https://doi.org/10.1016/j.cis.2014.10.007

Song H, Nor YA, Yu MH, Yang YN, Zhang J, Zhang HW et al (2016a) Silica nanopollens enhance adhesion for long-term bacterial inhibition. J Am Chem Soc 138:6455–6462. https://doi.org/10.1021/jacs.6b00243

Song Y, Li Y, Xu Q, Liu Z (2016b) Mesoporous silica nanoparticles for stimuli-responsive controlled drug delivery: advances, challenges, and outlook. Int J Nanomedicine 12:87–110. https://doi.org/10.2147/ijn.s117495

Stöber W, Fink A, Bohn E (1968) Controlled growth of monodisperse silica spheres in the micron size range. J Colloid Interface Sci 26(1):62–69. https://doi.org/10.1016/0021-9797(68)90272-5

Subramaniam T, Gustafsson J, Kidd SP, Prestidge CA (2019) Rifampicin-loaded mesoporous silica nanoparticles for the treatment of intracellular infections. Antibiotics 8(2):39. https://doi.org/10.3390/antibiotics8020039

Swihart MT (2003) Vapor-phase synthesis of nanoparticles. Curr Opin Colloid Interface Sci 8(1): 127–133. https://doi.org/10.1016/s1359-0294(03)00007-4

Tang L, Cheng J (2013) Nonporous silica nanoparticles for nanomedicine application. Nano Today 8(3):290–312. https://doi.org/10.1016/j.nantod.2013.04.007

Thétiot-Laurent SA-L, Boissier J, Robert A, Meunier B (2013) Schistosomiasis chemotherapy. Angew Chem Int Ed 52(31):7936–7956. https://doi.org/10.1002/anie.201208390

Torres-Sangiao E, Holban A, Gestal M (2016) Advanced nanobiomaterials: vaccines, diagnosis and treatment of infectious diseases. Molecules 21(7):867. https://doi.org/10.3390/molecules21070867

Ulijn RV (2006) Enzyme-responsive materials: a new class of smart biomaterials. J Mater Chem 16(23):2217. https://doi.org/10.1039/b601776m

Vallet-Regi M, Balas F, Colilla M, Manzano M (2008) Bone-regenerative bioceramic implants with drug and protein controlled delivery capability. Progr Solid State Chem 36:163–191. https://doi.org/10.1016/j.progsolidstchem.2007.10.002

Vinhas A, Rodrigues MT, Gonçalves AI, Reis RL, Gomes ME (2020) Magnetic responsive materials modulate the inflammatory profile of IL-1β conditioned tendon cells. Acta Biomater 117:235–245. https://doi.org/10.1016/j.actbio.2020.09.028

Walker M (2008) Conquering Common Breast-feeding Problems. J Perinatal Neonatal Nurs 22(4): 267–274. https://doi.org/10.1097/01.jpn.0000341356.454

Watanabe T, Miki Y, Masuda T, Deguchi H, Kanai H, Hosokawa S et al (2011) Synthesis of γ-Ga2O3–Al2O3 solid solutions by spray pyrolysis method. Ceramics Int 37(8):3183–3192. https://doi.org/10.1016/j.ceramint.2011.05.08

Williams DF (2008) On the mechanisms of biocompatibility. Biomaterials 29(20):2941–2953. https://doi.org/10.1016/j.biomaterials.2008.0

Wong C, Stylianopoulos T, Cui J, Martin J, Chauhan VP, Jiang W et al (2011) Multistage nanoparticle delivery system for deep penetration into tumor tissue. Proc Natl Acad Sci 108(6):2426–2431. https://doi.org/10.1073/pnas.1018382108

Wu X, Wu M, Zhao JX (2014) Recent development of silica nanoparticles as delivery vectors for cancer imaging and therapy. Nanomed Nanotechnol Biol Med 10(2):297–312. https://doi.org/10.1016/j.nano.2013.08.008

Xu C, Lei C, Yu C (2019) Mesoporous silica nanoparticles for protein protection and delivery. Front Chem 7:290. https://doi.org/10.3389/fchem.2019.00290

Yadav HKS, Mohammed A, Dibi M (2018) Advancements in exogeneous techniques for stimuli-sensitive delivery systems. In: Drug targeting and stimuli sensitive drug delivery systems, pp 447–481. https://doi.org/10.1016/b978-0-12-813689-8.00012-4

Yan F, Jiang J, Chen X, Tian S, Li K (2014) Synthesis and characterization of silica nanoparticles preparing by low-temperature vapor-phase hydrolysis of SiCl4. Indus Eng Chem Res 53(30): 11884–11890. https://doi.org/10.1021/ie501759w

Zhao M, Wang F, Fan Y, Raheem A, Zhou H (2019) Low-temperature alkaline pyrolysis of sewage sludge for enhanced H2 production with in-situ carbon capture. Int J Hydrog Energy 44(16): 8020–8027. https://doi.org/10.1016/j.ijhydene.2019.02.04

Nano Drug Delivery Approaches for Lymphatic Filariasis Therapeutics

12

Mukesh Soni, Mayank Handa, and Rahul Shukla

Abstract

Engineering and crafting of a functional system at the molecular level is coined as nanotechnology, ranging in the scale of 0.1–100 nm. This range of scale is helpful in the special characterization of the nanodrug delivery system (nDDS) which is helpful to overcome the biological barriers with an advancement in the bioreactivity and multiple functionalities. On the right hand, a wide range of the novel nano medications associated applications are in the window of development or hanging around the commercialization. Most of these endeavours are targeted on the life-threatening illness like AIDS and HIV, cancer and much more, not so major scrutiny related to LF (lymphatic filariasis) have been seen in the past couple of years. However, processing a desire of new and substitute tactic to improvise the pharmaceutical therapeutic efficiency of anti-filarial medications. In this informative data, an appropriate variety of predicaments manipulating the oral bioavailability of drugs supporting anti-filarial action, heading to unfortunate pharmacodynamics and pharmacokinetics problems in lymphatic filariasis chemotherapy has been known and a few significant nanodrug delivery system to pass out such jams have been emphasized. The concept is multi-times disciplined in nature and used to improvise insights, and facts and values for the sophisticated elimination of the human lymphatic filariasis have been instructed.

M. Soni · M. Handa · R. Shukla (✉)
Department of Pharmaceutics, National Institute of Pharmaceutical Education and Research-Raebareli, Lucknow, UP, India
e-mail: rahul.shukla@niperraebareli.edu.in

S. Hameed, S. Rehman (eds.), *Nanotechnology for Infectious Diseases*,
https://doi.org/10.1007/978-981-16-9190-4_12

Keywords

Lymphatic filariasis · Filaricidal · Nanotechnology · Lymphatic targeting · Infectious disorder · Neglected tropical disease

12.1 Introduction

Engineering and crafting of a functional system at the molecular level is coined as nanotechnology, regarding the scale of 0.1–100 nm (Shukla et al. 2020a). This range of scale is helpful in the special characterization of the nanodrug delivery system (nDDS) which is helpful to overcome the biological barriers with the advancement in the bioreactivity and multiple functionalities. So that it will open new dimensions to work on the structure inside the living body cells. In present life, a diversity of the hybrid or combinational therapeutics at the nano scale and nDDSs are targeted to almost each and every location of the body in order to treat the complex and crucial illness (Shukla et al. 2021a). On the right hand, a wide range of the novel nano medications associated applications are in the window of development or hanging around the commercialization. Most of these endeavours are targeted on the life threatening illness like AIDS and HIV, cancer and much more, not so major scrutiny related to LF (lymphatic filariasis) have been seen in the past couple of years (Shukla et al. 2021b). These data supportably evident the usage of nanotech in the diagnosis proposes, treatment purpose and control purpose of such melancholic illness. In contrast, some discussions came into consideration regarding the shortage of an appropriate pharmaceutical carrier of nano scale or nDDS to recuperate the pharmacodynamic and pharmacokinetic abilities of the anti-filarial medication to improvise their chemotherapeutic worth (Shukla et al. 2013). With the support of the past advances in the nanomedicinal sciences, parasitologists get to know the ideal solution in the window to heal lymphatic filariasis. It would be particularly targeting of anti-filarial medication by the usage of an appropriate nanotherapeutic carrier like polymer nanoparticles, SLNs (solid lipid nano particles), liposomes, pharmacosomes or reverse micelles, niosomes and many more.

Many companies from all around the world are working on nanomedicine which shows that nanoscience is building strong roots and receiving a global appraisal. However, most companies do not give attention in the formulation of anti-filiarial medications as the illness is present majorly in the poverty-attached region of the people of developed and developing countries (Shukla et al. 2015). Hence, it will not offer a profitable market to these companies. In these circumstances, many challenges arise for the health authorities and the government health sector to manage and control such kind of illness. The chief issue for health organizations in removing LF through mass drug administration (MDA) is that the parasite will show their existence continuously which lead to the revival of microfilariae (MFs) for numerous weeks after treatment. The risk of growth of tolerance against mainline drugs is seen as a chief subject. Hence possessing a desire of new and substitute tactic to improvise the pharmaceutical therapeutic efficiency of anti-filarial

medications. In this informative data, an appropriate variety of predicaments manipulating the oral bioavailability of drugs supporting anti-filarial action, heading to unfortunate pharmacodynamics and pharmacokinetics problems in lymphatic filariasis chemotherapy has been known and few significant nanodrug delivery system to pass out such jams have been emphasized (Ali et al. 2013a, 2014a). The concept is multi-times disciplined in nature and used to improvise insights, and facts and values for the sophisticated elimination of the human lymphatic filariasis have been instructed.

12.2 Pathophysiological Aspects of Lymphatic Filariasis and Drug Based Targeting

The pathological aspects of lymphatic filariasis are started with the life cycle which begins when stage-third infectious larvae tires in the course of wound caused due to the mosquito vector in the human body. The larva walks to the lymphatic system and stay out there for a long time to grow and develop into the adult worms. Nanodrug delivery system to lymphatic system holding the anti-filarial drug for adult worms can be utilized to smartly target such mature worms in human lymphatic system (Rajan 1990; Nutman 2016). Moreover, nanoDDS must be able to do the task before the mating condition developed by the worms so that the embryogenesis and generation of thousand to millions of microfilaria can be stopped which would discharge to the lymph and later to the main blood stream. This can be timely identified in the side-line blood. Then these microfilariae are sucked up by mosquito vector, where they molt two times to give birth to stage three larvae which is infectious. To the moment, when third-stage is attained, they delivered to the new host through the mosquito bite and roots lymphatic filarial illness. To further neglect this, nanodrug delivery systems with long circulating microfilaricidal activity seen as the potential agent which greatly prison the transmission of microfilaria. Hence disrupt the filarial life cycle (Taylor et al. 2010; Hoerauf 2008; Bockarie et al. 2009; Chandy et al. 2011; Szuba and Rockson 1998).

12.3 Nanomedicine as Advanced Tool for Targeting

In the window of nanodrug delivery system to treat lymphatic filariasis, most proportion of the drug must be delivered to the targeted parasites through the circulation in the blood system or by residing in the lymphatic system of the human body (Germain et al. 2020; Metselaar and Lammers 2020; Wu et al. 2020; Decuzzi et al. 2021). Unluckily, there is a range of specific barriers such as pharmacodynamic, pharmacokinetic, anatomical, and physiological that are linked with the anti-filarial medication which shows resistance from drug (Joardar et al. 2021). In the past period, nano scale medicine has grown at a faster rate and evident as the most promising and innovative approach in the modern healing asset. It will offer the tools to endorse good health by optimizing and modification of the

re-existing treatment windows for multifaceted and crucial disease and also tackle a lot of unmet therapeutic desires (Singh et al. 2010; Townson et al. 2007; Molyneux and Zagaria 2002; Geary and MacKenzie 2011). Nanodrug delivery system is one of the finest approaches in the nanomedical research, which plays an important drama in controlling the therapeutic effect of the API as this can alter the pharmacological and kinetic profiles such as drug release rate and target specific mechanics of the drug. These parameters and characteristics are the prime consideration and significant for the improvisation of the anti-filarial treatment tactics. Undeniably, this information recommends that nanodrug delivery systems are the best choice solution for countering this disease (Sanabria 2021). The nano scientific answer for the rounded off domino effect supposed in the anti-filarial chemical therapy of lymphatic filariasis is greatly specified here, clarifying that such rising technology carries a large diversity of solutions for lymphatic filariasis. In order to modify the medicaments of anti-filarial salt by nanodrug delivery system would surely produce the existing healing protocols of filarial defensive chemotherapy and positively offer foremost health advantages to the LF critical patients. Nevertheless, to attain such, it becomes necessary to acknowledged about the close limitations linked to the anti-filarial mediators which leads to poor pharmacodynamics and pharmacokinetics. This may also offer a transparent idea of the close characteristics of the drug delivery system for the selection of right nanodrug delivery system for the particular anti-filarial medication. This segment centred at the drawbacks in the wide promising anti-filarial agents in opposition to human lymphatic filariasis such as albendazole, rifampicin, diethylcarbamazine, ivermectin, doxycycline and tetracycline and at similar period suggests diverse nanodrug delivery system as an answer to the predicaments linked with those centralized human lymphatic filarial agents, initiates from bad solubility. It also signifies some pharmacodynamics and pharmacokinetics problems and also the worry of resistance against drug to structural barriers in filarial treatment chemical therapy (Ottesen 2006; Gyapong et al. 2005).

12.3.1 Nanotechnology for Overcoming Anatomical Barriers

12.3.1.1 In the Treatment of Adult Stage Filariasis Through Targeted Nano Drug Delivery System

The sophisticated framework of lymphatics acts as a barrier for anti-filarial medication and limits the targeting of parasites that resides in the core. This prolongs the treatment duration. Surprisingly, one of the topmost inventions in filarial research society is the identification of *Wolbachia* which is the intracellular α-proteobacteria. This is the newest approach for targeting this deadly disease. These kinds of organisms can live in symbiotic environment within adult stage filariasis in lymphatic system of the host. Therefore, the atypical framework of the lymphatic interstitial is the main confront to allocate the core located worms or *Wolbachia* through the filarial treating medications. In recent era, the relevancies of the nanodrug delivery system targeting by close endothelial crossing point of the junctions of the brain (which do not allow 98% of the drug to pass through and

only allows >30 nm particles to cross easily) importantly advise that it may focused on the anti-filarial to filarial parasite living down to the loosely packed lymphatic endothelium by which a particle size of >100 nm can pass through, using such advance technology. This differentiation can clarify the practical ability of accumulation of filarial treating prophylaxis. In addition to that, previously it was observed that drug accumulation can be increased about 5000 times than delivering drug through systemic circulation with nanoparticles targeting of the lymphatic system. To prove this fact, a study shows that the lymphatic concentration of the polymethylmethacrylate NPs and polyhexylcynoacrylate (both radio-labeled) after administered intraperitoneally in rats, it was found that 70 to more than 2000-times greater than the analogous rat's lymph nodes getting IV injection of the nanoparticles. These data evident that targeted nanodrug delivery system can be utilized to bump up the systemic availability of the anti-filarial medication 1000-times near to the lymphatic residence parasites and it can also be used to answer the complexity of the anatomical fences in the cure of lymphatic filariasis. Nanotechnology-mediated lymphatic delivery ensures the effective accumulation of chemotherapeutic in the lymphatics with an appended approach for neutralization of worm. The uptake of nanoparticles by lymphatic system differs in optimum size window of 20–70 nm showing effective uptake and also be the best suited size range for lymphatic accumulation of filarial curing medication using nanodrug delivery system. Lymphatic scheme acts as the central dogma in the maintenance of homeostasis and commonly answers in the partial uptake through the process of passive diffusion because of interstitial confrontation putted by osmotic pressure. Due to such condition, surface alteration of nanoparticles becomes important for boosting the uptake of anti-filarial medication loaded nanodrug delivery system and rewarding filarial killing effect. Superficial crafting of nanodrug delivery system can be performed either by linking the ligands unambiguous to increase the lymphatic aiming such as lectin, folate, hyaluron, dextrin, L-selectin, etc. or by hindering stabilization of nanoDDS by covering with PEG and associated co-polymers. It is already evident previously that superficially altered lymphatic aimed nanosystems with increased localization effect of pharmaceutical agents had been produced. Regrettably, none of such effect prophylaxis has been utilized for active transfer of the anti-filarial drugs. On the other hand, focusing on the pathological and physiological accumulation of worms or illness in the lymphatic system, it can be idolized that lymphatic aimed nanodrug delivery systems are charismatic, valuable and novel technology for the improvisation of anti-filarial therapeutics of lymphatic filariasis (Permana et al. 2019; Ali et al. 2013b, 2014b; Singh et al. 2016; Shrivastava et al. 2020; Binnebose et al. 2015).

12.3.1.2 Nanotherapeutic Amplification of MIF Effectiveness to Stop the Conduction of Lymphatic Filariasis

Majority of the removal concept for the filariasis are done by multi-drug administration (MDA) of singular doses of ivermectin and albendazole, diethylcarbamazine and albendazole or diethylcarbamazine/ivermectin solo that destruct microfilariae and block the spread of disease. Even so, the set of courses are efficacious, but later

by few weeks, microfilariae seen again and develop into a significant source of the broadcast of the illness in prevalent regions. To perk up the illness interruption scenario, the contribution of nanomedicine carriers will be the most suitable tactic. These nanomed carriers possess the ability to boost the bioavailability of MIF medications to the rotating microfilariae with a longer duration of action. Alongside, such kind of systems also necessitate anti-filarial therapeutics to run over many systemic hurdles like first-pass metabolism, reticuloendothelial system (RES), plasma bounding or phagocytic uptake to clean up microfilariae. The wholesome effect would be answered in the prolong period of suppression of microfilariae in the peripheral system and the blocking of lymphatic filariasis spread for later duration than the general cures in endemic region. For illustration, the improvisation in MIF effectiveness of diethylcarbamazine has been well written after making of diethylcarbamazine as liposomes only at a nearly optimum dosing of 25 mg per kg body weight that beneficially removes microfilariae from central circulation in animals imposed with *B. malayi* from 60 days of post-infective terms. Highlighting, the unused drug convey control about 30 days and the parasitic volume tend to increase after this duration of time. This shows that nanodrug delivery systems of MFs can take longer control over the growth of microfilaricides, which offer a lot of benefits for illness disruption protocol. Other than that, liposomes improvise the MIF's mechanism of action, their speedy removal from central circulation by reticuloendothelial system or immune units may self-symbolize most of the hurdles in eradication of microfilaricides from the blood. On that note, alongside these traditional nanodrug delivery systems, development in nanotherapeutic sciences also passes on the long traveling carriers in blood or stealth nanoparticles as forthcoming contender for the anti-filarial prophylaxis of lymphatic filariasis since such are acknowledged as principle circumvent recognized by the immune system and broaden the circulation time of blood for large period. Nanodrug delivery system sensitive to antibodies is also effective in evading mononuclear phagocytic clearance and stood strongly to spin out in latitude for forceful eradication of microfilaricides and disruption of lymphatic filariasis. Without a doubt, stealth-immuno-nanodrug delivery systems are the final and safest bullet to hit in nanoscience DDSs for this chemical cure that possess a potential to perform as a masterstroke over centrally traveling microfilaricides in endemic regions and may see as the advance technology to get rid of such problematic lymphatic filariasis talk (Ross et al. 2015; Jayalakshmi et al. 2017; McDougall and Waudby 1978; Bregani et al. 2003).

12.3.2 Nanotechnology as Enhancing the Physiochemical Properties

In the management of lymphatic filariasis through nanotechnological sciences, it is desired that the major proportion of the given dose should reach to the objective parasites either through the circulation system or dwelling in the lymphatic system. On a general note, when tradition treatment is given to a patient to cure LF, physiochemical properties of drug and dosage form greatly affect the targeting so to overcome such issue nanoscience plays a phenomenal role. Unluckily, there is a

wide range of physiological, pharmacokinetic, pharmacodynamical, and anatomical barricades linked with the anti-filarial drugs themselves which cause issues like drug resistance. In the past couple of years, nanoscience has swiftly become a novel and innovative technique in modern day treatment and also offers tools to endorse health by standardizing old therapy protocols of reoccurring and distressing illness, this also assist in deal with unmet therapeutic needs. Nanodrug delivery systems are the keenest nanoscience research which plays a powerful role in operating the pharmacological effects of therapeutic agents as they affect the kinetic profile, release rate of drug and mode of action of drug according to site specification. All these features are primarily significant and essentially needed for the improvisation of the anti-filarial prophylaxis. Moreover, it advises that nanodrug delivery systems are the vital answer for this morbid illness. Simply, nanotechnology will offer a lot of solution in order to treat LF. Improvising the predicaments (from solubility profiles and standardizing the kinetic and dynamic parameters) of anti-filarial fighters by nanodrug delivery systems would positively grow the old healing windows of anti-filarial prophylaxis and likely to offer the great health advantages to lymphatic filariasis sufferers. Nevertheless, to attain such, it is necessary to know the close drawbacks linked to anti-filarial medicaments leading to dull pharmacodynamics and pharmacokinetics features. It could also offer the unambiguous conception of the divided characteristics of drug delivery systems for the selection of suitable nanoDDSs for a particular agent to fight filariasis. This segment aims on the drawbacks regarding physiochemical properties in the most promising drugs which are generally give in filarial condition such as ivermectin (IVM), rifampicin (RIF), albendazole (ALB), diethylcarbamazine (DEC), tetracycline (TETRA) and doxycycline (DOXY) and in parallel way suggests diverse nanodrug delivery systems as the fine answer to the predicaments linked with such core anti-filarial agents, initiating from bad solubility. Biopharmaceutical issues hinder the chemotherapy for filariasis (Bremer-Hoffmann et al. 2018; Gao and Lowry 2018; Zhang et al. 2008; Samrot et al. 2020; Sudha et al. 2018).

12.3.2.1 Nanoscientific Elucidation for Poor Solubility of Anti-filarial Agents

Bad solubility is an intrinsic issue of most therapeutic agents which produce an outcome in terrible pharmacokinetic problems and alternatively poor availability of drug in the systemic circulation. Drugs administered via oral route encounters with biopharmaceutical issue of poor solubility and dissolution in GIT and net results cause a decrease in absorption of the drug. Majority of the therapeutic agents which are prescribed in the prophylaxis of lymphatic filariasis given in multidrug administration protocol possess low aqueous solubility and fall in BCS class II or class III according to biopharmaceutical classification system. On that note, these therapeutic agents explain the issue of bad gastro-intestinal absorption and reduced bioavailability. Complexation with cyclodextrin or o/w emulsion formulation can be helpful in improvising the filaricidal activity of pitiably soluble drug. Moreover, these kinds of changes or alteration do not assure specific aiming of therapeutic agents to the infection. On the other side, in the past two decades, nanoscience solution can be

employed as the most suitable and universal choice for clarifying the solubility problems of the lipophilic/hydrophobic compounds, thus providing some diversity of great nanotechniques like nanocrystalline materials, nanosuspensions, nanoemulsions, nanoparticles that can formulate the insoluble compound to soluble material and offer high encapsulating efficacy, drug aiming and advance working over traditional approaches. Anti-filarial agents with poor solubility to treat lymphatic filariasis are pretty fine aspirants to produce formulations through such materializing techniques to get better in their efficacy to fight parasitic infection for cure. At method selection time, solubilization property can be offered via self-emulsifying drug delivery system (SMEDDS), multiple micelles and mesophases which are greatly appropriate to formulate anti-filarial agents as these tactics offer solubilization quite analogous to the original principle of dietary-mixed micelles that assisting the solubilization of lipophilic-nature compounds like oils/fats in gastro-intestinal environment (Stegemann et al. 2007; Lipinski 2000; Da Silva et al. 2020; Shukla et al. 2020b; Handa et al. 2021; Mishra et al. 2021).

12.3.2.2 Pharmacokinetic Problems in NanoDDSs and Anti-filarial Agents

Orally administered anti-filarial agents undergo many pharmacokinetic problems such as complex formation, low dissolution rate, loss via luminal degradation and mucosal metabolism, inability to cross GIT, poor receptor binding and many more. Moreover, novel nanosciences have the capability to overcome such problems and will offer faster recover to patient. Here some significant discussion on nanodrug delivery system is done which considers a lot of chief pharmacokinetic problems in old anti-filarial agent absorption like over binding to plasma, broader biological distribution, faster metabolism and clearance (Edwards et al. 1994; Kalani et al. 2014; Goa et al. 1991).

12.3.2.3 Anti-filarial Agents Given Orally Showing Poor Absorption and Gastro-intestinal DDSs

Majority of the anti-filarial agents are easily accessible in oral dosage formulation and on that note, they exhibit poor GI absorption profile and poor bioavailability as well because of the degradation of drug in highly acidic pH of GIT and through the degrading or digesting enzymes present in GIT and because of the contact with endogenous molecules or their poor ability to dissolve in gastro-intestinal environment. By taking an example of tetracycline, it is used to eliminate filarial parasite to heal sufferer, it builds stable non-soluble complexes through complexation process in GIT with metal ions that will decrease its own gastro-intestinal absorption and ultimately lower the bioavailability of drug in systemic circulation. Analogous to that albendazole is an important drug to fight LF as well and offer great macro-filaricidal action, illustrates less targeting influence after mouth administration majorly because of its lower rate of dissolution in gastro-intestinal environment and bad absorption. Ivermectin is a potent filaricide as well, but it is also bungling to cross gastro-intestinal tract for better absorption. To overcome this issue, here liposomal drug delivery system techniques have been used which shows a generous

mechanism to enhance the absorptions of poorly soluble drug. For instance, liposomal formulation of ivermectin explains a greater absorption as evidenced by greater Tmax of Ivomec (Tmax—1.13 day) than Tmax of 0.23 day by a solo subcutaneous (SC) injection in rabbits. Equally, capture of albendazole inside liposomes has been studied to improvise the mouth bioavailability of albendazole in sigmodon hispidus (cotton rats) contaminated with metacestodes of Echinococcus multilocularis (cyclophyllid tapeworm). The liposomal formulation through encapsulation of tetracycline has shown greater results in combating this morbid parasite as well which might be because of the blocking of gastro-intestinal complex development and ultimately absorption is increased. Nevertheless, in order to improvise the process of absorption, the systemic way of working anti-filarial agents is an important feature as well before GIDDSs for the secure removal of MFs. For example, diethylcarbamazine is the therapeutic agent that kills microfilariae. Rather than being lipophilic nature, with faster absorption from GIT, it become difficult to be used in patients suffering from onchocerca infection because rapid killing of microfilariae by diethylcarbamazine produce outcome as critical skin complications, generally known as "Mazzotti reaction", can be seen as rashes on dermis. By looking ahead to these limitations, a systemic balance between absorption and systemic bioavailability of filaricidal fighters becomes necessary to shun adverse effects. Conversely, gastro-intestinal drug delivery system is the finest recommendation to get over systemic side effects and to get equilibrium towards major factors from GI path. Two different kinds of gastro-intestinal drug delivery systems provide extra intellectual and systemic tactics for anti-filarial agents in order to cure lymphatic filariasis. Mucoadhesive or floating drug delivery system increase the GIT transition time and enhances the systemic absorption. Therefore, it will offer security and effectiveness for anti-filarial agents or chemical agents. Drug delivery systems with mucoadhesive properties are the better choice to select for delivery because it is smooth to access and beneficial over other gastro-intestinal drug delivery systems like to avoid first-pass metabolism via liver, improvisation in the patient compliance, magnificent accessibility, one direction drug flow and increased penetration permeability. Nevertheless, the vision of such gastro-intestinal biomucoadhesive drug delivery systems for filaricidal drugs looking to be targeted in their aptitude for longer residence time of these agents at the location of absorption and allowing an overstated connection with the epithelial barricades, producing the suitable level to MFs bothered with central circulation lower than the minimum toxicity strength and upside the minimum effectual strength (Bhoj et al. 2019; Singh et al. 2012; Sangshetti et al. 2017; Brienne et al. 1987; Zafar et al. 2016; Katiyar and Singh 2011).

12.3.2.4 Significance of Controlled Release of nanodrug delivery Systems in Biotransformation and Clearance Half-life of Anti-filarial Agents

Generally, plasma binding can exert effect on the biological half-life of a therapeutic agent that states the time need to minimize the plasma concentration by 50% through biotransformation or elimination. Anti-filarial agents go through hepatic metabolism

(first-pass effect) and biotransformed especially after absorption, illustrating reduced bioavailability to the centrally circulating MFs or motile worms in tissues. Nanodrug delivery system can modify the metabolism of therapeutic agents, bypassing degradation via liver and improvising the elimination $t_{1/2}$ of medication. This can be attained by taking control over the release rate by a longer period that could be advantageous procedure for filaricidal agents that are quickly biotransformed and removed from the host after administration or by having little plasmatic half-life about 2–3 h like diethylcarbamazine (DEC). The main aim to build such CRDDS is to uphold the therapeutic bioavailability of the drug for extended term that is specifically beneficial in the healing procedure of lymphatic filariasis. For a case, if the production of an filaricidal agent from DDS is limited in such a way that the production is initiated for a longer duration, it could uphold the plasma strength for a broader function of time in the pharmaceutical and therapeutical range which in twist can minimize the regularity of dosing and extend the period of suppression of microfilariae. Positively, such effect can be seen in liposomal medication as well in the demonstration of doxycycline and rifampicin in a lenient *Brugia malayi, M.* coucha mice model of lymphatic filariasis. The highest strength of the liposomal medication of doxycycline is 8.8 microgram per millilitre and rifampicin is 8.6 microgram per millilitre, both are studied by 12 hours post-SC administration which seems to be more than their lowest inhibitory strengths (minimum effective concentration, 4 μg/mL) and illustrated sustained release for about 48 h for antibiotics which falls in therapeutic window. On the other hand, free forms of the drugs are administered through the similar pathway the peak concentrations are quickly attained by the antibiotics and lower down their minimum effective concentration (MICs) in duration of 6 h. On that note, by extended release through the liposomal concept, the plasma half-life of anti-filarial formulation can be improvised which will further produce outcome in prolong suppression and elimination of the filarial parasite from peripheral blood.

12.3.2.5 Pharmacodynamics Deliberation for Anti-filarial Agents

The perceptions of pharmacodynamics in filaricidal agents are inclusive of the lessons of physiological and biochemical results of these life saving agents on worm drug aims within the host, and the link associated with drug strength, systemic toxicity and pharmacodynamic effects. Moreover, the whole pharmacodynamics of filaricidal agents tends to be acceptable in phrases of their own toxicological data, but the responses associated with dose are contradicted. Diethylcarbamazine (DEC) is an appreciable filaricidal agent and studied as fractionally active to fight adult parasites. Ironically, anti-parasitic action of diethylcarbamazine with greater dose results in various adverse actions like liver toxicity and inflammation. Curiously, diethylcarbamazine was found to be zero toxic and appreciably effectual to kill motile adult parasites at a less optimum dose about 25 mg/kg in tuftsin (tetrapeptide consisting of Thr-Lys-Pro-Arg) accepted liposomal formulation. Such kind of systems offer endless pathways to optimize the dose–response data, receptor selectivity, reducing side effects and many more so on that note, it promises high yield values of anti-parasitic chemotherapy.

12.3.3 Nanotechnology to Counteract Drug Resistance

In the treatment of several diseases, drug resistance pop-up as a persistent issue. For a case, tumour cells are broadly studied for MDR (multidrug resistance). The hold up of resistance for drug has also materialized in parasitic illness like filariasis tuberculosis (TB) and malaria. For a case, ivermectin is the frontier of such resistance problem in the opposition to a wide range of helminth nematodes. A P-glycoprotein homologue which is expressed on surface of cell is an efflux pump participating in a significant drama in a variety of worms. In present day, nanotechnology is the novel advancement to take fight against drug resistance by decreasing the action of P-glycoprotein. In the initial data, light sensitive coupled nanomolecules loaded with doxycycline were resulted in the exertion of the additive and synergistic activity on the doxycycline resistant breast tumour MCF-7 cell line and a MDR transporter inhibitor, i.e., d-αtocopheryl poly (ethylene glycol) 1000 succinate (TPGS) was synchronized to increase the functionalities and therapeutic effects of this hybrid. The involvement of TPGS will decrease the activity of P-glycoprotein (P-gp) and enhance the functionalities of doxycycline in drug resistant MCF-7 breast tumour. Another good example can be seen in the studies reported by Wang X et al. on folate receptor aimed nanoparticles to reduce paclitaxel drug resistance in a P-glycoprotein cancer model with over expression, here multifunctional nanomolecules are used to overcome the issue of drug resistance. Furthermore, these nanoparticles are usually applied to participate in cancer applications for drug resistance but along with that they showed a marvelous scope for anti-filarial drugs in which P-glycoproteins are accountable for drug resistance like ivermectin (Bajpai et al. 2005; Dangi et al. 2010; García-Rodriguez et al. 2001; Gaur et al. 2007; Casulli et al. 2006; Bassissi et al. 2006; Wen et al. 1996; Guerra-Caceres et al. 1980; Luo et al. 2017; Haarbrink et al. 2000; Shieh et al. 2011).

A past report on nanofighters shows that they are able to overcome the bacterial antibiotic conflict. A panel of scientists and engineers guided by Dr. James Hedrick at IBM inclusively crafted a novel kind of astonishing nanoparticles which possess the ability to effectively bind and destroy the cell membrane of methicillin-resistant Staphylococcus aureus (MRSA), Gram positive bacteria. These kinds of live examples of nanomedication to come over the bacterial resistance is a superb marker of the wide point of view for anti-filarial antibiotics to get success in antibiotic conflict against wolbachia for the treatment of lymphatic filariasis (LF).

12.4 Conclusion and Futuristic Perspective

It is completely justified from the above conversation that the anti-wolbachial or anti-filarial drugs for human lymphatic filariasis are widely lipophilic and linked with a variety of pharmacodynamic and pharmacokinetic factors which leads to derisory chemotherapy for LF, insist higher dosing calendar with many side effects and decreased efficacy. On that note, the perfect answer for this prophylaxis is the nanodrug delivery systems (nanoDDSs) of nanomedicine which offers improvised

bioavailability, drug efficacy, safety, reduces dosing calendar and almost no toxicity as a result it holds diverse possibilities in the treatment of this horrible illness.

Rather than the great achievement pursued in MDA curriculum through efficacious chemical therapeutic technology, it has been understood that such programs on a singular note do not assure the destruction of lymphatic filariasis. In those regions where LF spread is very low about less than 1% in the population, eradication of this morbid disease still remains indescribable and furthermore circumstances, this illness has resurge because of reoccurrence of microfilaricides (MFs). Nevertheless, this occurs because of having little knowledge about the link between pharmacokinetics (circulating drug concentration) and pharmacodynamics (anti-microfilarial effect) properties on MFs and main focus to improvise such tactics is dedicated to gracious administration of the drug combination for LF, finalizing drug target and screening of the molecular compounds and then routing to the other facets like biochemical, immunological or parasitic genomic studies to gawk upgradation. Alternatively, except the answers for the pharmacokinetics misfortunes and the quantification of the truthful pharmacodynamic endpoints for the present anti-filarial drugs are not performed through germane approaches, health agencies/organizations will be unable to standardize cure for sufferers docking wide range of MFs or personalities who need morbidity turnaround consideration in endemic regions.

Successful events in nanomedical sciences have evident that the nanotherapeutical principle of a dosage form soberly assesses or influences pharmacodynamic and pharmacokinetic problems of medication and permits to guess, control and circumvent any complications present in a specific prophylaxis. Moreover, these potent burdens of this materializing nanoscience have not been familiar for the improvisation of anti-filarial chemical treatment. Hence, when little positive results have been pursued in the relation with clinical pharmacokinetics and pharmacodynamics of filarial treating agents, this vital phase of nanodrug delivery systems greatly stayed unseen. Nonetheless, many previously mentioned approaches are still in line to get satisfied sanction for these agents in the formulation making labs from literature to clinical practices at society stages. From the point of view of nanomedicine, society directed execution of this horrible disease can be sophisticatedly improvised by concerning nanotech suggestions to pharmacodynamic and pharmacokinetic concepts of anti-filarial prophylaxis. The greatly circulating microfilaricide nanodrug delivery system with extended mean residence time (MRT) has remarkable possibility for quick capture of lymphatic filariasis transmission in prevalent regions, whereas altered lymphatic aimed MFs nanoDDSs would be the finest point to finish the motile parasites or intracellular endosymbiont worm breathing deep in tissue mass in sufferer suffering from lymphatic filariasis. This evident information may propose a new trick in anti-filarial medication crafting of filaricides.

Moreover, a crystal clear insight of the classified individualities of different nanodrug delivery system and a core knowledge of the drawbacks linked with the lymphatic filarial illness and the pharmacokinetic and pharmacodynamic constraints of prophylaxis agents, etc., will magnify the approval of the appropriate approaches. A bequeathed designing for anti-filarial agent nanodrug delivery systems can be

made for optimize testing of pharmacodynamic and pharmacokinetic parameters of orally suitable anti-filarial agent. With this approach, a novel range of anti-filarial medicine to fight filarial parasite with improvised dose-response curve, very little side effects and greater interaction with the specific sites or boost uptake by the parasite are majorly expected. To the end, the notion may contract the illness and the financial burden across the world, pushing this horrible disease to the ending phases.

In a previous study, silver nanoparticles have been acknowledged to bring out great loss in the microfilarial functionality of B. malayi obtained from the backwash of peritoneal cavities of infected jirds (meriones unguiculatus), proposing nanoparticles of silver as a deadly drug aspirant that has been revealed for liberal value in medical treasure like fullerene, carbon nanotubes, etc. are tend to be screened in high throughput (HTP) anti-filarial screening window for the recognition of new nano filaricidal lead agents that would perfectly have an innovative mechanism of action to oppose MFs and motile worms. These may be the better choices to replace the old drugs and offer a foundation in anti-filarial fighters crafting or chemical treatment with a novel point of view to guarantee a bright future where such morbid disease does not exist.

Acknowledgments The authors acknowledge the Department of Pharmaceuticals, Ministry of Chemical and Fertilizers, Government of India, for financial support. The NIPER-R communication number for this article is NIPER-R/Communication/248.

Conflict of Interest The authors declare no conflict of interest among themselves.

References

Ali M, Afzal M, Bhattacharya SM, Ahmad FJ, Dinda AK (2013a) Nanopharmaceuticals to target antifilarials: a comprehensive review. Expert Opin Drug Deliv 10(5):665–678

Ali M, Afzal M, Verma M, Misra-Bhattacharya S, Ahmad FJ, Dinda AK (2013b) Improved antifilarial activity of ivermectin in chitosan-alginate nanoparticles against human lymphatic filarial parasite, Brugia malayi. Parasitol Res 112(8):2933–2943

Ali M, Afzal M, Kaushik U, Bhattacharya SM, Ahmad FJ, Dinda AK (2014a) Perceptive solutions to anti-filarial chemotherapy of lymphatic filariasis from the plethora of nanomedical sciences. J Drug Target [Internet] 22(1):1–13. http://www.tandfonline.com/doi/full/10.3109/1061186X.2013.832766

Ali M, Afzal M, Verma M, Bhattacharya SM, Ahmad FJ, Samim M et al (2014b) Therapeutic efficacy of poly (lactic-co-glycolic acid) nanoparticles encapsulated ivermectin (nano-ivermectin) against brugian filariasis in experimental rodent model. Parasitol Res [Internet] 113(2):681–91. http://link.springer.com/10.1007/s00436-013-3696-5

Bajpai P, Vedi S, Owais M, Sharma SK, Saxena PN, Misra-Bhattacharya S (2005) Use of liposomized tetracycline in elimination of Wolbachia endobacterium of human lymphatic filariid Brugia malayi in a rodent model. J Drug Target [Internet] 13(6):375–81. https://www.tandfonline.com/doi/full/10.1080/10611860500285439

Bassissi F, Lespine A, Alvinerie M (2006) Assessment of a liposomal formulation of ivermectin in rabbit after a single subcutaneous administration. Parasitol Res 98(3):244–249

Bhoj P, Togre N, Bahekar S, Goswami K, Chandak H, Patil M (2019) Immunomodulatory activity of sulfonamide Chalcone compounds in mice infected with filarial parasite, Brugia malayi.

Indian J Clin Biochem [Internet] 34(2):225–9. http://link.springer.com/10.1007/s12291-017-0727-5

Binnebose AM, Haughney SL, Martin R, Imerman PM, Narasimhan B, Bellaire BH (2015) Polyanhydride nanoparticle delivery platform dramatically enhances killing of filarial worms. PLoS Negl Trop Dis 9(10):e0004173

Bockarie MJ, Taylor MJ, Gyapong JO (2009) Current practices in the management of lymphatic filariasis. Expert Rev Anti Infect Ther 7(5):595–605

Bregani ER, Balzarini L, Ghiringhelli C, Tarsia P (2003) Transfusional Mansonella perstans microfilariasis. Parassitologia [Internet] 45(2):71–2. http://www.ncbi.nlm.nih.gov/pubmed/15266999

Bremer-Hoffmann S, Halamoda-Kenzaoui B, Borgos SE (2018) Identification of regulatory needs for nanomedicines. J Interdiscip Nanomed [Internet] 3(1):4–15. https://onlinelibrary.wiley.com/doi/10.1002/jin2.34

Brienne MJ, Varech D, Leclercq M, Jacques J, Radembino N, Dessalles C et al (1987) New antifilarial agents. 1. Epoxy sulfonamides and ethynesulfonamides. J Med Chem [Internet] 30(12):2232–9. https://pubs.acs.org/doi/abs/10.1021/jm00395a010

Casulli A, Gomez Morales MA, Gallinella B, Turchetto L, Pozio E (2006) 2-Hydroxypropyl-β-cyclodextrin improves the effectiveness of albendazole against encapsulated larvae of Trichinella spiralis in a murine model. J Antimicrob Chemother 58(4):886–890

Chandy A, Thakur AS, Singh MP, Manigauha A (2011) A review of neglected tropical diseases: filariasis. Asian Pac J Trop Med 4(7):581–586

Da Silva FLO, Marques MBDF, Kato KC, Carneiro G (2020) Nanonization techniques to overcome poor water-solubility with drugs. Expert Opin Drug Discov [Internet] 15(7):853–64. https://www.tandfonline.com/doi/full/10.1080/17460441.2020.1750591

Dangi A, Dwivedi V, Vedi S, Owais M, Misra-Bhattacharya S (2010) Improvement in the antifilarial efficacy of doxycycline and rifampicin by combination therapy and drug delivery approach. J Drug Target [Internet] 18(5):343–50. http://www.tandfonline.com/doi/full/10.3109/10611860903450007

Decuzzi P, Peer D, Di Mascolo D, Palange AL, Manghnani PN, Moghimi SM et al (2021) Roadmap on nanomedicine. Nanotechnology [Internet] 32(1):012001. https://iopscience.iop.org/article/10.1088/1361-6528/abaadb

Edwards G, Winstanley PA, Ward SA (1994) Clinical pharmacokinetics in the treatment of tropical diseases. Clin Pharmacokinet [Internet] 27(2):150–65. http://link.springer.com/10.2165/00003088-199427020-00006

Gao X, Lowry GV (2018) Progress towards standardized and validated characterizations for measuring physicochemical properties of manufactured nanomaterials relevant to nano health and safety risks. NanoImpact 9:14–30

García-Rodriguez JJ, Torrado J, Bolás F (2001) Improving bioavailability and anthelmintic activity of albendazole by preparing albendazole-cyclodextrin complexes. Parasite [Internet] 8:S188–S190. http://www.parasite-journal.org/10.1051/parasite/200108s2188

Gaur RL, Dixit S, Sahoo MK, Khanna M, Singh S, Murthy PK (2007) Anti-filarial activity of novel formulations of albendazole against experimental brugian filariasis. Parasitology 134(Pt 4):537–544

Geary TG, MacKenzie CD (2011) Progress and challenges in the discovery of macrofilaricidal drugs. Expert Rev Anti Infect Ther 9(8):681–695

Germain M, Caputo F, Metcalfe S, Tosi G, Spring K, Åslund AKO et al (2020) Delivering the power of nanomedicine to patients today. J Control Release [Internet] 326:164–71. https://linkinghub.elsevier.com/retrieve/pii/S0168365920303825

Goa KL, McTavish D, Clissold SP (1991) Ivermectin. Drugs [Internet] 42(4):640–58. http://link.springer.com/10.2165/00003495-199142040-00007

Guerra-Caceres JG, Bryceson ADM, Quakyi I, Spry CJF (1980) Studies on the mechanisms of adverse reactions produced by diethylcarbamazine in patients with onchocerciasis—Mazzotti

reaction. Parasite Immunol [Internet] 2(2):121–31. https://onlinelibrary.wiley.com/doi/10.1111/j.1365-3024.1980.tb00048.x

Gyapong JO, Kumaraswami V, Biswas G, Ottesen EA (2005) Treatment strategies underpinning the global programme to eliminate lymphatic filariasis. Expert Opin Pharmacother 6(2): 179–200

Haarbrink M, Abadi GK, Buurman WA, Dentener MA, Terhell AJ, Yazdanbakhsh M (2000) Strong association of interleukin-6 and lipopolysaccharide-binding protein with severity of adverse reactions after diethylcarbamazine treatment of microfilaremic patients. J Infect Dis 182(2):564–569

Handa M, Tiwari S, Yadav AK, Almalki WH, Alghamdi S, Alharbi KS et al (2021) Therapeutic potential of nanoemulsions as feasible wagons for targeting Alzheimer's disease. Drug Discov Today [Internet]. https://linkinghub.elsevier.com/retrieve/pii/S1359644621003251

Hoerauf A (2008) Filariasis: new drugs and new opportunities for lymphatic filariasis and onchocerciasis. Curr Opin Infect Dis 21(6):673–681

Jayalakshmi J, Hafeez M, Kumar MU (2017) Prevalence of bovine microfilariasis in Rayalaseema region of Andhra Pradesh. J Parasit Dis [Internet] 41(3):874–9. http://link.springer.com/10.1007/s12639-017-0909-3

Joardar N, Mukherjee N, Sinha Babu SP (2021) Nanopharmaceuticals to target antifilarials: administration of old age drugs in a novel way. In: Applications of nanobiotechnology for neglected tropical diseases [Internet]. Elsevier, p 329–56. https://linkinghub.elsevier.com/retrieve/pii/B9780128211007000157

Kalani K, Kushwaha V, Sharma P, Verma R, Srivastava M, Khan F et al (2014) In vitro, in silico and in vivo studies of ursolic acid as an anti-filarial agent. Munirathinam G, editor. PLoS One [Internet] 9(11):e111244. https://dx.plos.org/10.1371/journal.pone.0111244

Katiyar D, Singh LK (2011) Filariasis: current status, treatment and recent advances in drug development. Curr Med Chem [Internet] 18(14):2174–85. http://www.eurekaselect.com/openurl/content.php?genre=article&issn=0929-8673&volume=18&issue=14&spage=2174

Lipinski CA (2000) Drug-like properties and the causes of poor solubility and poor permeability. J Pharmacol Toxicol Methods 44(1):235–249

Luo S, Luo Y, Wu H, Li M, Yan L, Jiang K et al (2017) Self-assembly of 3D carbon nanotube sponges: a simple and controllable way to build macroscopic and ultralight porous architectures. Adv Mater 29(1):1603549

McDougall AC, Waudby H (1978) Dermal microfilariasis and leprosy. Lepr Rev 49(2):168

Metselaar JM, Lammers T (2020) Challenges in nanomedicine clinical translation. Drug Deliv Transl Res [Internet] 10(3):721–5. http://link.springer.com/10.1007/s13346-020-00740-5

Mishra P, Handa M, Ujjwal RR, Singh V, Kesharwani P, Shukla R (2021) Potential of nanoparticulate based delivery systems for effective management of alopecia. Colloids Surfaces B Biointerfaces [Internet] 208:112050. https://linkinghub.elsevier.com/retrieve/pii/S0927776521004944X

Molyneux DH, Zagaria N (2002) Lymphatic filariasis elimination: progress in global programme development. Ann Trop Med Parasitol 96(Suppl 2):S15–S40

Nutman TB (2016) Immunology of lymphatic filariasis. In: Encyclopedia of immunobiology [internet]. Elsevier, p 142–9. https://linkinghub.elsevier.com/retrieve/pii/B9780123742797130140

Ottesen EA (2006) Lymphatic filariasis: treatment, control and elimination. Adv Parasitol 61:395–441

Permana AD, Tekko IA, McCrudden MTC, Anjani QK, Ramadon D, McCarthy HO et al (2019) Solid lipid nanoparticle-based dissolving microneedles: a promising intradermal lymph targeting drug delivery system with potential for enhanced treatment of lymphatic filariasis. J Control Release 316:34–52

Rajan TV (1990) Molecular biology of human lymphatic filariasis. Exp Parasitol [Internet] 70(4): 500–3. https://linkinghub.elsevier.com/retrieve/pii/0014489490901372

Ross KA, Brenza TM, Binnebose AM, Phanse Y, Kanthasamy AG, Gendelman HE et al (2015) Nano-enabled delivery of diverse payloads across complex biological barriers. J Control Release 219:548–559

Samrot A V., Sean TC, Kudaiyappan T, Bisyarah U, Mirarmandi A, Faradjeva E et al (2020) Production, characterization and application of nanocarriers made of polysaccharides, proteins, bio-polyesters and other biopolymers: a review. Int J Biol Macromol [Internet] 165:3088–105. https://linkinghub.elsevier.com/retrieve/pii/S0141813020347346

Sanabria R (2021) Nanotechnological improvement of veterinary anthelmintics. Pharm Nanotechnol [Internet] 9(1):5–14. https://www.eurekaselect.com/182245/article

Sangshetti JN, Shinde DB, Kulkarni A, Arote R (2017) Two decades of antifilarial drug discovery: a review. RSC Adv 7:20628–20666

Shieh MJ, Hsu CY, Huang LY, Chen HY, Huang FH, Lai PS (2011) Reversal of doxorubicin-resistance by multifunctional nanoparticles in MCF-7/ADR cells. J Control Release 152(3): 418–425

Shrivastava S, Gupta A, Kaur CD (2020) The epitome of novel techniques and targeting approaches in drug delivery for treating lymphatic filariasis. Curr Drug Targets [Internet] 21(12):1250–63. https://www.eurekaselect.com/183297/article

Shukla R, Kumar J, Dwivedi P, Gatla P, Mishra PR (2013) Microparticles of diethylcarbamazine citrate for the treatment of lymphatic filariasis. Asian J Chem 25:S302–S304

Shukla R, Gupta J, Shukla P, Dwivedi P, Tripathi P, Bhattacharya SM et al (2015) Chitosan coated alginate micro particles for the oral delivery of antifilarial drugs and combinations for intervention in Brugia malayi induced lymphatic filariasis. RSC Adv 5:69047–69056

Shukla R, Handa M, Pardhi VP (2020a) Introduction to pharmaceutical product development. In: Pharmaceutical drug product development and process optimization [internet]. Includes bibliographical references and index. Apple Academic Press, p 1–32. https://www.taylorfrancis.com/books/9781000731323/chapters/10.1201/9780367821678-1

Shukla R, Sethi A, Handa M, Mohan M, Tripathi PK, Kesharwani P (2020b) Dendrimer-based drug delivery systems for tuberculosis treatment. In: Nanotechnology based approaches for tuberculosis treatment

Shukla R, Thok K, Kakade S, Handa M, Beg S (2021a) Clinical translation status of nanoformulations. In: Nanoformulation strategies for cancer treatment [internet]. Elsevier, p 303–38. https://linkinghub.elsevier.com/retrieve/pii/B9780128210956000124

Shukla R, Mourya A, Handa M, Ujjwal RR (2021b) Role of nanomedicines in neglected tropical diseases. In: Nanopharmaceutical advanced delivery systems [internet]. Wiley, p 407–46. https://onlinelibrary.wiley.com/doi/10.1002/9781119711698.ch18

Singh PK, Ajay A, Kushwaha S, Tripathi RP, Misra-Bhattacharya S (2010) Towards novel antifilarial drugs: challenges and recent developments. Future Med Chem 2(2):251–283

Singh SK, Goswami K, Sharma RD, Reddy MVR, Dash D (2012) Novel microfilaricidal activity of nanosilver. Int J Nanomedicine 7:1023–1030

Singh Y, Srinivas A, Gangwar M, Meher JG, Misra-Bhattacharya S, Chourasia MK (2016) Subcutaneously administered ultrafine PLGA nanoparticles containing doxycycline hydrochloride target lymphatic filarial parasites. Mol Pharm 13(6):2084–2094

Stegemann S, Leveiller F, Franchi D, de Jong H, Lindén H (2007) When poor solubility becomes an issue: from early stage to proof of concept. Eur J Pharm Sci 31(5):249–261

Sudha PN, Sangeetha K, Vijayalakshmi K, Barhoum A (2018) Nanomaterials history, classification, unique properties, production and market. In: Emerging applications of nanoparticles and architectural nanostructures: current prospects and future trends

Szuba A, Rockson SG (1998) Lymphedema: classification, diagnosis and therapy. Vasc Med [Internet] 3(2):145–56. http://journals.sagepub.com/doi/10.1177/1358836X9800300209

Taylor MJ, Hoerauf A, Bockarie M (2010) Lymphatic filariasis and onchocerciasis. Lancet 376(9747):1175–1185

Townson S, Ramirez B, Fakorede F, Mouries MA, Nwaka S (2007) Challenges in drug discovery for novel antifilarials. Expert Opin Drug Discov [Internet] 2(suppl 1):S63–S73. http://www.tandfonline.com/doi/full/10.1517/17460441.2.S1.S63

Wen H, New RRC, Muhmut M, Wang JH, Wang YH, Zhang JH et al (1996) Pharmacology and efficacy of liposome-entrapped albendazole in experimental secondary alveolar echinococcosis and effect of co-administration with cimetidine. Parasitology [Internet] 113(2):111–21. https://www.cambridge.org/core/product/identifier/S003118200006635X/type/journal_article

Wu LP, Wang D, Li Z (2020) Grand challenges in nanomedicine. Mater Sci Eng C [Internet] 106:110302. https://linkinghub.elsevier.com/retrieve/pii/S0928493118325773

Zafar A, Ahmad I, Ahmad A, Ahmad M (2016) Copper(II) oxide nanoparticles augment antifilarial activity of Albendazole: in vitro synergistic apoptotic impact against filarial parasite Setaria cervi. Int J Pharm [Internet] 501(1–2):49–64. https://linkinghub.elsevier.com/retrieve/pii/S037851731630059X

Zhang L, Gu F, Chan J, Wang A, Langer R, Farokhzad O (2008) Nanoparticles in medicine: therapeutic applications and developments. Clin Pharmacol Ther [Internet] 83(5):761–9. http://doi.wiley.com/10.1038/sj.clpt.6100400

Nanodrug Delivery Systems for Infectious Diseases: From Challenges to Solutions

13

Vijaya Ravinayagam and B. Rabindran Jermy

Abstract

Nanotherapeutics research has been rising steadily in interdisciplinary core area for the development of targeted drug delivery system. The poor gastrointestinal stability, low bioavailability, poor transport behavior limitations are overcome by nanodrug delivery system. The design of an effective drug delivery system depends on the particle size, functional moieties, textural characteristics (surface area, pore size, and pore volume), targeting ligand, cleavable and non-cleavable linker, drug sensitivity to environment stimuli, etc. The functionalization of drugs over nanocarriers needs to improve the stability of drug components, increase the bioavailability, reduce metabolization and excretion. Different types of nanocarriers broadly based on structured silica, carbon, polymers, liposome, metal nanoparticles, gel, nanocage, clay, and viral carriers have been developed for drug delivery system. Infectious diseases are rising globally prompting the need for an advanced drug delivery to overcome the present challenges to solutions. One best option is to utilize the advantages of individual components in mutual nanocomposites. Porous silica and carbon nanocarriers are advantageous with high textural uniformity, non-toxic with high drug loading capabilities. However, several linkers and polymeric wrappings are required for developing a stimuli responsive drug delivery system. Polymeric nanogels are attractive drug delivery options for treating lung infections. However, the

V. Ravinayagam (✉)
Deanship of Scientific Research and Department of Nano-Medicine Research, Institute for Research and Medical Consultations, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia
e-mail: vrnayagam@iau.edu.sa

B. R. Jermy
Department of Nano-Medicine Research, Institute for Research and Medical Consultations, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia

S. Hameed, S. Rehman (eds.), *Nanotechnology for Infectious Diseases*,
https://doi.org/10.1007/978-981-16-9190-4_13

hydrogels are affected by mechanical based stress leading to disorientation of porous network by ruptures. Developing a hybrid variants involving silica/hydrogel and carbon/hydrogel nanocomposites could be interesting due to increased stability with similar tissue morphological features that are favorable for microenvironment. Nanocomposites design based on the pharmacological metals, recognition receptors, green nanoparticles, virus mimicking structures, and polyphenols can improve the delivery of vaccines, peptide/antiviral drugs.

Keywords

Biocompatibility · Drug delivery system · Infectious diseases · Nanocarriers · Nanotherapeutics

13.1 Mesostructured Silica-Based Drug Delivery System

Pathogens such as bacteria, viruses, parasite, fungi represent a major health issue causing various diseases, spread rapidly and result in huge mortality (14–16 million) worldwide (Jiang et al. 2020; Singh et al. 2020; Fatima et al. 2021). Recently, most of the outbreaks are caused by pathogens. The confirmed cases and deaths are continuously rising across the globe (202 countries) making this infectious spread deadliest for humankind in modern times. Re-emergence of infectious diseases is mostly of zoonotic including Ebola, Human Immunodeficiency Virus (HIV), Hepatitis C with high proliferation rate. Coronavirus belonging to large family of viruses causes illnesses of a wide range of severity. In the first instance, the illness of variable severity was caused by coronavirus at China in 2003 with Severe Acute Respiratory Syndrome (SARS). Later in 2012, the outbreak occurred in Saudi Arabia with Middle East Respiratory Syndrome (MERS). Coronavirus disease (COVID-19) is caused by the severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) (Khan et al. 2020). The genomic sequence analysis of current virus COVID-19 revealed that virus contains spike proteins, a receptor-binding domain, termed as RBD, that used to hold the host cells and a cleavage site used to crack open and enter the host cells. The proteins evolution of virus effectively targets the receptor termed as angiotensin-converting enzyme (ACE2) that is involved in maintaining blood pressure (Ni et al. 2020). The angiotensin system has been reported to play a vital role in cardiovascular homeostasis, acute inflammation, and autoimmune disorders (Shil et al. 2014; Nehme and Zouein 2019; Rehman et al. 2020a, 2021a).

In the present generation, a tremendous technological advancement is taking place in stimuli responsive biomedical applications such as targeted oriented drug therapy, diagnostic purpose, stem cell, and bioengineering (Das et al. 2020). The nanotherapeutics has broadened the scope for effective and efficient therapeutic approach on deadly diseases such as cancer, diabetic, and other metabolic disorders. In particular, the targeted drug delivery can be very effective for cancer treatment, which poses a major challenge worldwide. Nanobiotechnology based research has

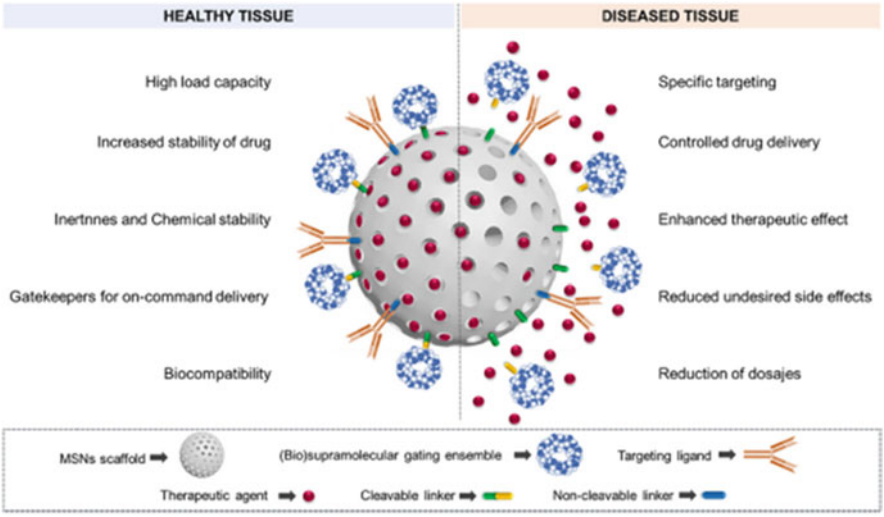

Fig. 13.1 Schematic representation of structured silica and their modifications as drug delivery system (García-Fernández et al. 2021)

been rising steadily in interdisciplinary core area for the development of targeted drug delivery system (Zhang et al. 2021). The conventional drugs are effective but also remains unselective eventually causing unavoidable side effects to normal tissues. Highly crystalline nature of drugs is insoluble in water and leads to diminutive bioavailability (Wilson et al. 2020). The advantage of using nanocarrier is that it has the capability to transform crystalline drugs to nano form (i.e., in amorphous state) itself due to nano sized pores that eventually converts them from insoluble state of drug to soluble form of drug. Nanotherapeutics offers critical advantages of delivering drug to the targeted cells. Nanomedicine research is currently on the rise focusing target-oriented drug therapy (Rehman et al. 2019, 2020a, b, c). The functionalization of drugs over nanocarriers improves the stability of drug components, increases the bioavailability, and reduces metabolization and excretion (Wilson et al. 2020).

Several smart nanocarriers based on structured silica, metal oxides, carbon, liposomes, and polymeric species based therapeutic tools have been reported for treating chronic diseases such as cancer, cardiovascular, and diabetics (Yang et al. 2021; Nahvi et al. 2021; Qureshi et al. 2021).

Porous structured silicas such as hexagonal shaped mesoporous silica (SiMCM-41 and SBA-15) and cubic shaped mesoporous silica (SBA-16 and MCM-48) are reported for targeted nanotherapeutics. The preference for such silicas is due to their superior textural properties (surface area ranging between 500 and 1100 m^2/g), stable framework channels, chemical stability, and biocompatibility and on-demand drug delivery capabilities (Fig. 13.1) (García-Fernández et al. 2021). In

addition, the nature of drug characteristics is changed leading to high solubility of drugs, release of drugs can be controlled and correlated to the type of pore diameter of silica. If the drug molecular size matches to that of pore size of silica, the drug release is found to be more efficient for longer duration due to confined molecular stacking inside the pores of silica. However, if the pore size is larger than the molecular size of drug, the releasing rate was found to be higher and uncontrolled. The hexagonal pores with straight channels are reported to release at faster rate than cubic shaped silica due to obstructive 3D pores. In addition, the potential charge matching, surface hydrophobic and hydrophilic property of silica are additional properties which have to be considered for enhanced drug release. The nano type silica is found to have high drug loading character and releasing ability (Vallet-Regí et al. 2007; Argyo et al. 2014). The drug support shown to lessen the denaturation of drug and improve target-oriented drug release. There are several types of nanoparticle silica reported to be synthesized using different types of templates including ionic (cetyltrimethyl ammonium bromide) and non-ionic copolymers (Pluronic F123 and Pluronic P123). The template mediated synthesis of silica is an added advantage as the template loaded silica can be exchanged in tumor pH condition while remains as such at normal pH condition. These characteristics of silica make them a perfect host to delivery drugs at tumor while remain passive at normal tissues.

13.2 Porous Carbon Based Drug Delivery System

Porous carbon has been attractive and is explored in multifunctional scientific applications (Rahman et al. 2021).

The nanoporous carbon is non-toxic and has controlled drug release property. It is structurally robust and has high capability to adsorb and store drugs. The high surface area has large prospective for chemical functionalization. The art of synthesizing several shaped porous carbon has been evolved over the years due to continuous improvisation and modification of synthesis techniques. The attention is usually related to the superior textural characteristics. Conventional porous carbons (activated carbon and molecular sieves) were prepared through pyrolysis and using organic precursors like wood, coal, and polymers at high temperatures. The pores of such carbon range between micropores and mesopores (Rehman et al. 2021b). The mesoporous carbon is preferred for several applications based on the disadvantage of microporous carbon such as diffusion constrain, defects, and low stability of pores during high temperature modification and graphitization process (Fig. 13.2) (Rahman et al. 2021). The formation of mesoporous carbon is achieved by the process of pyrolysis (Liu et al. 2017), polymeric templates (Javed et al. 2021). The mesoporous carbon using mesoporous silica has been reported in various structured forms including hexagonal MCM-41 (Lin et al. 2006), HMS (Zhou et al. 2009), SBA-15 (Yang and Sayari 2005), MSU-1 (Ania and Bandosz 2006), cubic SBA-16, SBA-1, and SBA-7 (Im3m, Pm3n) (Ryoo and Joo 2004), cubic Ia3d (MCM-48) (Kruk et al. 2000), cubic I a3d KIT-6 (Dai et al. 2010), and cubic Fm3m KIT-5 (Vinu et al. 2006). Recently, the synthesis of nitrogen doped mesoporous carbon has been

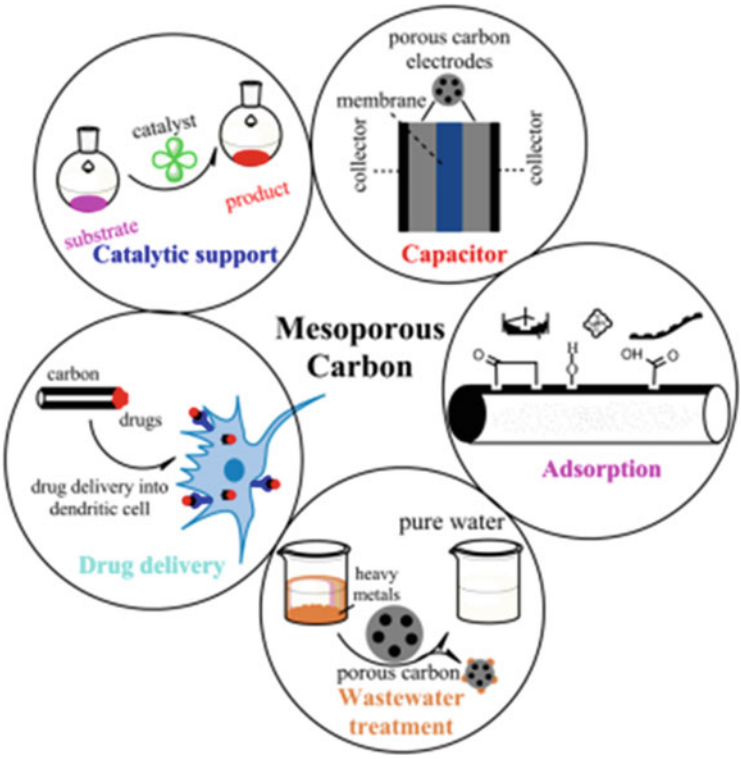

Fig. 13.2 Schematic representation of mesoporous carbon and their applications (Rahman et al. 2021)

reported using spray drying-vapor deposition method (Sun et al. 2020), mechano-chemical assembly using copolymers and metal complexes (Zhang et al. 2017), evaporative induced self-assembly technique (Azhar et al. 2020), mixture of polyphenol (phloroglucinol) and non-ionic template (F127) (Kawigraha et al. 2020). Converting large graphene into ultrasmall GO is termed as top-down approach (Yan et al. 2020) The most common approach involves exfoliation or hydrothermal synthesis or oxidation through electrochemical process or lithography technique. The oxidation of graphite leads to graphene oxide formation composing mono, bi, or multiple layers of graphene along with functional groups (hydroxyl, epoxide, and COOH groups). The preparation of graphene oxide (GO) is usually carried out using Hummers synthesis method. The presence of such functional group on GO offers variable bonding interactions (H-bonding, weak bonding) leading to the solubilization in aqueous and polar solvents. Recently the nano sized graphitized/mesoporous carbon is very attractive due to their ordered graphitic carbon layers and therefore tried in wide areas including fuel cells and batteries. The structure of graphitized carbon is composed of pentagonal structure with diameter of 30–35 nm. The unique properties of graphitized/mesoporous carbon include high crystallinity,

homogeneous structure with high degree of graphite domains, less surface functional groups, and less structural defects (Janekarn et al. 2020). The siliceous and carbon frameworks can be transformed from single drug delivery system to multifunctional therapeutics with doping of pharmacological active metal oxide system (ZnO, SPIONs, CeO_2, TiO_2).

13.3 Potential of Nanogel (Hydrogel) as Drug Delivery System

Hydrogels (nanogel) are composed of polyethylene oxide and polyvinylpyrrolidone three-dimensional (3D) hydrophilic polymers interrelating via crosslinks. The polymeric linkage interacts along with water medium in the form of colloidal gel. The structure of such gel is flexible and remains similar to that of tissues with high biocompatibility. Therefore, such hydrogels were extensive researched for the application of tissue engineering, pH, temperature stimuli, and blood glucose sensitive drug release (Shi et al. 2019; Mantha et al. 2019). The gels also applied as biosensors for antigens identification and in medical electrodes (Fig. 13.3).

However, such hydrogels are affected by mechanical based stress leading to disorientation of porous network by ruptures. In recent years, hybrid variants

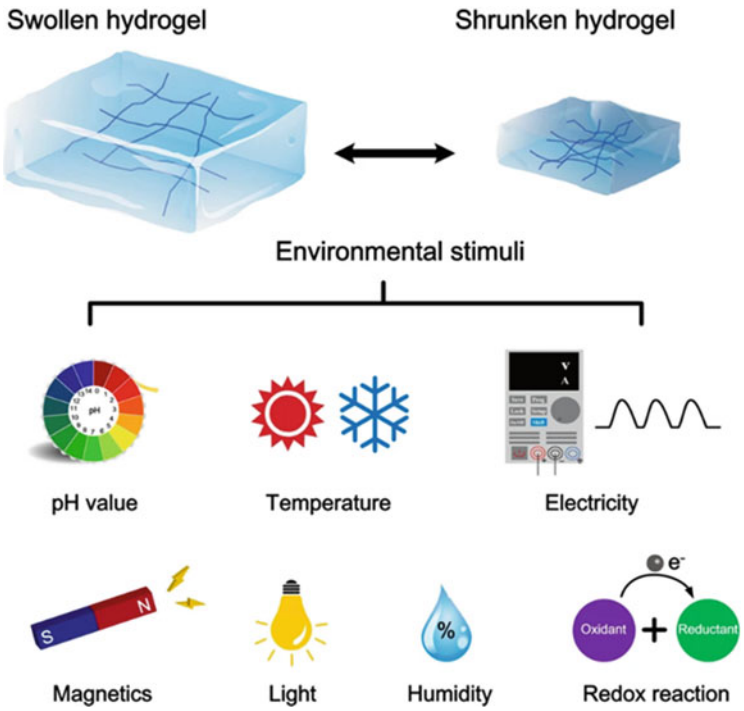

Fig. 13.3 Schematic representation of stimuli responsive hydrogels and applications as drug delivery system (Shi et al. 2019)

involving hydrogels/silica nanocomposite have attracting researchers' interest due to increased stability with similar tissue morphological features that are favorable for microenvironment. The polymeric gel is super flexible, cheap, highly biocompatible and look alike natural tissue with high water adsorption ability in form of colloidal silica (Wahid et al. 2020).

13.4 Potential of Nanogel/Silica or Nanogel/Carbon Nanocomposites as Drug Delivery System

The combination of biocompatible hydrogel/GO and hydrogel/silica nanocomposite is gaining importance in biomedical application (Yi et al. 2020). GO are termed as next generation material for biomedical applications (Fig. 13.4). In case of silicalite based silica, the presence of zeolite features in mesostructures tends to overcome the disadvantage of amorphous framework mesoporous to transform into robust materials due to presence of crystalline zeolite. Carboxymethylcellulose, graphene oxide, and zinc-based metal organic framework have been reported to be an effective composite through solvothermal technique for doxorubicin drug delivery and anti-cancer activity (K562 cells). Compared to graphene oxide, the composite showed pH sensitive DOX release due to presence of pi-pi stacking, hydrogen and electrostatic interactions (Javanbakht et al. 2019). Hydrogel in sodium form has been reported to act as capping agent for mesoporous pore channels for the delivery of doxorubicin release. The presence of glutathione modulates disulfide groups that act as pH

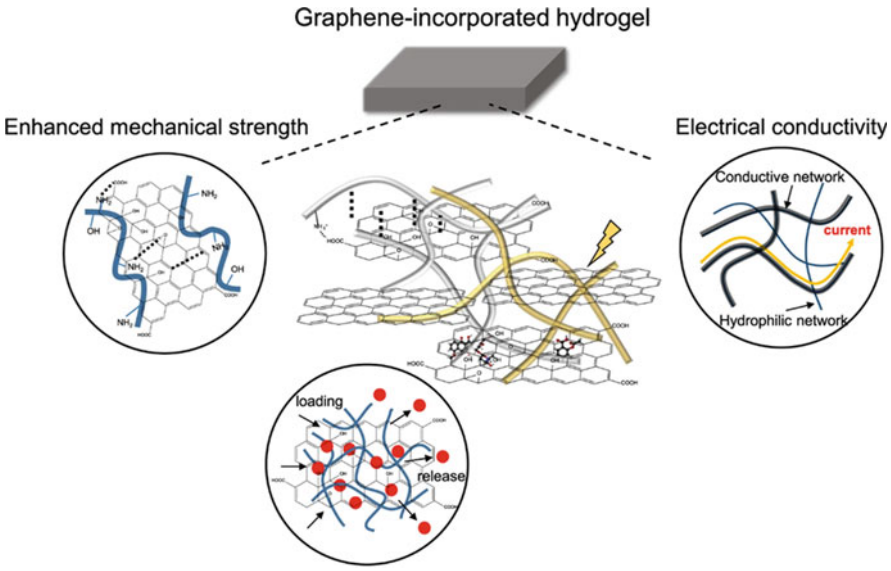

Fig. 13.4 Schematic representation of hybrid nanocomposite based on graphene and hydrogel for multifunctional drug delivery system (Yi et al. 2020)

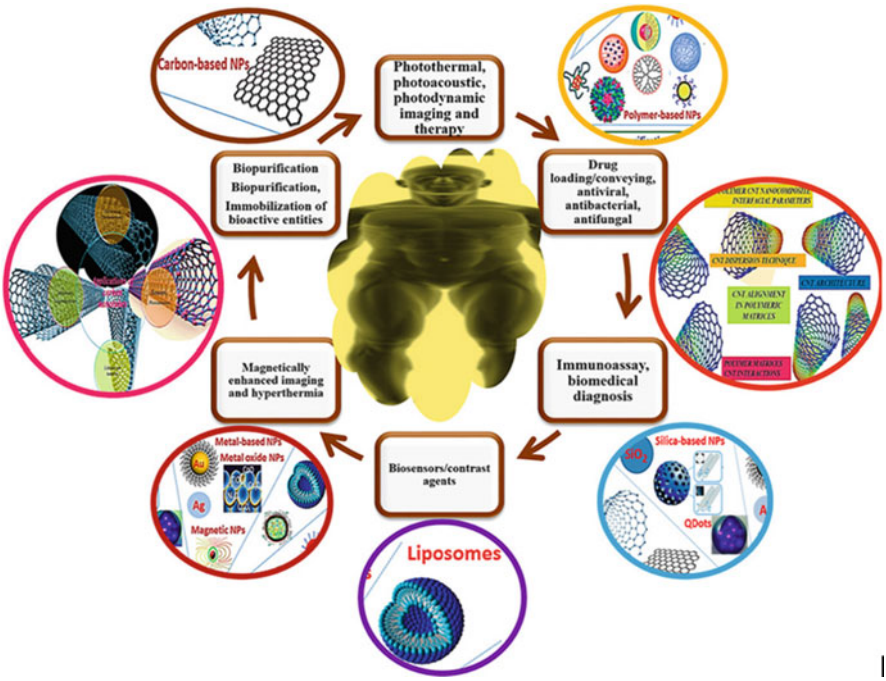

Fig. 13.5 Schematic representation of hybrid nanocomposite based on hydrogel and different nanoparticles for multifunctional drug delivery system (Idumah et al. 2021)

sensitive gate keeper at physiological conditions (pH 7.4 and 5.0), where intracellular specific release was observed through MTT assay of HeLa cells (Yuan et al. 2018).

The ability of hydrogel fabrications was reported with various metal oxides, quantum dots (QD) for imaging, magnetic nanoparticles (MRI response, hyperthermia), silica, liposomes, and carbon nanotubes (Idumah et al. 2021) (Fig. 13.5).

In continuation of advancement in the field of nanotechnology, the therapeutic chances have been broadened on chronic cancer, diabetic, autoimmunity, neurological disorders, and infectious diseases (Chountoulesi and Demetzos 2020; Majumder and Minko 2021; Rehman et al. 2021a, b, c). The multifunctional applications and fabrications potential of hydrogel along with drugs in a single platform could be used for effective drug release in tumor acidic condition. Therefore, such technologically viable drug carrier can enable more target-oriented drug release, improve drug bioavailability. The biocompatible nanocomposite with large stable surface of silica and carbon is expected to improve the stability of hydrogel, utilize engineered pore system, to reduce burst release, and improve target efficiency at the tumor acidic microenvironment to release antioxidant/drug. Silica and mesoporous carbon with high surface area and pore size uniformity can be loaded with high payload drugs for antiviral drug delivery applications. This engineering of nanocomposite with fluorescence nanoparticles increases the potential to act as a targeted nanovehicle in

sensitive organs like lungs. The present QD that used in imaging purpose are cadmium selenide (CdSe) quantum dots. Though they are best QD available, it is still marred by certain critical issues like passivation due to inorganics, pH sensitive and can induce cytotoxicity. In order to reduce the toxicity, several modifications like using ZnS and biocompatible polymers are reported. Even though such modifications are effective, the diffusion of Cd ions through defective shell occurs leading to destruction of biological system due to nanoparticle destruction and light irradiation. Several preventive measures were reported like replacing the ligands with thiols (mercaptoacetic acid) and carboxyl terminated ligands. However, the presence of week thiol-ZnS link and pH-based dispersion limit the ligand exchange efficiency. Quantum dots are excellent fluorescent probe and are biocompatible. The application of semiconducting nanocrystals in optical and magnetic resonance imaging is expected to be an effective solution for antiviral drug delivery applications. QD is reported to be an efficient semiconductor with imaging capability. The dots can be doped selectivity and are biodegradable and non-toxic. QD encapsulated hydrogel can be effectively used as image guided drug delivery applications in infectious diseases including COVID-19 (Manivannan and Ponnuchamy 2020). In the case of gold nanoparticle, different shape (sphere, rod, and shell) and diameter (1–100 nm) of gold nanoparticles can be used for dual purpose of bioimaging and photothermal therapy. Au/hydrogel and SPIONs/hydrogel are effectively used for treating infectious tissue wounds (Zhang et al. 2021; Patwa et al. 2020). The provision of tumor treatment accompanied with dual antimicrobial photodynamic therapy with dual capability of photosensitizer and magnetic resonance was found to be added advantage in treating non-local bacterial infections (Toledo et al. 2020). Therefore, such technologically viable hydrogel/silica or mesoporous carbon hybrid drug carriers can be more target-oriented drug release, improve drug bioavailability at infectious sites.

Delivery of drugs to lungs is attractive but clinical phase is limited by dose formulation and drug stability. Further, delivering drugs to lungs is quite challenging and complicated. The administered drugs inside the lungs can be either cleared by mucus patches and macrophages. Pulmonary drug delivery system has been developed to treat of lung infection including. The targeted nanodrug delivery can reduce the dose and help for bioavailability at large lung surface area (Al-Obaidi et al. 2021). Most striking advantages of such pulmonary nanocarriers based on silica/polymer and mesoporous carbon/polymer nanocomposites are attributed to the presence of large external surface area, high stability in physiological conditions, and high functionalization of multifunctional moieties (García-González et al. 2021). An effective pulmonary drug delivery system needs to stabilize the loaded drugs through effective encapsulation and loading efficiency, improve the biocompatibility, strong adhesion ability, rapid diffusion through mucus, sustained drug release capacity, and delivering drugs specifically to bronchi and alveoli (Bonam et al. 2020). Nanocarriers based on liposomes, polymeric micelles, silica nanoparticles, and metal organic frameworks were used for infectious diseases (Tang et al. 2021). Magnetically active micro or nanoparticles, superparamagnetic iron oxide nanoparticles (SPIONs) based drug delivery system has shown to improve the

targeted delivery to desired position with the help of external magnetic field (Saadat et al. 2020). Magnetic nanoparticles fabricated with poly (ethylene oxide) and poly (l-lactide) polymers are shown to increase the drug accumulation and improve targeted release of drugs at the lower airways (Nikolaou et al. 2021). Hydrogels and related composites were reported to be effect in improving the nasal delivery of vaccines, peptide/drugs (Salatin et al. 2016).

The delivery of the drugs to a particular site such as the pulmonary system, as it forms the main site for SARS-CoV-2 invasion, is important as poor absorption and low bioavailability effects are present. Recently, many studies have shown promising results for pulmonary delivery of nanostructured carriers for delivery antiviral agent. Recently, nanotechnology has been utilized as immunomodulators (Brain et al. 2021), delivering vaccine (Hoseini et al. 2021), vulvovaginal infections (Vanić et al. 2021), point of care diagnostic for infectious disease (Wang et al. 2021a, b). Bacterial infection (Shabana et al. 2021; Najafi et al. 2021), bimodal antibacterial (Bai et al. 2019), sofosbuvir delivery potential against hepatitis C virus (Mehmood et al. 2020), polymicrobial infections using combination therapy (Gounani et al. 2019), epsilon-poly-L-lysine coated SBA-15 as antifungal agent (Song et al. 2019). Bacterial imaging (Kirla et al. 2020) and biosensors were used for detection of infectious diseases (Sheikhzadeh et al. 2021), immunotherapeutic engineering to fight covid and infectious disease (Zarubova et al. 2021), and nanofibrous membranous as bactericide (Shan et al. 2020).

Mesoporous materials are explored for delivering antiviral drugs to infectious diseases (Whittle et al. 2021). Hexagonal shaped SBA-15 has exhibited a high loading efficacy of linear peptide Bactofencin A (~95%) than periodic mesoporous prepared using organosilanes for antimicrobial nanotherapeutics. The pore size of SBA-15 and periodic mesoporous were in the range between 60 and 68 Å, while Bactofencin A was less than of 30 Å. In spite of a larger accommodable pore size, periodic mesoporous exhibited a lower peptide loading efficacy and burst drug release (20% in 6 h). SBA-15 showed a high loading capacity with loading of 93 μg/mg. The main factor for such an effective adsorption is ascribed to the hydrophilic property due to silanols on the surface and electrostatic interaction of SBA-15 (Durack et al. 2019). The insertion of non-toxic metal oxides such as Zn, Au, Cu, and SPIONs into the silica nanoparticles modulates monofunctional to multifunctionality roles against bacteria (Baptista et al. 2018).

Hexagonal pore shaped MCM-41 with silane functionalization (aminopropyl) and polyvinyl alcohol showed high loading of sofosbuvir and sustained release for potential treatment of infectious liver disease. Sofosbuvir is a nucleotide based antiviral drug used to treat hepatitis C virus. The drug has low solubility, disintegrate rapidly and low permeability. Drug loading followed by functionalization on mesoporous silica nanoparticles (MSN) with particle size of 200 nm led to high drug loading (100 mg/mL) with a maximum loading of about 29%. Characterization of drug adsorbed MSN using X-ray diffraction technique reveals an amorphous transformation of drugs with some crystalline drugs adsorbed on the silanes at the external surface of MSN. Such adsorption tends to control the drug release ability of sofosbuvir. MSN in the absence of silane functionalization showed faster release of

drug with 4 h, while with silane functionalization a sustained drug release of about 30% in 1 h and 100% over 16 h. However, the initial surface area of MSN of about 602 m^2/g reduced significantly to 31 m^2/g with drug loading, silane and polyvinyl multilayer coating (Mehmood et al. 2020). ε-poly-L-lysine capped mesoporous silica-based nanoparticles for antibacterial activity (Velikova et al. 2017), antibiotic loaded mesoporous bioactive glass (Anand et al. 2020). Virus-like mesoporous silica-coated plasmonic Ag nanocube with strong bacteria adhesion for diabetic wound ulcer healing (Wang et al. 2021a, b), direct synthesis of Ag modified mesoporous silica as efficient antibacterial materials (Joardar et al. 2021), silver-loaded MSN for thin film coating for long-lasting antibacterial activity (Catalano et al. 2016), rattle-type magnetic mesoporous silica adsorption of single and binary antibiotics (Xu et al. 2011), MSN loaded with doxycycline as antimicrobial agent (Labban et al. 2021).

Recently, several antiviral drugs like dexamethasone, favipiravir, ribavirin, interferons, hydroxychloroquine combined with antibiotic azithromycin, lopinavir/ritonavir in combination with interferon were found to be effective in treatment of COVID-19. In particular, dexamethasone causes diabetes related complications, immune suppressor and hypertension. Poor gastrointestinal stability, low bioavailability, poor transport behavior, and side effect on other organs like kidney limit its therapeutic effectivity. Carboxymethylcellulose, graphene oxide, and metal organic framework have been reported to be an effective for antiviral drug delivery (Seifi and Kamali 2021; Wang et al. 2020). Compared to graphene oxide, the composite showed pH sensitive drug release due to presence of pi-pi stacking, hydrogen and electrostatic interactions (Hoseini-Ghahfarokhi et al. 2020). Therefore, such composites can be effectively used for delivery antiviral drugs. The nanotechnology-based cancer drug delivery system can be potentially developed and upscaled for possible treatment of COVID-19. The system can be used in inhaled drug delivery of nanomedicine like dexamethasone and other potential antiviral drugs. The combination of dexamethasone/ halloysite based nanoformulation with tumor imaging capable zinc ferrite are expected to improve the treatment efficacy by overcoming drug resistance and increase site-specific onsite drug delivery to the lung (Jermy et al. 2022). The nanocarrier particles with sizes between 60 and 300 nm are reported to diffuse freely in tracheal mucus of mouse. Biocompatible polymer polyethylene glycol coating of nanoparticle of 100 and 200 nm is shown to penetrate respiratory mucus.

The antiviral therapeutics will be critical in combating the disease mortality. In present medical emergency, pulmonary route of treatment using nanotechnology can be one of the innovative ways to treat viral infection. Nanodrug inhalation, encapsulation of antiviral drugs/vaccines, delivery system based on cell, viral, exosomes, bacterial membrane, Zn-based drug delivery systems, engineered DNA/RNA origami are some of the nanotechnology strategic designs against SARS-CoV-2 (Tang et al. 2021). Delivery of drugs to lungs is attractive but clinical phase is limited by dose formulation and drug stability. Further, delivering drugs to lungs is quite challenging and complicated. The administered drugs inside the lungs can be either

cleared by mucus patches and macrophages (Bustamante-Marin and Ostrowski 2017).

Then design a highly efficient, biocompatible, angiotensin-converting enzyme 2 (ACE2) functionalized interferon-silica biocomposite expected to block entry of CoVID-19. Epithelial cells present in the surface of body are the prime target for virus infection. Recombinant human interferon-α can be used for the management of chronic viral disease. The effectivity of developed nanocomposites can be studied in vitro model using lung epithelial cell lines. The targeted efficacy of drug formulations can be evaluated. Ps-COVID-19 could be generated using a lentivirus scaffold. To establish this, second-generation matched and unmatched lentiviral vectors, pseudotyped for spike protein, can be selected as a safe alternative method to mimic the structure and surfaces of COVID-19, and efficacy can be tested of nanocarriers as entry inhibitors and modulators of cytokine/chemokine response in the cells transduced with pseudotyped particles.

The therapeutic protein interferon-α is used to treat viral infections. However, the poor gastrointestinal stability, low bioavailability, poor transport behavior limit its therapeutic effectivity. PEGylated nanoparticles were found to be effective for interferon-α therapeutics (Moncalvo et al. 2020). A biocompatible silica/hydrogel or mesocarbon hydrogel nanocomposites based on pharmacologically active metal ions (Zn, Au, Ti, Fe etc.) coated with biocompatible polymers (chitosan, PEG, PLGA) can be developed with fabrication of interferon-α. The presence of high surface area nanocarriers expected to improve the oral biocompatibility of protein. The ratio of active metal ions/hydrogel ratios could be varied for high therapeutic efficiency. The loading effect of metal oxides, loading techniques (impregnation, isomorphous substitution) can be optimized and explored with effect of particle size between 5 and 20 nm, different functional groups such as silanes, vinyl, carboxylic acid, and biotin with angiotensin-converting enzyme 2 (ACE2) and interferon-α. The wrapping of nanocomposites with chitosan, poly (D, L-lactide-co-glycolide), and polyethylene glycol can be explored for improving biocompatibility and help drug delivery systems to reduce burst release and improve target efficiency at the infectious microenvironment to release antiviral drugs. Wang et al. (2017) have designed a mesoporous silica mimicking the structure of virus and shown to improve the cellular internalization thereby increasing the prospect of applicable of such nanocarriers to antiviral therapeutic strategies.

Mannose receptor present at the macrophages, endothelial cells, and dendritic cells (immature) is a C-type lectin and reported to play a critical role in receptor mediated endocytosis (Fig. 13.6). Several mannosylated nanocarriers based on liposomes, solid lipids, polymers (microspheres, micelles), quantum dots, cyclodextrin, and dendrimers were reported to exert target specificity, high binding affinity, internalization, pharmacokinetics profiles for infectious diseases, and cancer treatment (Patil and Deshpande 2020).

Endoplasmic reticulum sessile protein (STING) involved in signaling process with several receptors and is responsible for interferon (type 1) production against DNA viruses (Fig. 13.7). The protein senses infectious pathogens in cytosol and responds through signaling molecule cyclic dinucleotides to pathogen derived

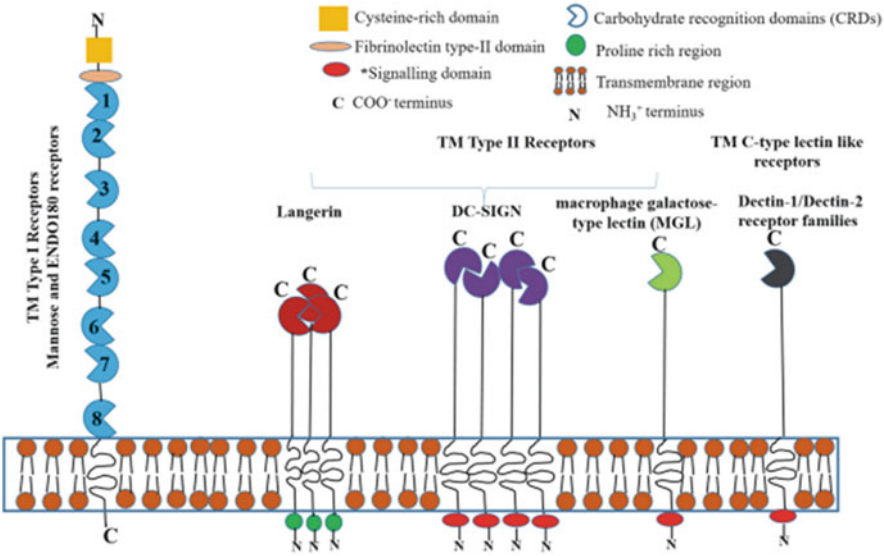

Fig. 1. Structural features of different transmembrane C-type lectin receptors (TM-CLRs).

Fig. 13.6 Schematic representation of mannosylated nanocarriers as drug delivery system to infectious diseases (Patil and Deshpande 2020)

cytosolic DNA. STING role is important in antitumor and antiviral immunity. Recently, several STING based nanoformulations are developed against infectious diseases (hepatitis B virus, adenovirus, Human alphaherpesvirus 3, and herpes viruses) (Zhou et al. 2021). The common STING agonists with binding affinity for STING include cyclic dinucleotides, xanthones, flavonoids, dispiro diketopiperzine were instrumental in developing vaccine adjuvants. However, the limited drug absorption (bioavailability), degradation within endosomes, and efficacy limited their clinical applications. Nanotechnology with ability to tune the size, shape, and functional moieties has potential to overcome such shortcomings of conventional vaccines. Vaccines in nanosize can be engineered to the pathogens size for improved cellular update and lymphatic drainage. However, the shape of nanoparticles is critical and determines the efficiency in cellular update. Spherical shaped nanoparticles induce a less toxicity and has high feasibility of consumption up by immune cells. Ellipsoid shaped nanoparticles get along well with surface membrane, while toxicity observed with nanorods and nanostars shaped nanoparticles. Therefore, the shape and size of nanovaccines are critical and define the advantages of nanovaccines.

Subunit vaccines are safe but still have issues related to lower efficiency and poor immunogenicity. Immune response stimulant, adjuvants are unable to attain the desired immune response. Therefore, a suitable designing of nanocarriers is important to delivery antigens crossing mucosal barrier. Nanocarriers such as nanogel, alginate, liposomes, mesoporous silica (MSN) have been reported to play a key role

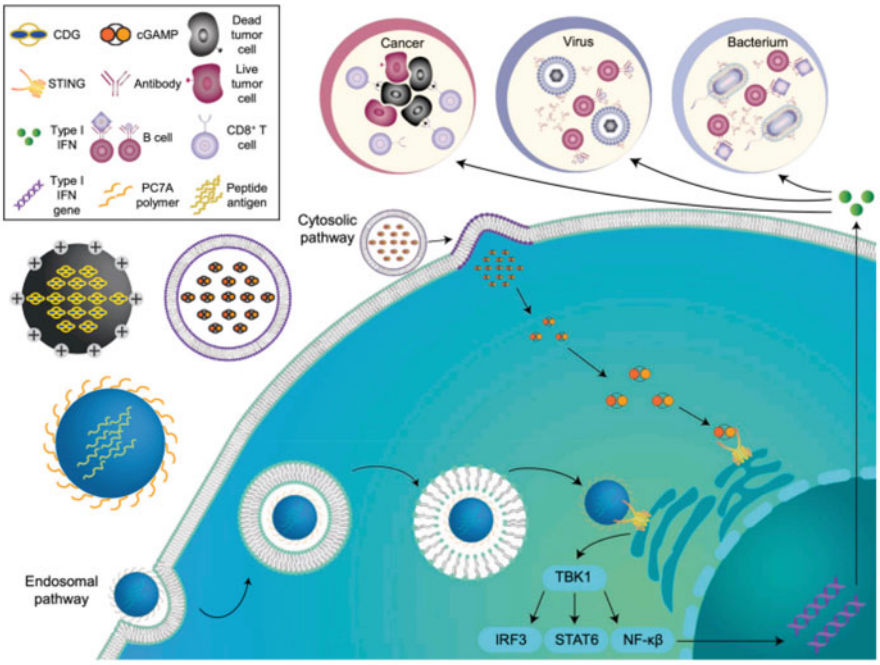

Fig. 1. STING-activating nanovaccines against cancers, viruses and bacteria. Nanovaccines can be synthesized from various nanomaterials to deliver STING payloads intracellularly. After endocytosis, pH-responsive nanovaccines can escape from the endosome and engage with STING in the cytosol. In the cytosolic pathway, the nanovaccines can fuse directly with the plasma membrane to release the encapsulated payload into the cytosol. Once activated, STING complexes with TANK-binding kinase 1 (TBK1) and phosphorylates interferon regulatory factor 3 (IRF3), signal transducer and activator of transcription 6 (STAT6), or nuclear factor-κB (NF-κB) to stimulate the production of type I interferons (IFNs).

Fig. 13.7 Schematic representation of Endoplasmic reticulum sessile protein (STING) involved in signaling process with several receptors and responsible for interferon (type 1) production against DNA viruses (Zhou et al. 2021)

in vaccine delivery against infectious lung diseases (Pan et al. 2021). Cationic nanogels in a monodispersed form with particle size of about 50 nm were interacted with serum albumin protein (Bovine serum albumin) for protein delivery. Presence of colloidal stabilization tends to increase the protein stabilization than cationic liposomes and assist effective internalization into cells (Ayame et al. 2008). Delivery of vaccine through intranasal route shown to restrict pneumococci colonization at the nasal cavity. To improve antigen specific immune responses, a toxin based mucosal adjuvants needs to be co-administered. However, such toxins can affect the central nervous system (CNS) or divert the vaccine antigen to CNS (Kong et al. 2013). Cationic nanogels are attractive non-toxin mucosal antigen carriers for delivering vaccines through nasal route. Nochi et al. (2010) have prepared a cationic cholesteryl pullulan based nanogel for delivery of pneumococcal vaccine.

Green synthesis of NPs is an important recent field of research, where nanomaterial synthesis is reported to be more pharmacologically active than chemical route-based NPs. The controllability of mono dispersion of NPs can be easily

Fig. 13.8 Schematic representation of green synthesis of nanoparticles as drug delivery system (Dutta and Das 2020)

controlled by varying the synthesis parameters like pH, temperature, precursor ratios, and incubation period (Dutta and Das 2020).

For drug delivery application, the green synthesis of nanoparticles, clay composites could be engineered with antiviral drug and biocompatible copolymers, for targeted drug delivery application (Liang et al. 2014). The technique is cheap, simple, reproducible, and scalable (Fig. 13.8). Natural halloysite (kaolin type) built with a tubular type of clay and nanocomposites have attracted nanobiotechnology field of research (Danyliuk et al. 2020) Silane functionalization of halloysite tends to be highly efficient in drug delivery inflammatory drug ibuprofen. In particular, temperature pretreatment of amine functionalized clay tube between 0.1 and 0.3 mmol/g at 120 °C and 400 °C has been shown to improve the ibuprofen loading to about 15%. The increased loading was attributed to an increase in the electrostatic attraction between silane amino group and COOH group of ibuprofen (Tan et al. 2013). The tubular layers are on mesoscopic scale ranging from meso to macrolevel larger than nanotubes and exhibited drug delivery ability of Aspirin. Biocompatible polymer chitosan fabricated tubular clay reported to show a steady state of aspirin release. In case of such tubular clay, an initial burst release was observed of about 58 wt%, while steadied in case of chitosan composite with about 27 wt% release. An extended period of drug release was observed with chitosan that reached about 65 wt % for 24 h (Li et al. 2016). Pseudotyped for spike protein can act as a safe alternative method to mimic the structure and surfaces of COVID-19, and test the efficacy of

nanocarriers as entry inhibitors and modulators of cytokine/chemokine response in the cells transduced with pseudotyped particles.

Natural antioxidants such as curcumin, resveratrol, etc. exhibit antiviral properties (Murthy et al. 2021). The polyphenol compounds are safe and non-toxic and have been reported to prevent viral replication and budding (Obata et al. 2013). However, the stability of such natural compounds under physiological conditions is less leading to poor bioavailability. Nanotechnology can be an alternative solution to improve the stability, bioavailability, and increase the therapeutic target efficacy on infectious site. Yang et al. (2016) have reported the curcumin/AgNPs nanocomposite using green synthesis condition. The chemical precursors that have been used as reducing agents, metal sources, and capping agents were avoided while replaced with curcumin to play the dual role of reducing and capping agent. Using such antioxidant produced monodispersed AgNPs (11.95 nm) and mutually stabilized the curcumin thermal stability. Curcumin interacts with AgNPs with carbonyl group, while phenolic hydroxyl group of curcumin induce antiviral activity against respiratory syncytial virus infection. Therefore, designing a silica/polymer, mesoporous carbon/silica nanocomposites will be effective in benefiting mutual characteristics of nanocarriers for antiviral delivery. Hydrogel textural characteristics were improved with nanocomposite formation with silica and GO. Preparation of ZnO or Au/hydrogel-GO with high biocompatibility for novel surfactant protein A loading could be effective for antiviral applications. Further, the developed drug design could be compared with hydrogel-silica composite and with various other metal oxides such as TiO_2 and metal silver. Presence of silica will improve the textural natures of nanocomposite (large surface area, pore size distributions), while polymer presence can improve, the stimuli responsive characteristics are based on infectious environments. The presence of functional moieties will enhance the smart drug carrier capabilities that play a critical role in sensing infectious sites, improve the host–guest interactions, and deliver antiviral drugs.

13.5 Conclusions

Pathogenic microorganisms cause infectious diseases leading to high mortality. Nanotherapeutics involving developing an advanced drug delivery could overcome the present challenges to solutions. A hybrid nanocomposite based on silica/polymer and carbon/polymers is advantageous with high textural uniformity, biocompatibility, providing mutual structural stability with high drug loading capabilities. Nanodrug inhalation, loading of pharmacologically active nanoparticles (Zn, Ag, Au, Cu, SPIONs), antiviral drugs/vaccines (encapsulation), delivery system based on structured silica, clay, cell, viral, exosomes, bacterial membrane, and engineered DNA/RNA origami are some of the nanotechnology strategic designs against infectious diseases. The receptors (angiotensin-converting enzyme 2 (ACE-2)), mannose, endoplasmic reticulum sessile protein (STING) based drug delivery design could play a critical role in receptor mediated endocytosis of nanoparticles. Green synthesis of nanoparticles based on biocompatible metal oxides, clay, and structural design

of nanocarriers mimicking the structure of virus could improve the cellular internalization. The functionalization of polyphenols is safe, non-toxic and has potential to prevent viral replication and budding.

References

Al-Obaidi H, Granger A, Hibbard T, Opesanwo S (2021) Pulmonary drug delivery of antimicrobials and anticancer drugs using solid dispersions. Pharmaceutics. 13(7):1056

Anand A, Das P, Nandi SK, Kundu B (2020) Development of antibiotic loaded mesoporous bioactive glass and its drug release kinetics. Ceram Int 46(4):5477–5483

Ania CO, Bandosz TJ (2006) Highly mesoporous carbons obtained using a dynamic template method. Microporous Mesoporous Mater 89(1–3):315–324

Argyo C, Weiss V, Bräuchle C, Bein T (2014) Multifunctional mesoporous silica nanoparticles as a universal platform for drug delivery. Chem Mater 26(1):435–451

Ayame H, Morimoto N, Akiyoshi K (2008) Self-assembled cationic nanogels for intracellular protein delivery. Bioconjug Chem 19(4):882–890. https://doi.org/10.1021/bc700422s

Azhar MR, Arafat Y, Zhong Y, Khiadani M, Tade MO, Wang S, Shao Z (2020) An adsorption–catalysis pathway toward sustainable application of mesoporous carbon nanospheres for efficient environmental remediation. ACS ES & T Water 1(1):145–156

Bai YM, Mao J, Li DX, Luo XJ, Chen J, Tay FR, Niu LN (2019) Bimodal antibacterial system based on quaternary ammonium silane-coupled core-shell hollow mesoporous silica. Acta Biomater 85:229–240. https://doi.org/10.1016/j.actbio.2018.12.037

Baptista PV, McCusker MP, Carvalho A, Ferreira DA, Mohan NM, Martins M, Fernandes AR (2018) Nano-strategies to fight multidrug resistant bacteria—"a battle of the titans". Front Microbiol 9:1441

Bonam SR, Kotla NG, Bohara RA, Rochev Y, Webster TJ, Bayry J (2020) Potential immuno-nanomedicine strategies to fight COVID-19 like pulmonary infections. Nano Today 36:101051

Brain D, Plant-Hately A, Heaton B, Arshad U, David C, Hedrich C, Owen A, Liptrott NJ (2021) Drug delivery systems as immunomodulators for therapy of infectious disease: relevance to COVID-19. Adv Drug Deliv Rev 178:113848

Bustamante-Marin XM, Ostrowski LE (2017) Cilia and mucociliary clearance. Cold Spring Harb Perspect Biol 9(4):a028241

Catalano PN, Pezzoni M, Costa C, Soler GJ, Bellino MG, Desimone MF (2016) Optically transparent silver-loaded mesoporous thin film coating with long-lasting antibacterial activity. Microporous Mesoporous Mater 236:158–166

Chountoulesi M, Demetzos C (2020) Promising nanotechnology approaches in treatment of autoimmune diseases of central nervous system. Brain Sci 10(6):338

Dai W, Zheng M, Zhao Y, Liao S, Ji G, Cao J (2010) Template synthesis of three-dimensional cubic ordered mesoporous carbon with tunable pore sizes. Nanoscale Res Lett 5(1):103–107

Danyliuk N, Tomaszewska J, Tatarchuk T (2020) Halloysite nanotubes and halloysite-based composites for environmental and biomedical applications. J Mol Liq 309:113077

Das SS, Bharadwaj P, Bilal M, Barani M, Rahdar A, Taboada P, Bungau S, Kyzas GZ (2020 Jun) Stimuli-responsive polymeric nanocarriers for drug delivery, imaging, and theragnosis. Polymers 12(6):1397

Durack E, Mallen S, O'Connor PM, Rea MC, Ross RP, Hill C, Hudson S (2019) Protecting bactofencin A to enable its antimicrobial activity using mesoporous matrices. Int J Pharm 558:9–17. https://doi.org/10.1016/j.ijpharm.2018.12.035

Dutta D, Das BM (2020) Scope of green nanotechnology towards amalgamation of green chemistry for cleaner environment: a review on synthesis and applications of green nanoparticles. Environ Nanotechnol Monit Manag 15:100418

Fatima Z, Kumari P, Rehman S, Hameed S (2021) Virulence traits of Candida spp.: an overview. In: Recent trends in mycological research, pp 439–55

García-Fernández A, Sancenón F, Martínez-Máñez R (2021) Mesoporous silica nanoparticles for pulmonary drug delivery. Adv Drug Deliv Rev 177:113953. https://doi.org/10.1016/j.addr.2021.113953

García-González CA, Sosnik A, Kalmár J, De Marco I, Erkey C, Concheiro A, Alvarez-Lorenzo C (2021) Aerogels in drug delivery: from design to application. J Control Release 332:40–63

Gounani Z, Asadollahi MA, Pedersen JN, Lyngsø J, Skov Pedersen J, Arpanaei A, Meyer RL (2019) Mesoporous silica nanoparticles carrying multiple antibiotics provide enhanced synergistic effect and improved biocompatibility. Colloids Surf B Biointerfaces 175:498–508. https://doi.org/10.1016/j.colsurfb.2018.12.035

Hoseini ZS, Hajizade A, Razmyar J, Ahmadian G, Arpanaei A (2021) Mesoporous silica nanoparticles-based formulations of a chimeric proteinous vaccine candidate against necrotic enteritis disease. Mater Sci Eng C Mater Biol Appl 128:112316. https://doi.org/10.1016/j.msec.2021.112316

Hoseini-Ghahfarokhi M, Mirkiani S, Mozaffari N, Sadatlu MA, Ghasemi A, Abbaspour S, Akbarian M, Farjadain F, Karimi M (2020) Applications of graphene and graphene oxide in smart drug/gene delivery: is the world still flat? Int J Nanomedicine 15:9469

Idumah CI, Nwuzor IC, Odera RS (2021) Recent advances in polymer hydrogel nanoarchitectures and applications. Curr Res Green Sustain Chem 4:100143

Janekarn I, Hunt AJ, Ngernyen Y, Youngme S, Supanchaiyamat N (2020) Graphitic mesoporous carbon-silica composites from low-value sugarcane by-products for the removal of toxic dyes from wastewaters. R Soc Open Sci 7(9):200438

Javanbakht S, Pooresmaeil M, Namazi H (2019) Green one-pot synthesis of carboxymethylcellulose/Zn-based metal-organic framework/graphene oxide bio-nanocomposite as a nanocarrier for drug delivery system. Carbohydr Polym 208:294–301

Javed H, Pani S, Antony J, Sakthivel M, Drillet JF (2021) Synthesis of mesoporous carbon spheres via a soft-template route for catalyst supports in PEMFC cathodes. Soft Matter 17(33):7743–7754

Jermy BR, Ravinayagam V, Almohazey D, Alamoudi WA, Dafalla H, Akhtar S, Tanimu G (2022) PEGylated green halloysite/spinel ferrite nanocomposites for pH sensitive delivery of dexamethasone: a potential pulmonary drug delivery treatment option for COVID-19. Appl Clay Sci 216:106333

Jiang C, Yao X, Zhao Y, Wu J, Huang P, Pan C, Liu S, Pan C (2020) Comparative review of respiratory diseases caused by coronaviruses and influenza A viruses during epidemic season. Microbes Infect 22(6–7):236–244. https://doi.org/10.1016/j.micinf.2020.05.005

Joardar S, Adams ML, Biswas R, Deodhar GV, Metzger KE, Deweese K, Davidson M, Richards RM, Trewyn BG, Biswas P (2021) Direct synthesis of silver nanoparticles modified spherical mesoporous silica as efficient antibacterial materials. Microporous Mesoporous Mater 313:110824

Kawigraha AD, Krisnandi YK, Abdullah I (2020) Synthesis of impregnated nickel-mesoporous carbon and its application for reaction of acetylene with carbon dioxide. In: IOP conference series: materials science and engineering, vol. 763, no. 1. IOP Publishing, p 012040

Khan S, Tombuloglu H, Hassanein SE, Rehman S, Bozkurt A, Cevik E, Abdel-Ghany S, Nabi G, Ali A, Sabit H (2020) Coronavirus diseases 2019: current biological situation and potential therapeutic perspective. Eur J Pharmacol 886:173447

Kirla H, Hughes L, Henry DJ (2020) Carbohydrate coated fluorescent mesoporous silica particles for bacterial imaging. Colloids Surf B Biointerfaces 188:110751. https://doi.org/10.1016/j.colsurfb.2019.110751

Kong IG, Sato A, Yuki Y, Nochi T, Takahashi H, Sawada S, Mejima M, Kurokawa S, Okada K, Sato S, Briles DE, Kunisawa J, Inoue Y, Yamamoto M, Akiyoshi K, Kiyono H (2013) Nanogel-based PspA intranasal vaccine prevents invasive disease and nasal colonization by Streptococcus pneumoniae. Infect Immun 81(5):1625–1634. https://doi.org/10.1128/IAI.00240-13

Kruk M, Jaroniec M, Ryoo R, Joo SH (2000) Characterization of ordered mesoporous carbons synthesized using MCM-48 silicas as templates. J Phys Chem B 104(33):7960–7968
Labban N, Al-Otaibi HN, Binrayes A, Aljamhan AS, Alfouzan AF, Al Taweel SM, Assery MK (2021) Mesoporous nanosilica loaded with doxycycline to enhance antimicrobial property and mechanical strength at hybrid layer. Int J Adhes Adhes 111:102975
Li X, Ouyang J, Yang H, Chang S (2016) Chitosan modified halloysite nanotubes as emerging porous microspheres for drug carrier. Appl Clay Sci 126:306–312
Liang JJ, Wei JC, Lee YL, Hsu SH, Lin JJ, Lin YL (2014) Surfactant-modified nanoclay exhibits an antiviral activity with high potency and broad spectrum. J Virol 88(8):4218–4228
Lin CF, Zhang X, Lin H, Wang N, Li JB, Yang XZ (2006) Synthesis of ordered mesoporous carbon using MCM-41 mesoporous silica as template. In: Advanced materials research, vol. 11. Trans Tech Publications Ltd, pp 543–546
Liu L, Zhang H, Wang G, Du J, Zhang Y, Fu X, Chen A (2017) Synthesis of mesoporous carbon nanospheres via "pyrolysis-deposition" strategy for CO2 capture. J Mater Sci 52(16): 9640–9647
Majumder J, Minko T (2021) Targeted nanotherapeutics for respiratory diseases: cancer, fibrosis, and coronavirus. Adv Therap 4(2):2000203
Manivannan S, Ponnuchamy K (2020) Quantum dots as a promising agent to combat COVID-19. Appl Organomet Chem 34(10):e5887
Mantha S, Pillai S, Khayambashi P, Upadhyay A, Zhang Y, Tao O, Pham HM, Tran SD (2019) Smart hydrogels in tissue engineering and regenerative medicine. Materials 12(20):3323
Mehmood Y, Khan IU, Shahzad Y, Khan RU, Khalid SH, Yousaf AM, Hussain T, Asghar S, Khalid I, Asif M, Shah SU (2020) Amino-decorated mesoporous silica nanoparticles for controlled sofosbuvir delivery. Eur J Pharm Sci 143:105184. https://doi.org/10.1016/j.ejps.2019.105184
Moncalvo F, Martinez Espinoza MI, Cellesi F (2020) Nanosized delivery systems for therapeutic proteins: clinically validated technologies and advanced development strategies. Front Bioeng Biotechnol 8:89
Murthy NT, Agrahari V, Chauhan H (2021) Polyphenols against infectious diseases: controlled release nano-formulations. Eur J Pharm Biopharm 161:66–79
Nahvi I, Belkahla S, Asiri SM, Rehman S (2021) Overview and prospectus of algal biogenesis of nanoparticles. In: Microbial nanotechnology: green synthesis and applications. Springer, Singapore, pp 121–134
Najafi A, Khosravian P, Validi M, Daryasari MP, Drees F, Gholipour A (2021) Antimicrobial action of mesoporous silica nanoparticles loaded with cefepime and meropenem separately against multidrug-resistant (MDR) Acinetobacter baumannii. J Drug Deliv Sci Technol 65: 102757
Nehme A, Zouein FA (2019) An update on the tissue renin angiotensin system and its role in physiology and pathology. J Cardiovasc Dev Dis 6:14
Ni W, Yang X, Yang D, Bao J, Li R, Xiao Y, Hou C, Wang H, Liu J, Yang D, Xu Y (2020) Role of angiotensin-converting enzyme 2 (ACE2) in COVID-19. Crit Care 24(1):422
Nikolaou E, Jochems SP, Mitsi E, Pojar S, Blizard A, Reiné J, Solórzano C, Negera E, Carniel B, Soares-Schanoski A, Connor V (2021) Experimental human challenge defines distinct pneumococcal kinetic profiles and mucosal responses between colonized and non-colonized adults. mBio 12(1):e02020
Nochi T, Yuki Y, Takahashi H, Sawada S, Mejima M, Kohda T, Harada N, Kong IG, Sato A, Kataoka N, Tokuhara D, Kurokawa S, Takahashi Y, Tsukada H, Kozaki S, Akiyoshi K, Kiyono H (2010) Nanogel antigenic protein-delivery system for adjuvant-free intranasal vaccines. Nat Mater 9(7):572–578. https://doi.org/10.1038/nmat2784
Obata K, Kojima T, Masaki T, Okabayashi T, Yokota S, Hirakawa S, Nomura K, Takasawa A, Murata M, Tanaka S, Fuchimoto J (2013) Curcumin prevents replication of respiratory syncytial virus and the epithelial responses to it in human nasal epithelial cells. PLoS One 8(9):e70225

Pan C, Yue H, Zhu L, Ma GH, Wang HL (2021) Prophylactic vaccine delivery systems against epidemic infectious diseases. Adv Drug Deliv Rev 176:113867. https://doi.org/10.1016/j.addr.2021.113867

Patil TS, Deshpande AS (2020) Mannosylated nanocarriers mediated site-specific drug delivery for the treatment of cancer and other infectious diseases: a state of the art review. J Control Release 320:239–252

Patwa R, Zandraa O, Capáková Z, Saha N, Sáha P (2020) Effect of iron-oxide nanoparticles impregnated bacterial cellulose on overall properties of alginate/casein hydrogels: potential injectable biomaterial for wound healing applications. Polymers 12(11):2690

Qureshi A, Blaisi NI, Abbas AA, Khan NA, Rehman S (2021) Prospectus and development of microbes mediated synthesis of nanoparticles. In: Microbial nanotechnology: green synthesis and applications. Springer, Singapore, pp 1–15

Rahman M, Ara MG, Alim MA, Uddin M, Najda A, Albadrani GM, Sayed AA, Mousa SA, Abdel-Daim MM (2021) Mesoporous carbon: a versatile material for scientific applications. Int J Mol Sci 22(9):4498

Rehman S, Jermy BR, Akhtar S, Borgio JF, Abdul Azeez S, Ravinayagam V, Al Jindan R, Alsalem ZH, Buhameid A, Gani A (2019) Isolation and characterization of a novel thermophile; Bacillus haynesii, applied for the green synthesis of ZnO nanoparticles. Artif Cells Nanomed Biotechnol 47(1):2072–2082

Rehman S, Asiri SM, Khan FA, Jermy BR, Ravinayagam V, Alsalem Z, Al Jindan R, Qurashi A (2020a) Anticandidal and in vitro anti-proliferative activity of sonochemically synthesized indium tin oxide nanoparticles. Sci Rep 10(1):1–9

Rehman S, Jermy R, Asiri SM, Shah MA, Farooq R, Ravinayagam V, Ansari MA, Alsalem Z, Al Jindan R, Reshi Z, Khan FA (2020b) Using Fomitopsis pinicola for bioinspired synthesis of titanium dioxide and silver nanoparticles, targeting biomedical applications. RSC Adv 10(53): 32137–32147

Rehman S, Majeed T, Ansari MA, Ali U, Sabit H, Al-Suhaimi EA (2020c) Current scenario of COVID-19 in pediatric age group and physiology of immune and thymus response. Saudi J Biol Sci 27(10):2567–2573

Rehman S, Almessiere MA, Al-Jameel SS, Ali U, Slimani Y, Taskhandi N, Al-Saleh NS, Manikandan A, Khan FA, Al-Suhaimi E, Baykal A (2021a) Designing of Co0. 5Ni0. 5GaxFe2-xO4 ($0.0 \leq x \leq 1.0$) microspheres via hydrothermal approach and their selective inhibition on the growth of cancerous and fungal cells. Pharmaceutics 13(7):962

Rehman S, Ravinayagam V, Nahvi I, Aldossary H, Al-Shammari M, Amiri MS, Kishore U, Al-Suhaimi EA (2021b) Immunity, sex hormones, and environmental factors as determinants of COVID-19 disparity in women. Front Immunol 12:68084

Rehman S, Ansari MA, Al-Dossary HA, Fatima Z, Hameed S, Ahmad W, Ali A (2021c) Current perspectives on mycosynthesis of nanoparticles and their biomedical application. In: Modeling and control of drug delivery systems. Academic, pp 301–311

Ryoo R, Joo SH (2004) Nanostructured carbon materials synthesized from mesoporous silica crystals by replication. In: Studies in surface science and catalysis, vol. 148. Elsevier, pp 241–260

Saadat M, Manshadi MK, Mohammadi M, Zare MJ, Zarei M, Kamali R, Sanati-Nezhad A (2020) Magnetic particle targeting for diagnosis and therapy of lung cancers. J Control Release 328: 776–791

Salatin S, Barar J, Barzegar-Jalali M, Adibkia K, Milani MA, Jelvehgari M (2016) Hydrogel nanoparticles and nanocomposites for nasal drug/vaccine delivery. Arch Pharm Res 39(9): 1181–1192

Seifi T, Kamali AR (2021) Antiviral performance of graphene-based materials with emphasis on COVID-19: a review. Med Drug Discov:100099

Shabana MS, Taju G, Majeed A, Karthika M, Ramasubramanian V, Sahul Hameed AS (2021) Preparation and evaluation of mesoporous silica nanoparticles loaded quercetin against bacterial infections in Oreochromis niloticus. Aquac Rep 21:100808

Shan H, Si Y, Yu J, Ding B (2020) Flexible, mesoporous, and monodispersed metallic cobalt-embedded inorganic nanofibrous membranes enable ultra-fast and high-efficiency killing of bacteria. Chem Eng J 382:122909

Sheikhzadeh E, Beni V, Zourob M (2021) Nanomaterial application in bio/sensors for the detection of infectious diseases. Talanta 230:122026. https://doi.org/10.1016/j.talanta.2020.122026

Shi Q, Liu H, Tang D, Li Y, Li X, Xu F (2019) Bioactuators based on stimulus-responsive hydrogels and their emerging biomedical applications. NPG Asia Mater 11(1):64

Shil PK, Kwon KC, Zhu P, Verma A, Daniell H, Li Q (2014) Oral delivery of ACE2/Ang-(1-7) bioencapsulated in plant cells protects against experimental uveitis and autoimmune uveoretinitis. Mol Ther 22:2069–2082

Singh S, Rehman S, Fatima Z, Hameed S (2020) Protein kinases as potential anticandidal drug targets. Front Biosci (Landmark Ed) 25:1412–1432

Song Y, Zhu P, Wu Y, Tan L, Wei W, Liu S, Huang Q, Chen J (2019) Epsilon-poly-l-lysine decorated ordered mesoporous silica contributes to the synergistic antifungal effect and enhanced solubility of a lipophilic drug. Mater Sci Eng C Mater Biol Appl 99:231–240. https://doi.org/10.1016/j.msec.2019.01.077

Sun X, Kong Y, Liu Y, Zhou L, Nanjundan AK, Huang X, Yu C (2020) Nitrogen-doped mesoporous carbon microspheres by spray drying-vapor deposition for high-performance supercapacitor. Front Chem 8:1018

Tan D, Yuan P, Annabi-Bergaya F, Yu H, Liu D, Liu H, He H (2013) Natural halloysite nanotubes as mesoporous carriers for the loading of ibuprofen. Microporous Mesoporous Mater 179:89–98

Tang Z, Ma Q, Chen X, Chen T, Ying Y, Xi X, Wang L, Ma C, Shaw C, Zhou M (2021) Recent advances and challenges in nanodelivery systems for antimicrobial peptides (AMPs). Antibiotics 10(8):990

Toledo VH, Yoshimura TM, Pereira ST, Castro CE, Ferreira FF, Ribeiro MS, Haddad PS (2020) Methylene blue-covered superparamagnetic iron oxide nanoparticles combined with red light as a novel platform to fight non-local bacterial infections: a proof of concept study against Escherichia coli. J Photochem Photobiol B Biol 209:111956

Vallet-Regí M, Balas F, Arcos D (2007) Mesoporous materials for drug delivery. Angew Chem Int Ed 46(40):7548–7558

Vanić Ž, Jøraholmen MW, Škalko-Basnet N (2021) Nanomedicines for the topical treatment of vulvovaginal infections: addressing the challenges of antimicrobial resistance. Adv Drug Deliv Rev 178:113855

Velikova N, Mas N, Miguel-Romero L, Polo L, Stolte E, Zaccaria E, Cao R, Taverne N, Murguía JR, Martinez-Manez R, Marina A, Wells J (2017) Broadening the antibacterial spectrum of histidine kinase autophosphorylation inhibitors via the use of ε-poly-L-lysine capped mesoporous silica-based nanoparticles. Nanomedicine 13(2):569–581. https://doi.org/10.1016/j.nano.2016.09.011

Vinu A, Miyahara M, Mori T, Ariga K (2006) Carbon nanocage: a large-pore cage-type mesoporous carbon material as an adsorbent for biomolecules. J Porous Mater 13(3–4):379–383

Wahid F, Zhao XJ, Jia SR, Bai H, Zhong C (2020) Nanocomposite hydrogels as multifunctional systems for biomedical applications: current state and perspectives. Compos Part B 200:108208

Wang W, Wang P, Tang X, Elzatahry AA, Wang S, Al-Dahyan D, Zhao M, Yao C, Hung CT, Zhu X, Zhao T (2017) Facile synthesis of uniform virus-like mesoporous silica nanoparticles for enhanced cellular internalization. ACS Central Sci 3(8):839–846

Wang Y, Hu Y, He Q, Yan J, Xiong H, Wen N, Cai S, Peng D, Liu Y, Liu Z (2020) Metal-organic frameworks for virus detection. Biosens Bioelectron 169:112604

Wang C, Liu M, Wang Z, Li S, Deng Y, He N (2021a) Point-of-care diagnostics for infectious diseases: from methods to devices. Nano Today 37:101092. https://doi.org/10.1016/j.nantod.2021.101092

Wang P, Jiang S, Li Y, Luo Q, Lin J, Hu L, Liu X, Xue F (2021b) Virus-like mesoporous silica-coated plasmonic Ag nanocube with strong bacteria adhesion for diabetic wound ulcer healing. Nanomedicine 34:102381. https://doi.org/10.1016/j.nano.2021.102381

Whittle E, Martín-Illana A, Cazorla-Luna R, Notario-Perez F, Veiga-Ochoa MD, Rubio J, Tamayo A (2021) Silane modification of mesoporous materials for the optimization of antiviral drug adsorption and release capabilities in vaginal media. Pharmaceutics 13(9):1416

Wilson VR, Lou X, Osterling DJ, Stolarik DF, Jenkins GJ, Nichols BL, Dong Y, Edgar KJ, Zhang GG, Taylor LS (2020) Amorphous solid dispersions of enzalutamide and novel polysaccharide derivatives: investigation of relationships between polymer structure and performance. Sci Rep 10(1):18535

Xu L, Dai J, Pan J, Li X, Huo P, Yan Y, Zou X, Zhang R (2011) Performance of rattle-type magnetic mesoporous silica spheres in the adsorption of single and binary antibiotics. Chem Eng J 174(1):221–230

Yan Y, Nashath FZ, Chen S, Manickam S, Lim SS, Zhao H, Lester E, Wu T, Pang CH (2020) Synthesis of graphene: potential carbon precursors and approaches. Nanotechnol Rev 9(1): 1284–1314

Yang Y, Sayari A (2005) SBA-15 templated-ordered mesoporous carbon: effect of SBA-15 microporosity. In: Studies in surface science and catalysis, vol. 156. Elsevier, pp 543–550

Yang XX, Li CM, Huang CZ (2016) Curcumin modified silver nanoparticles for highly efficient inhibition of respiratory syncytial virus infection. Nanoscale 8(5):3040–3048

Yang Y, Zeng W, Huang P, Zeng X, Mei L (2021) Smart materials for drug delivery and cancer therapy. View 2(2):20200042

Yi J, Choe G, Park J, Lee JY (2020) Graphene oxide-incorporated hydrogels for biomedical applications. Polym J 52(8):823–837

Yuan L, Fan W, Han L, Guo C, Yan Z, Zhu M, Mo X (2018) Evaluation of hydrogels for soft tissue adhesives in vitro and in vivo analyses. Front Mater Sci 12(1):95–104

Zarubova J, Zhang X, Hoffman T, Hasani-Sadrabadi MM, Li S (2021) Biomaterial-based immunoengineering to fight COVID-19 and infectious diseases. Matter 4(5):1528–1554. https://doi.org/10.1016/j.matt.2021.02.025

Zhang P, Wang L, Yang S, Schott JA, Liu X, Mahurin SM, Huang C, Zhang Y, Fulvio PF, Chisholm MF, Dai S (2017) Solid-state synthesis of ordered mesoporous carbon catalysts via a mechanochemical assembly through coordination cross-linking. Nat Commun 8(1):15020

Zhang X, Tan B, Wu Y, Zhang M, Liao J (2021) A review on hydrogels with photothermal effect in wound healing and bone tissue engineering. Polymers 13(13):2100

Zhou H, Li G, Wang X, Jin C, Chen Y (2009) Preparation of a kind of mesoporous carbon and its performance in adsorptive desulfurization. J Nat Gas Chem 18(3):365–368

Zhou J, Ventura CJ, Fang RH, Zhang L (2021) Nanodelivery of STING agonists against cancer and infectious diseases. Mol Asp Med:101007

Part IV

Nanotechnology and Drug Resistance

Nanotechnology and Multidrug Resistance 14

Insha Nahvi, Irum Nahvi, and Suriya Rehman

Abstract

Antibiotics play an important role in fighting against deadly diseases. However, the development of multidrug resistance among microbes has raised public health concerns around the globe and this is due to the improper use of antibiotics. These health crises need new strategies and development of new drugs to overcome multidrug resistance in microorganisms. Nano particles (NPs) are the powerful strategy to manage infections caused by multidrug resistant organisms (MDROs). Variety of nanomaterials (NMs) are being designed and used to combat the alarming situation of multidrug resistance (MDR) among microbes. Some of them are polymeric nanomaterials, liposomes, micelles, ferritin, metallic NPs, solid lipid nanoparticles, and many more. These nanomaterials with updated physiochemical properties provide a useful platform in forming agents against microbes thus providing a better solution for bacterial resistance. NPs use numerous mechanisms like CHT-NMs, metal oxide NMs, etc. to bypass the progress of resistance by microorganisms. In addition, the combination of NPs and antibiotics has prevented the emergence of resistance by bacteria.

I. Nahvi (✉)
Department of Basic Sciences, Preparatory Year Deanship, King Faisal University, Al Hofuf, Saudi Arabia

Amity Institute of Biotechnology, Amity University, Manesar, Haryana, India
e-mail: iahmad@kfu.edu.sa

I. Nahvi
College of Computer Engineering and Science, Prince Mohammad Bin Fahd University, Al Khobar, Saudi Arabia

S. Rehman
Department of Epidemic Diseases Research, Institute for Research and Medical Consultations (IRMC), Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia

S. Hameed, S. Rehman (eds.), *Nanotechnology for Infectious Diseases*,
https://doi.org/10.1007/978-981-16-9190-4_14

Keywords

Antibiotics · Bactericidal · Biofilms · Multidrug Resistance · Nanomaterials · Nanotechnology

14.1 Introduction

Antibiotics are also called as antimicrobials as they are the powerful medications that fight infection caused by bacteria by either stopping them from reproducing or by destroying them. Antibiotics hinder the genetic flow, i.e., DNA to RNA to protein synthesis, biofilm formation, and obstruction of synthesis of cell wall in bacteria. Penicillin was the first antibiotic to be discovered by scientists and penicillin-based antibiotics like amoxicillin and ampicillin are still being used to treat against a variety of bacterial infections. Nevertheless, a major growing concern nowadays which is turning out to be a public health threat is the development of resistance against antibiotics. Antibiotics that were earlier termed as miracle drugs are now losing their effectiveness. The excessive and inappropriate use of antibiotics has accelerated their emergence of resistance and consequence of this is the proliferation of pathogenic bacteria that are resistant to multiple drugs (Singh et al. 2014).

Nanotechnology is a promising platform that is used now a days against multidrug resistance of various infection causing microorganisms like bacteria. Multidrug resistance (MDR), also known as pleiotropic drug resistance is a situation where one agent confers resistance to that drug and other drugs of the same class even it causes resistance to several other unrelated agents (Markman et al. 2013). *Staphylococcus aureus* strains have been found resistant to methicillin. Similarly, *E. coli, Enterococcus, Enterobacteriaceae, Pseudomonas, and Acinetobacter* have gained resistance towards different antibiotics (Rehman et al. 2019a, b).

Many factors are responsible for MDR. Some of them are DNA repair mechanism, overexpression of efflux transporters, upregulation of enzymatic repair systems, reshuffling of the genetic code, etc. (Yanga et al. 2016). Bacteria continually evolve by mutating with all these mechanisms. MDR has imposed a major threat even in cancer therapy although variety of ways have been developed to overcome this issue. Few such methods include protein kinase C inhibitor, P-glycoprotein inhibitor, etc. but due to high dosage administration, it caused many side effects in patients that were sometimes lethal as well (Al-Jameel et al. 2021). To confront such a variation between antibiotics and bacterial evolution, nanomaterials were the best solution for the researchers (Muzammil et al. 2018). Nanotechnology provides an interesting action plan to alter physico-chemical properties of materials as compared to their bulk counterpart that can be employed for bioapplications (Nahvi et al. 2021; Rehman et al. 2021). Variety of nanoparticles have been proved to be an alternative to antibiotics and helped to control multidrug resistant organism infections. Nano particles (NPs) are the powerful strategy to manage infections caused by MDROs. Variety of nanomaterials (NMs) are being designed and used to combat this alarming situation of MDR. Some of them are polymeric nanomaterials, liposomes, micelles,

ferritin, metallic NPs, solid lipid nanoparticles, and many more. These nanomaterials with updated physiochemical properties provide a useful platform in forming agents against microbes thus providing a better solution for bacterial resistance. Nanomaterials have different ways of being bactericidal. Some NPs directly get attached to cell wall of microbes while others like metal oxides work through reactive oxygen species (ROS) (Schröfel et al. 2014). Some NMs are environment friendly as well. They disrupt the membrane of microorganisms and make them favorable against drug resistant bacteria by various methods like chemical conjugation, physical encapsulation, adsorption (Zhang et al. 2010).

14.2 Mechanism of Bacterial Antibiotic Resistance

(1) Intrinsic/Natural Mechanism. (2) Acquired Mechanism.

14.2.1 Intrinsic/Natural Mechanism

It is an inherited resistance that includes the low-affinity targets, less cell permeability, antibiotic inactivation, and the appearance of efflux mechanisms. It is a natural resistance of a bacterial species.

14.2.2 Acquired Mechanism

This mechanism is acquired when mutation in the genes that are targeted by antibiotics occurs. Sometimes it happens due to the transfer of resistance determinants via plasmids, bacteriophages, etc. (Alekshun and Levy 2007). Various processes are involved to accomplish this exchange. Some of them are conjugation via plasmids, transformation via incorporation into the chromosome of chromosomal DNA and transduction via bacteriophages (Rehman et al. 2019a, b).

To study the progression of resistance to antibiotics, the microorganism starts its gene expression followed by choice of resistance gene that is expressed. Transduction, conjugation, and transformation occur for the transfer of this gene and this microorganism develops resistance against the drugs. The microorganism that has more resistance gene results in MDR. When microbe is exposed to the specific drug, only then that resistance gene is expressed (Davies and Davies 2010).

14.2.3 Efflux Mechanism

Elevated efflux of drugs and decreased uptake of drugs is one of the reasons of MDR. There are some bacteria like *P. aeruginosa* with a thin layer of peptidoglycan inside the cell wall and in the periplasmic space their inner membrane protein is linked to a linker protein. Regulatory protein coding for efflux protein suppresses the

gene that results in mutation of this regulatory protein thereby causing overexpression of efflux protein and MDR of *P. aeruginosa* (Nikaido 2009). A similar kind of mechanism occurs in *E. coli* where many antibiotics are expelled out and thus causing antibiotic resistance in *E. coli*.

14.2.4 Substrate Modification

Modification of substrate also causes MDR by expressing the resistance gene to which the drug usually binds. The binding affinity of the drug to modified substrate is lower as compared to the original substrate. One such resistance gene, i.e. Mec A resistance gene causes resistance against beta lactams. This resistance gene codes for modified penicillin binding protein (PBP) which in turn has a low binding affinity to beta lactams resulting in tolerance against them (Alkharsah et al. 2018; Alkharsah et al. 2019). Similarly VanA resistance gene causes resistance against vancomycin antibiotic. In the same way, quinolone resistance is caused due to modified DNA gyrase or topoisomerase IV (Zignol et al. 2018).

14.2.5 Bacterial Biofilms

Formation of biofilms causes chronic bacterial infections. Bacteria that form biofilm show more tolerance to antibiotics in comparison to bacteria that do not form biofilm

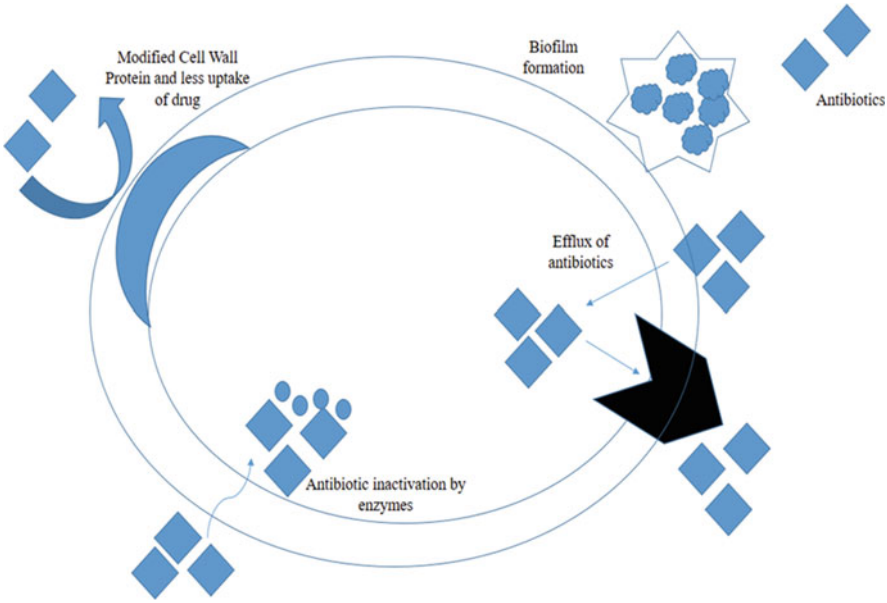

Fig. 14.1 Antibiotic resistance by bacteria

(Zhang et al. 2022). Mechanism of antibiotic resistance in bacteria is shown in Fig. 14.1.

14.3 Mode of Action of Nanomaterials to Battle Against Microbial Antibiotic Resistance

Different mechanisms exist in NMs to fight microbial resistance. For example, metallic nanomaterials, nitric oxide releasing NMs, and chitosan derived NMs use numerous processes to block the progression of pathogen resistance (Liu et al. 2019) (Fig. 14.2). NMs possess antimicrobial activities that help in overcoming resistance mechanisms like enzyme inactivation, enzyme modification, cell membrane disruption, triggering of host immune response, prevention of biofilm formation, etc. (Lee et al. 2019). NPs now known as next generation antibiotics play a significant role in health care system. NPs have gained importance even as NP-based drug delivery system. One of the best example of such system is use of NP coating on implants devices, dressing used for wounds, dental, and bone cement materials (Wang et al. 2017).

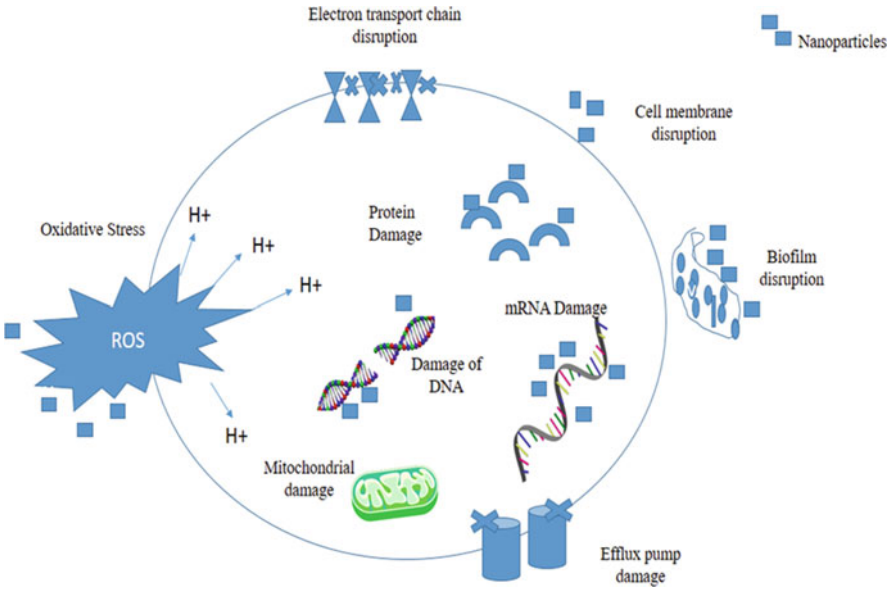

Fig. 14.2 Mechanism of antimicrobial activities by nanoparticles

14.3.1 Metallic Nanomaterials (Metallic NMs)

Variety of NMs with variety of mechanisms are being used to prevent bacterial resistance pattern (Qureshi et al. 2021). They not only prevent bacterial resistance but also play an important role in MDR in anticancer therapies. Some of these are NMs of gold (Au), silver (Ag), aluminum oxide (Al_2O_3), titanium (Ti), magnesium (Mg), bismuth (Bi), zinc oxide (ZnO), and many more.

14.3.1.1 Gold Nanoparticles (Au-NPs)

Au-NPs alone do not possess antimicrobial activity but it works when conjugated with antibiotic and other antimicrobial substances (Feng et al. 2016). Au-NPs with size of 1–100 nm target *methicillin resistant staphylococcus aureus* (MRSA) using different mechanisms like generating holes in the wall of the cell reduced ATPase activity, disruption of the respiratory chain, membrane disruption, etc. (Zaidi et al. 2017). Kanamycin and levofloxacin upon conjugation with Au-NMs exhibited improved antimicrobial activity (Bagga et al. 2017). Due to some unknown methods like endocytosis (in those bacteria who do not have ability of endocytosis) and drug outflow (Au-AMP-NPs hinders transmembrane pump), the effect of Au-NPs is questionable. To overcome these issues, functionalization of Au-NMs is enabled which has proved to be a competent tool in penetrating cell membranes of bacteria. One such example is Au-NMs functionalized with vancomycin (Van) against *Vancomycin resistant enterococci* (Gu et al. 2003). Au-NPs can also be used as a delivery platform for cancer drugs. This drug delivery was developed by binding doxycycline (DOX) on Au-NPs surface via acid labile linkage (Wang et al. 2011a, b). When compared to free DOX, this study found that the medication was retained and accumulated more in cancer cells.

14.3.1.2 Silver Nanoparticles (Ag-NPs)

Ag-NPs have been studied extensively as they have multiple mechanisms of bactericidal action with upgraded biocompatibility and functionalized potential (Baranwal et al. 2018). Silver when mixed in aqueous solution forms silver ions (Ag+) and has the antimicrobial action. Due to the positive charge on Ag, Ag ions bind to the negative parts (sulfur and phosphate) of cell membrane of bacteria and create a hole resulting in cytoplasm content of the cell to spread. This may lead to the cell death. Otherwise, Ag+ ions penetrate cytoplasm via cell membrane that can lead to stronger action by Ag+ ions against bacteria. Having a thin cell wall, gram-negative bacteria may be more sensitive to Ag+ ions. But due to this, they become more vulnerable to Ag+ ions as they may easily attach to LPS. Thus, due to bounding of Ag+ ion and LPS, there are less chances of Ag+ ions to penetrate gram-negative bacteria (Acharya et al. 2018). According to Song et al., it was reported that silver nanoparticle interacts with thiol groups of some enzymes and DNA (because it contains phosphorus) causes death of cell by preventing replication of DNA and cell division (Song et al. 2019). Ag-NPs exhibit effective bactericidal activity on *M. tuberculosis,* multidrug resistant, extensive drug resistant, and other *mycobacterium* strains (Agarwal et al. 2013). Furthermore, with the help of bioreduction process by

Aspergillus flavus, Ag-NPs can be more bactericidal against MDR strains of *Klebsiella pneumonia, Pseudomonas aeruginosa, and Escherichia coli (*Ninganagouda et al. 2013).

Ag-NMs in combination with drugs (e.g., penicillin, clindamycin, and erythromycin) boost the performance of drugs against bacteria like *staphylococcus aureus and E. coli.* Some researchers loaded silver nanoparticles with antibiotics and that resulted in growth inhibition of *P. aeruginosa, S. aureus,* and *Micrococcus* spp. Wang et al. studied the consequence of Ag-NMs combined with an antibiotic like levofloxacin and came to the conclusion that it shows synergistic action in animal study (Wang et al. 2016). According to Mottais et al.., nano-articulated complexes of silver carbene show unfavorable effects on MDR bacteria like *P. aeruginosa, K. pneumoniae,* and MRSA (Mottais et al. 2019). According to one more study Ag-NPs are known to have an antiviral action against hepatitis B virus and HIV-I (Rai et al. 2016). Bimetallic NPs are stable and have also gained importance. Ag-Au NPs when functionalized with tetracycline impose a synergetic effect in turn attributing to generation of reactive oxygen species (ROS) (Fakhri et al. 2017). Analysis via transmission electron microscope and proteomics explained that Ag-NPs damage cell membrane and collapse proton motive force that in turn results in bacterial death (Rehman et al. 2020). According to other study, disruption of respiration of *E. coli* cell by copper and Ag-NPs was caused due to decrease in efflux pump activity (Christena et al. 2015).

14.3.1.3 Metal Oxide NPs

Various metal oxides like Zinc Oxide (ZnO), Nitric Oxide (NO), Magnesium Oxide (MgO), Titanium oxide (TiO_2) and Aluminum Oxide (Al_2O_3) have been reported to have antibactericidal properties. Most commonly, it is their photocatalytic activity with wide band gap.

14.3.1.4 Zinc Oxide Nanomaterials (ZnO NMs)

ZnO nanoparticles have the ability to produce reactive oxygen species (ROS) and react with DNA and RNA (Ghasemi and Jalal 2016). ZnO-NPs overcome bacterial resistance as it is shows synergistic effects with various antibiotics like amoxicillin, gentamicin, vancomycin, erythromycin, clindamycin, tetracycline, etc. But these nanoparticles sometimes decrease the antibacterial activity of some antibiotics while it increases the antibacterial activity of others. One of the reasons that scientists proposed was that ZnO NPs block the efflux pump to overcome resistance towards these antibiotics in some bacteria. Also, ZnO reacts with membrane proteins that are responsible for the restriction in the insertion of quinolones to the membrane which causes an increase in antibiotic absorption in the cell (Banoee et al. 2010; Akhtar et al. 2020).

Various mechanisms are being used by ZnO-NPs to tackle resistance of bacteria. Some of them are as follows:

(a) Causing the rupture of the cells of bacteria by producing ROS and Zn^{2+} ions with hydrogen peroxide.

(b) By creating oxidative stress when covered with polyvinyl alcohol.
(c) ZnO-NPs destroy some molecules of the bacterial membrane that results in high penetrability of membrane and release of cytoplasmic substances from the cell and finally causing death of the cell (Siddiqi et al. 2018).

The toxic nature of ZnO-NPs is concentration dependent. At low concentration, they are mildly toxic. According to one of the investigations done by Raghupatti et al., ZnO-NPs have shown bactericidal properties against bacteria. In this study it was found that growth of *Enterococcus faecalis, MRSA,* and *Staphylococcus epidermidis* was inhibited by colloidal suspension of ZnO (Raghupathi et al. 2011).

14.3.1.5 Nitric Oxide Nanoparticles (NO-NPs)

One of the mechanisms used by NO-NPs to react against bacteria is reactive nitrogen oxide species production (RNOS) like S-nitrosothiols, peroxynitrite, and nitrogen dioxide. RNOS damages bacteria by interacting with their proteins. Tyrosyl radicals, sulfur-iron combination, amines, phenolic amino acids, heme, and reactive thiols are all involved in NO interactions with proteins. Peroxynitrite and NO2 oxidize proteins in a variety of ways that are nonspecific (Singh et al. 2014).

RNOS also inhibits DNA repair enzymes of bacteria. Even low concentrations of NO-NPs can exhibit antibacterial activity against many multiresistant bacteria. NO-NPs act against bacteria due to their significant broad spectrum antimicrobial activity and by releasing nitric oxide, these NPs can change the integrity of proteins and lipids of bacterial plasma membrane (Hajipour et al. 2012). MDR displayed by microbial species might be effectively combated using efficient NO releasing NPs. Using soft tissue models, it was investigated that NO-NPs can hamper the immune responses that aid in the reduction of bacterial load. Also, NO-NPs treatment resulted in healing by induction of collagen deposition.

14.3.1.6 Magnesium Oxide Nanoparticles (MgO-NPs)

MgO-NPs cause the bacterial cell death by stimulating ROS formation. The efficacy of MgO-NPs in antibacterial activity increases when halogens like Cl_2, Br_2, F_2 get adsorbed to these NP. Nanoparticles of $MgCl_2$, $MgBr_2$, MgF_2 show bactericidal and anti-biofilm activity against *Bacillus subtilis* and *S. aureus.* Several antimicrobial methods used by MgO-NPs are explained below:

1. Mg-halogen can cause lipid peroxidation in microbial cell membranes by triggering the generation of reactive oxygen species (ROS), hence damaging the cytoplasmic cell content outside.
2. Certain bacterial enzymes are inhibited by metal halide compounds.
3. MgF_2-NMs cause peroxidation of lipids traveling across the microbial cell membrane thus lowering the pH of the cytoplasm and increasing the membrane's potential. MgF_2-NPs inhibit the formation and growth of biofilms of *E. coli* and *S. aureus*.
4. MgO activity against microorganisms is based on the adsorption of halogen molecules on its surface. There is a fivefold increase in the amount of halogen

molecules adsorbed in MgO when MgO is packed in MgO-NM, enhancing the microbicide effect of halogens (Munir et al. 2020).

14.3.1.7 Titanium Dioxide Nanoparticles (TiO_2-NPs)

Using two mechanisms against bacteria by TiO_2-NPs, the chances of developing resistance to these NPs become low. These two mechanisms are as follows:

1. Photocatalysis
2. Absence of irradiation

Photocatalysis helps by rupturing the bacterial cell membrane because of ROS (containing OH and H_2O_2 radicals) produced by TiO_2 (Huang et al. 2016; Venkatasubbu et al. 2016).

TiO_2-NMs can be combined with other materials to create antibacterial composites. Numerous bacteria like *E. coli, P. aeruginosa, S. aureus, Enterococcus faecium*, and *Candida albicans* are attacked and killed by TiO_2-NMs (Liu et al. 2017; Chen et al. 2014).

Other metal oxide-NPs like copper oxide (CuO-NPs) also exhibit bactericidal action although it is weaker if compared with others like Ag-NPs but they also have microbicidal action towards fungi like *Saccharomyces cerevisiae.* Microbes like *Listeria monocytogenes, S. aureus* are affected by CuO-NPs and their action depends on form and concentration (activity increases by higher doses of Cu-NPs) (Laha et al. 2014). One of the metallic nanoparticles that may raise the chances of developing multidrug resistance is aluminum oxide nanoparticles (Al_2O_3-NPs) although they also render toxic effects to E. coli. This might be due to oxidative destruction of the bacterial cell membrane caused by Al_2O_3-NPs and increased gene expression that promotes conjugation and decreased expression of genes that prohibit conjugation are both caused by Al_2O_3-NPs (Qiu et al. 2012).

14.3.2 Chitosan Based Nanomaterials (CHT-NMs)

Chitosan is a polysaccharide derived from chitin. At acidic pH, chitosan carries a positive charge due to which it can damage the bacterial cell wall with a negative charge. This causes osmotic damage, increased penetrability through microbe cell envelopes, and apparent movement of cytoplasm contents, such as proteins and ions. The antimicrobial activity by CHT-NMs in both fungal and bacterial cells is by interference with mRNA transcription leading to inhibition of translation of their proteins. Antibiotics like doxycycline and acetic acid were found to be more effective against *E. coli* and *S. aureus* when NMs were encapsulated with chitosan. In a study of the antimicrobial action of chitosan (polymeric beta-1, 4-N-acetylglucosamine) on *E. coli*, *E. coli* was shown to be more vulnerable to the inhibitory effects of dyes and bile acids used in selective medium but *S. typhimurium* highly cationic mutants were shown to be more resistant to chitosan than the parent mutants. Under electron microscopy, chitosan causes alterations in

the cell surface resulting in binding of NPs to the outer membrane of cell thus explaining the loss of barrier function (Ma et al. 2017). CHT-NMs with high molecular mass are effective in gram-positive bacteria and low molecular mass CHT-NMs are more effective in gram-negative bacteria. But CHT shows more efficacy against gram-negative bacteria due to its envelope's negative charge. The CHT amino group displaces the Mg^{++} and Ca^{++} ions that are responsible for gram-negative bacteria lipopolysaccharide (LPS) stability and coordination (Wang et al. 2011a, b).

14.4 Resistance to Nanoparticles and Multidrug Loading

Antibiotics conjugated with nanoparticles are the best option to combat resistance. This option of combining nanoparticles with antibiotics has widened the avenue of probing the impact of this combination on many pathogens. As nanoparticles have variety of mechanisms to attack bacteria, their conjugation with antibiotics allows for recovery of antimicrobial efficacy. Incorporation of multiple drugs in the same NP may give better results. Vancomycin when coated with chitosan NMs has known to be active against *Vancomycin resistant staphylococcus aureus* (VRSA) and vancomycin coated with Au-NM has been found to be active against VRSA and *E.coli* both with a high fold when compared with vancomycin alone (Payne et al. 2016; Xu et al. 2015). According to one of the researches, CHT when loaded with Ag-NMs (CHT@Ag-NMs) stops the growth of many pathogens like A. *baumannii, Proteus mirabilis, P. aeruginosa,* and *MRSA*. When CHT and Ag-NMs were used alone, their antimicrobial action was significantly low as compared with CHT@Ag-NMs conjugation. The reason behind it is that CHT makes it easy for Ag-NMs to enter into the cells to induce high bactericidal action (Yoksan and Chirachanchai 2010). In a similar way, nanomaterials of TiO_2 and Ag when combined together (TiO_2-Ag-NMs) showed more efficacy against *Aspergillus* and *C. albicans* (Huh and Kwon 2011).

Resistance of bacteria towards nanoparticles is a major concern. This resistance might be caused due to the outer membrane permeability changes and increased expression of efflux pumps. *K. pneumoniae* and *Enterobacter cloacae* developed resistance against Ag-NPs in burn cases (Finley et al. 2015). Resistance may be due to the genetic changes in bacteria.

14.5 Software Tool, Machine Learning, and Nanotechnology in Prediction of Antimicrobial Resistance

To recognize drug resistant genes in bacteria, machine learning approach can be utilized (https://www.sciencedaily.com/releases/2020/07/200706140843.htm n.d.). Considering pathogens that are unable to culture in the lab, such approaches are supportive. A simple program has been developed by Researchers in Washington State University to recognize fatal drug-resistant genes in bacteria. Applying the

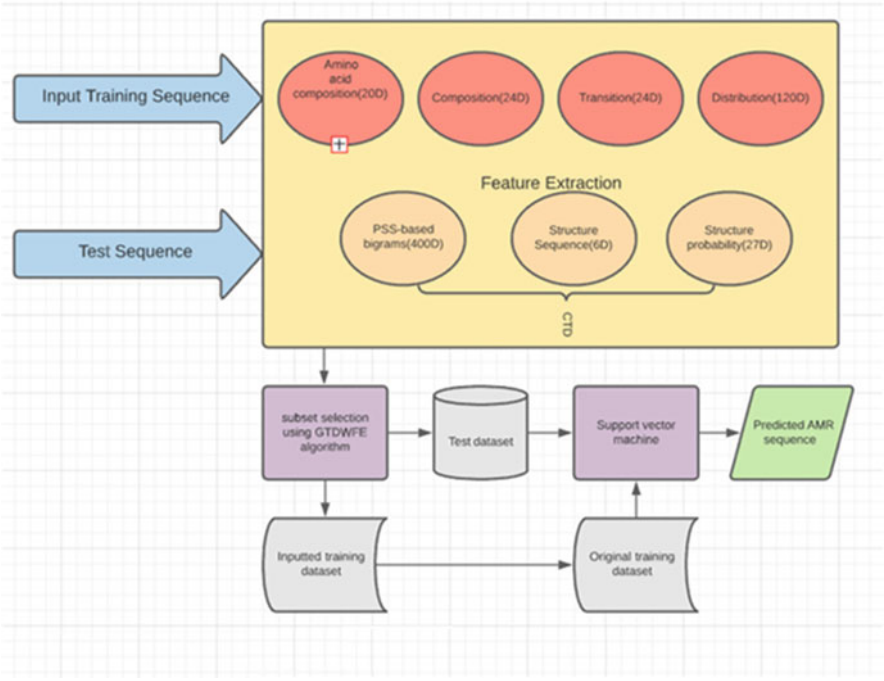

Fig. 14.3 The components of PARGT

game theory feature and machine learning algorithm, the research workers recognized antimicrobial resistance genes in gram-negative bacteria. However, gram-positive bacteria expressed accuracies up to 90% for genes encoding reluctance to the antibiotics, vancomycin and bacitracin. As long as more useful data will be accessible, improvements can be made to the software (Chowdhury et al. 2020). SVM machine learning model was trained to use the GTDWFE algorithm and was then used for prediction. In PARGT as shown in Fig. 14.3, the SVM was adapted by inputting the training data set. Thus chose the best support vector model to foresee the antimicrobial resistance proteins in the test-sequences. Different operating systems like MAC and Windows can adapt PARGT. PARGT is an open source application software and is created by using R and Python. To increase the efficiency, execution time is reduced by using the UniProt database. This database contains 538,585 protein sequences to create Position Specific Scoring Matrix (PSSM) and secondary structure features (Zhang et al. 2017; Jones 1999). Due to multidrug resistance (MDR), the unspecific drug distribution, and the systematic variability of cancer, the potentiality of chemotherapy in conjunction with nanotechnology has escalated the therapeutic effectiveness. Integration of diagnosis with treatment process and nano-theranostics can accomplish real time track of tumor and precise pre-detection (Wu et al. 2014; Pérez-Herrero and Fernández-Medarde 2015).

14.6 Theranostic Nanoparticles Build on Multimodal Imaging and Combination Therapy

Some of the imaging procedures are tomography (PET/CT), photoacoustic imaging (PA), computed tomography (CT), ultrasound imaging (USI), magnetic resonance imaging (MRI), single photon emission computed tomography (SPECT), and positron emission computed fluorescence imaging (FI) (Hou et al. 2009; Pelaz et al. 2017; Elgqvist 2017). In the emergence of nanotechnology, integration of imaging modalities has not only amplified the correctness of images but its details too. (Deng et al. 2017) (Liang et al. 2016) (Luo et al. 2016) (Lv et al. 2017) (Yan et al. 2016) (Park et al. 2011). In contrast to traditional clinic application, theranostic nanoparticles in combination with diagnostic agents and chemo-based therapy can identify the condition of the disease and with real time observation of drug pharmacokinetics provides therapeutic agents to pick out sites in control.

14.6.1 Prediction of Antibacterial Resistance in *Pseudomonas Aeruginosa* with Machine Learning Entitled Molecular Conjecture

Multiple random samples (genomic and transcriptomic data) from isolate collection were used to be trained by SVM build susceptibility/resistance classifier to ciprofloxacin, meropenem, tobramycin, and ceftazidime. The evaluation was repeated 10 times in rotation estimation technique to access the data set. SVM predicted some samples correctly and some were misclassified. One and all drugs, isolates were allocated indiscriminately to an input training sequence that incorporates 80% of the susceptible/resistant isolates and to a test set is 20% which is outstanding. Machine learning outcome can be dominated by the bacterial population composition; therefore, rotation estimation technique's execution was compared to logistic regression (logistic regression: 0.84 _ 0.06 vs macro F1-score for the SVM: 0.83 _ 0.06). Significantly one step ahead than random forest classifiers. The execution on extended data set was in considerable range (random forest 0.71 _ 0.16; logistic regression: 0.90 _ 0.04; SVM: 0.87 _ 0.07) (Khaledi et al. 2020).

14.7 Conclusion

Antibiotic resistance, a global threat, is evolving due to the lack of antibacterial agents and emergence of multidrug resistant bacteria. Various resistance mechanisms have been adapted by bacteria such as higher efflux pump and reduced drug uptake, biofilm formation, antibiotic inactivation and antibiotic modification, drug resistance gene modification, etc. In the era of antibiotic resistance, nanomaterials may become a foundation for treating bacterial infections with sustained research and development. Nanomaterials have played an important role in overcoming these bacterial antibiotic resistance mechanisms. They use numerous

mechanisms like CHT-NMs, metal oxide NMs, etc. to bypass the progress of resistance by microorganisms. Also, the combination of NPs and antibiotics has prevented the emergence of resistance by bacteria.

Acknowledgement Authors extend their acknowledgment to the Deanship of Scientific Research at King Faisal University, Al Hofuf, Saudi Arabia under project number: GRANT341 for financial support.

References

Acharya D, Singha KM, Pandey P, Mohanta B, Rajkumari J, Singha LP (2018) Shape dependent physical mutilation and lethal effects of silver nanoparticles on bacteria. Sci Rep 8(1):201. https://doi.org/10.1038/s41598-017-18590-6

Agarwal P, Mehta A, Kachhwaha S, Kothari SL (2013) Adv Sci Eng Med 5:709

Akhtar S, Rehman S, Asiri SM et al (2020) Evaluation of bioactivities of zinc oxide, cadmium sulfide and cadmium sulfide loaded zinc oxide nanostructured materials prepared by nanosecond pulsed laser. Mater Sci Eng C Mater Biol Appl 116:111156. https://doi.org/10.1016/j.msec.2020.111156. PMID: 32806284

Alekshun MN, Levy SB (2007) Cell 128:1037

Al-Jameel SS, Rehman S, Almessiere MA, Khan FA, Slimani Y, Al-Saleh NS, Manikandan A, Al-Suhaimi EA, Baykal A (2021) Anti-microbial and anti-cancer activities of Mn0. 5Zn0. 5DyxFe2-xO4 ($x \leq 0.1$) nanoparticles. Artif Cells Nanomed Biotechnol 49(1):493–499

Alkharsah KR, Rehman S, Alkhamis F, Alnimr A, Diab A, Al-Ali AK (2018) Comparative and molecular analysis of MRSA isolates from infection sites and carrier colonization sites. Ann Clin Microbiol Antimicrob 17(1):1–1

Alkharsah KR, Rehman S, Alnimr A, Diab A, Hawwari A, Tokajian S (2019) Molecular typing of MRSA isolates by spa and PFGE. J King Saud Univ Sci 31(4):999–1004

Bagga P, Hussain Siddiqui H, Akhtar J, Mahmood T, Zahera M, Sajid Khan M (2017) Gold nanoparticles conjugated levofloxacin: for improved antibacterial activity over levofloxacin alone. Curr Drug Deliv 14(8):1114–1119. https://doi.org/10.2174/1567201814666170316113432

Banoee M et al (2010) ZnO nanoparticles enhanced antibacterial activity of ciprofloxacin against *Staphylococcus aureus* and *Escherichia coli*. J Biomed Mater Res B Appl Biomater 93B(2):557–561

Baranwal A, Srivastava A, Kumar P, Bajpai VK, Maurya PK, Chandra P (2018) Prospects of nanostructure materials and their composites as antimicrobial agents. Front Microbiol 9:422. https://doi.org/10.3389/fmicb.2018.00422

Chen S, Guo Y, Zhong H et al (2014) Synergistic antibacterial mechanism and coating application of copper/titanium dioxide nanoparticles. Chem Eng J 256:238–246. https://doi.org/10.1016/j.cej.2014.07.006

Chowdhury AS, Call DR, Broschat SL (2020) PARGT: a software tool for predicting antimicrobial resistance in bacteria. Sci Rep 10:11033. https://doi.org/10.1038/s41598-020-67949-9

Christena LR et al (2015) Copper nanoparticles as an efflux pump inhibitor to tackle drug resistant bacteria. RSC Adv 5(17):12899–12909

Davies J, Davies D (2010) Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev 74(3):417–433. https://doi.org/10.1128/MMBR.00016-10

Deng L, Cai X, Sheng D, Yang Y, Strohm EM, Wang Z, Ran H, Wang D, Zheng Y, Li P (2017) A laser-activated biocompatible theranostic nanoagent for targeted multimodal imaging and photothermal therapy. Theranostics 7:4410–4423

Elgqvist J (2017) Nanoparticles as theranostic vehicles in experimental and clinical applications-focus on prostate and breast cancer. Int J Mol Sci 18:1102

Fakhri A, Tahami S, Naji M (2017) Synthesis and characterization of core–shell bimetallic nanoparticles for synergistic antimicrobial effect studies in combination with doxycycline on burn specific pathogens. J Photochem Photobiol B 169:21–26. https://doi.org/10.1016/j.jphotobiol.2017.02.014

Feng Y, Chen W, Jia Y et al (2016) N-heterocyclic molecule-capped gold nanoparticles as effective antibiotics against multi-drug resistant bacteria. Nanoscale 8(27):13223–13227. https://doi.org/10.1039/C6NR03317B

Finley PJ, Norton R, Austin C, Mitchell A, Zank S, Durham P (2015) Unprecedented silver resistance in clinically isolated Enterobacteriaceae: major implications for burn and wound management. Antimicrob Agents Chemother 59:4734–4741. https://doi.org/10.1128/AAC.00026-15

Ghasemi F, Jalal R (2016) Antimicrobial action of zinc oxide nanoparticles in combination with ciprofloxacin and ceftazidime against multidrug-resistant *Acinetobacter baumannii*. J Glob Antimicrob Resist 6:118–122

Gu H, Ho PL, Tong E, Wang L, Xu B (2003) Presenting vancomycin on nanoparticles to enhance antimicrobial activities. Nano Lett 3(9):1261–1263. https://doi.org/10.1021/nl034396z

Hajipour MJ et al (2012) Antibacterial properties of nanoparticles. Trends Biotechnol 30(10):499–511

Hou AH, Swanson D, Barqawi AB (2009) Modalities for imaging of prostate cancer. Adv Urol 2009:818065

https://www.sciencedaily.com/releases/2020/07/200706140843.htm (n.d.)

Huang Y-Y, Choi H, Kushida Y, Bhayana B, Wang Y, Hamblin MR (2016) Broad-spectrum antimicrobial effects of photocatalysis using titanium dioxide nanoparticles are strongly potentiated by addition of potassium iodide. Antimicrob Agents Chemother 60(9):5445–5453. https://doi.org/10.1128/AAC.00980-16

Huh AJ, Kwon YJ (2011) "Nanoantibiotics": a new paradigm for treating infectious diseases using nanomaterials in the antibiotics resistant era. J Control Release 156(2):128–145. https://doi.org/10.1016/j.jconrel.2011.07.002

Jones DT (1999) Protein secondary structure prediction based on position-specific scoring matrices. J Mol Biol 292:195–202

Khaledi A, Weimann A, Schniederjans M, Asgari E, Kuo T-H, Oliver A, Cabot G, Kola A, Gastmeier P, Hogardt M, Jonas D, Mofrad MRK, Bremges A, McHardy AC, Häussle S (2020) Predicting antimicrobial resistance in Pseudomonas aeruginosa with machine learning-enabled molecular diagnostics. EMBO Mol Med 12(3):e10264

Laha D, Pramanik A, Laskar A, Jana M, Pramanik P, Karmakar P (2014) Shape-dependent bactericidal activity of copper oxide nanoparticle mediated by DNA and membrane damage. Mater Res Bull 59:185–191. https://doi.org/10.1016/j.materresbull.2014.06.024

Lee N-Y, Ko W-C, Lee N-Y, Ko W-C, Hsueh P-R (2019) Nanoparticles in the treatment of infections caused by multidrug resistant organisms. Front Pharmacol 10:1153. https://doi.org/10.3389/fphar.2019.01153

Liang C, Kamkaew A, Sun H, Jiang D, Valdovinos HF, Hua G, England CG, Goel S, Barnhart TE, Cai W (2016) Dual-modality positron emission tomography/optical image-guided photodynamic cancer therapy with Chlorin e6-containing nanomicelles. ACS Nano 10:7721–7730

Liu N, Chang Y, Feng Y et al (2017) {101}–{001} Surface heterojunction-enhanced antibacterial activity of titanium dioxide nanocrystals under sunlight irradiation. ACS Appl Mater Interfaces 9(7):5907–5915. https://doi.org/10.1021/acsami.6b16373

Liu Y, Shi L, Su L et al (2019) Nanotechnology-based antimicrobials and delivery systems for biofilm-infection control. Chem Soc Rev 48(2):428–446. https://doi.org/10.1039/C7CS00807D

Luo Z, Zheng M, Zhao P, Chen Z, Siu F, Ping G, Gao G, Sheng Z, Zheng C, Ma Y (2016) Self-monitoring artificial red cells with sufficient oxygen supply for enhanced photodynamic therapy. Sci Rep 6:23393

Lv R, Yang P, Chen G, Gai S, Xu J, Prasad PN (2017) Dopamine-mediated photothermal theranostics combined with up-conversion platform under near infrared light. Sci Rep 7:13562

Ma Z, Garrido-Maestu A, Jeong KC (2017) Application, mode of action, and in vivo activity of chitosan and its micro-and nanoparticles as antimicrobial agents: a review. Carbohydr Polym 176:257–265. https://doi.org/10.1016/j.carbpol.2017.08.082

Markman JL, Rekechenetskiy A, Holler E, Ljubimova JY (2013) Nanomedicine therapeutic approaches to overcome cancer drug resistance. Adv Drug Deliv Rev 65:1866–1879

Mottais A, Berchel M, Le Gall T, Sibiril Y (2019) Antibacterial and transfection activities of nebulized formulations incorporating long n-alkyl chain silver n-heterocyclic carbene complexes. Int J Pharm 567:118500. https://doi.org/10.1016/j.ijpharm.2019.118500

Munir MU, Ahmed A, Usman M, Salman S (2020) Recent advances in nanotechnology-aided materials in combating microbial resistance and functioning as antibiotics substitutes. Int J Nanomedicine 15:7329–7358

Muzammil S, Hayat S, Fakhar EAM, Aslam B, Siddique MH, Nisar MA et al (2018) Nanoantibiotics: future nanotechnologies to combat antibiotic resistance. Front Biosci (Elite Ed) 10:352–374. https://doi.org/10.2741/e827

Nahvi I, Belkahla S, Asiri SM, Rehman S (2021) Overview and prospectus of algal biogenesis of nanoparticles. In: Microbial nanotechnology: green synthesis and applications. Springer, Singapore, pp 121–134

Nikaido H (2009) Multidrug resistance in bacteria. Annu Rev Biochem 78(1):119–146. https://doi.org/10.1146/annurev.biochem.78.082907.145923

Ninganagouda S, Rathod V, Jyoti H, Singh D, Prema K, Ul-Haq M (2013) Int J Pharm Bio Sci 4: 222

Park JK, Jung J, Subramaniam P, Shah BP, Kim C, Lee JK, Cho JH, Lee C, Lee KB (2011) Graphite-coated magnetic nanoparticles as multimodal imaging probes and cooperative therapeutic agents for tumor cells. Small 7:1647–1652

Payne JN, Waghwani HK, Connor MG et al (2016) Novel synthesis of kanamycin conjugated gold nanoparticles with potent antibacterial activity. Front Microbiol 7:607. https://doi.org/10.3389/fmicb.2016.00607

Pelaz B, Alexiou C, Alvarezpuebla RA, Alves F, Andrews AM, Ashraf S, Balogh LP, Ballerini L, Bestetti A, Brendel C (2017) Diverse applications of nanomedicine. ACS Nano 11:2313–2381

Pérez-Herrero E, Fernández-Medarde A (2015) Advanced targeted therapies in cancer: drug nanocarriers, the future of chemotherapy. Eur J Pharm Biopharm 93:52–79

Qiu Z, Yu Y, Chen Z et al (2012) Nanoalumina promotes the horizontal transfer of multiresistance genes mediated by plasmids across genera. Proc Natl Acad Sci U S A 109(13):4944–4949

Qureshi A, Blaisi NI, Abbas AA, Khan NA, Rehman S (2021) Prospectus and development of microbes mediated synthesis of nanoparticles. In Microbial nanotechnology: green synthesis and applications. Springer, Singapore, pp 1–15

Raghupathi KR, Koodali RT, Manna AC (2011) Langmuir 27:4020

Rai M, Deshmukh SD, Ingle AP, Gupta IR, Galdiero M, Galdiero S (2016) The protective nanoshield against virus infection. Crit Rev Microbiol 42(1):46–56. https://doi.org/10.3109/1040841X.2013.879849

Rehman S, Ansari MA, Alzohairy MA, Alomary MN, Jermy BR, Shahzad R, Tashkandi N, Alsalem ZH (2019a) Antibacterial and antifungal activity of novel synthesized neodymium-substituted cobalt ferrite nanoparticles for biomedical application. Processes 7:714. https://doi.org/10.3390/pr7100714

Rehman, S., Al Salem, Z., Al Jindan, R. and Hameed, S., 2019b. Microbial natural products: exploiting microbes against drug-resistant bugs. In: Pathogenicity and drug resistance of human pathogens. Springer, Singapore, pp 393–404

Rehman S, Farooq R, Jermy R, Mousa Asiri S, Ravinayagam V, Al Jindan R, Alsalem Z, Shah MA, Reshi Z, Sabit H, Alam KF (2020) A wild fomes fomentarius for biomediation of one pot synthesis of titanium oxide and silver nanoparticles for antibacterial and anticancer application. Biomolecules 10(4):622

Rehman S, Ansari MA, Al-Dossary HA, Fatima Z, Hameed S, Ahmad W, Ali A (2021) Current perspectives on mycosynthesis of nanoparticles and their biomedical application. In: Modeling and control of drug delivery systems. Academic, pp 301–311

Schröfel A, Kratošová G, Šafařík I, Šafaříková M, Raška I, Shor LM (2014) Applications of biosynthesized metallic nanoparticles—a review. Acta Biomater 10(10):4023–4042. https://doi.org/10.1016/j.actbio.2014.05.022

Siddiqi KS, Ur Rahman A, Husen A (2018) Properties of zinc oxide nanoparticles and their activity against microbes. Nanoscale Res Lett 13(1):1–13. https://doi.org/10.1186/s11671-018-2532-3

Singh R, Smitha MS, Singh SP (2014) The role of nanotechnology in combating multi-drug resistant bacteria. J Nanosci Nanotechnol 14:1–12

Song Z, Wu Y, Wang H, Han H (2019) Synergistic antibacterial effects of curcumin modified silver nanoparticles through ROS-mediated pathways. Mater Sci Eng C 99:255–263. https://doi.org/10.1016/j.msec.2018.12.053. the future of chemotherapy. Eur J Pharm Biopharm. 2015, 93, 52–79

Venkatasubbu GD, Baskar R, Anusuya T, Seshan CA, Chelliah R (2016) Toxicity mechanism of titanium dioxide and zinc oxide nanoparticles against food pathogens. Colloids Surf B Biointerfaces 148:600–606. https://doi.org/10.1016/j.colsurfb.2016.09.04

Wang F, Wang YC, Dou S, Xiong MH, Sun TM, Wang J (2011a) Doxorubicin-tethered responsive gold nanoparticles facilitate intracellular drug delivery for overcoming multidrug resistance in cancer cells. ACS Nano 5:3679–3692

Wang JJ, Zeng ZW, Xiao RZ et al (2011b) Recent advances of chitosan nanoparticles as drug carriers. Int J Nanomedicine 6:765. https://doi.org/10.2147/IJN.S25646

Wang Y, Ding X, Chen Y et al (2016) Antibiotic-loaded, silver core-embedded mesoporous silica nanovehicles as a synergistic antibacterial agent for the treatment of drug-resistant infections, pp 207–216

Wang L, Hu C, Shao L (2017) The antimicrobial activity of nanoparticles: present situation and prospects for the future. Int J Nanomedicine 12:1227–1249. https://doi.org/10.2147/IJN.S121956

Wu Q, Yang Z, Nie Y, Shi Y, Fan D (2014) Multi-drug resistance in cancer chemotherapeutics: mechanisms and lab approaches. Cancer Lett. 347:159–166

Xu J, Xu B, Shou D, Xia X, Hu Y (2015) Preparation and evaluation of vancomycin-loaded N-trimethyl chitosan nanoparticles. Polymers (Basel). 7(9):1850–1870. https://doi.org/10.3390/polym7091488

Yan S, Min LU, Ding X, Fei C, He X, Xu C, Hang Z, Qi W, Lan H, Zou J (2016) HematoPorphyrin Monomethyl Ether polymer contrast agent for ultrasound/photoacoustic dual-modality imaging-guided synergistic high intensity focused ultrasound (HIFU) therapy. Sci Rep 6:31833

Yanga J, Zhanga H, Chen B (2016) Application of nanoparticles to reverse multi-drug resistance in cancer. Nanotechnol Rev 5(5):489–496

Yoksan R, Chirachanchai S (2010) Silver nanoparticle-loaded chitosan–starch based films: fabrication and evaluation of tensile, barrier and antimicrobial properties. Mater Sci Eng C 30(6):891–897. https://doi.org/10.1016/j.msec.2010.04.004

Zaidi S, Misba L, Khan AU (2017) Nano-therapeutics: a revolution in infection control in post antibiotic era. Nanomedicine 13:2281–2301. https://doi.org/10.1016/j.nano.2017.06.015

Zhang L, Pornpattananangkul D, Hu C-M, Huang C-M (2010) Development of nanoparticles for antimicrobial drug delivery. Curr Med Chem 17(6):585–594. https://doi.org/10.2174/092986710790416290

Zhang P et al (2017) PROFEAT update: a protein features web server with added facility to compute network descriptors for studying omics-derived networks. J Mol Biol 429:416–425

Zhang JF, Rehman S, Alghamdi T, Sheikh FA, Hassan MS, Amna T (2022) Bionanotechnology approaches to combat biofilms and drug resistance. In: Innovative approaches for nanobiotechnology in healthcare systems. IGI Global, pp 230–248

Zignol M, Cabibbe AM, Dean AS et al (2018) Genetic sequencing for surveillance of drug resistance in tuberculosis in highly endemic countries: a multi-country population-based surveillance study. Lancet Infect Dis 18(6):675–683. https://doi.org/10.1016/S1473-3099(18)30073-2

Nanoparticles: Warheads to Overcome the Resistance Mechanism of Bacterial Superbugs

15

Rajashree Sahoo, A. Swaroop Sanket, Sanghamitra Pati, Rajni Kant, and Gaurav Raj Dwivedi

Abstract

In 2020–2021, communicable diseases became one of the major causes of morbidity and mortality worldwide. Widespread and unaware use of antibiotics, along with lack of novel antibiotics and novel vaccines in current situations have increased the level of resistance in bacterial pathogens. The drug resistance phenomenon in bacteria helps in the evolvement of specific drug-resistant, multidrug-resistant, and pan drug-resistant bacteria. It is need of the hour to find out a novel drug for various life-threatening pathogens. Nanoparticles (NPs) having unique physical and chemical properties may be considered as one of the promising platforms in this aspect. Nanoparticles with 1–100 nm are considered as the most emerging warhead to overcome bacterial drug resistance. Nano-technology has different applications like drug carriers, synergetics in antibacterial therapy, diagnostics, and preventives. NPs should also be explored some novel modes of action like increased uptake, decreased efflux pump, inhibition of biofilm formation, synthesis of porins, and degradation of metallo β lactamases to reverse the multidrug resistance.

R. Sahoo · A. S. Sanket · S. Pati
ICMR—Regional Medical Research Centre, Bhubaneswar, Odisha, India

R. Kant
ICMR—Regional Medical Research Centre, Gorakhpur, Uttar Pradesh, India

G. R. Dwivedi (✉)
ICMR—Regional Medical Research Centre, Gorakhpur, Uttar Pradesh, India

Microbiology Department, ICMR—Regional Medical Research Centre, Gorakhpur, Uttar Pradesh, India
e-mail: grd.rmrcb@gov.in

S. Hameed, S. Rehman (eds.), *Nanotechnology for Infectious Diseases*,
https://doi.org/10.1007/978-981-16-9190-4_15

Keywords

Drug resistance · MDR · Nanoparticle · Warhead · Efflux pumps

15.1 Introduction

Diseases can be defined as any physical, mental, and social illness which possess certain specific signs (WHO). Diseases can be categorized as infectious or non-infectious based on the mode of transmission. Globally communicable diseases are one of the main causes of sickness and casualty. Antibiotics are the marvel of drugs to fight against microbes. Rampant and unaware use of drugs with limited knowledge of targets, lack of novel antibiotics and vaccines have increased the level of resistance in pathogens. The continuous burden of antibiotics on microbes helps in the evolvement of single drug-resistant, multidrug-resistant, and pan drug-resistant bacteria.

Bacterial infections refer to the proliferation of a harmful strain on the surface or inside of the host body. They can infect any part of the body. Some Gram-positive and Gram-negative bacteria cause many deadliest diseases. *Enterobacteriaceae* family, *Pseudomonas, Acinetobacter, Mycobacterium, Helicobacter, Treponema spp*. are some of the life-threatening disease-causing bacteria. Some of the common diseases caused by these bacteria are urinary tract infection, gastroenteritis, sepsis, food poisoning, lungs infections, cystic fibrosis, wound infections, plague, and tuberculosis (Lowy 1884).

In a 2017 report, WHO identifies a list of dozen antibiotic-resistant bacteria (Dirty dozen) based on the severity of infection, treatment cost, and need for novel antibiotics. These dozen bacteria were further placed under critical, high, and medium priority groups. *Pseudomonas aeruginosa, Acinetobacter baumannii,* and *Enterobacteriaceae* members were categorized into critical groups. To date, antibiotics are the only major treatment option to control critical pathogens. The onset of antibiotic resistance enhances the loss of the antibiotic ability to inhibit these bacterial pathogens, and these resistant bacteria multiply in the presence of antibiotics. As a result of this, few of the existing antibiotics are available to the medicare for treatment of nosocomial infections. Some of those are used in combination, such as ceftolozane/tazobactam, ceftazidime/avibactam, and meropenem/vaborbactam. However, bacteria acquire different modes of resistance such as genetic mutation, production of hydrolyzing enzymes (β-lactamase), change in permeability (downregulation of porins and overexpression of efflux pumps), biofilm formation, etc. (Alanis 2005).

Nanoparticles (1–100 nm) are supposed to be one of the emerging warheads to counter bacterial drug resistance. Given the problem posed by critical superbugs and the absence of proper therapy, the present book chapter has been conceptualized to evaluate nanoparticles as a novel antibacterial treatment system.

15.1.1 Infectious Diseases

Infectious diseases can be defined as dangerous difference from the normal condition of an individual, which is associated with specific symptoms and signs (William Burrows). According to the mode of transmission, diseases can be divided into two groups, i.e., infectious diseases and non-infectious diseases. Many diseases are caused by external factors such as microorganisms, pollutants while some diseases are caused by abnormal functions of genes and dysfunction of organs. There are many infectious diseases, which do not show immediate signs or symptoms while some show. The ability of an organism to associate, invade, survive, and multiply inside the host cell is termed as infectivity, but the infectiveness of a disease indicates whether the infection is contagious or not (Rudan et al. 2008).

Microorganisms (bacteria, fungi, viruses, protozoa, or parasites) are responsible for infection in humans. Infections are called subclinical until they perceptively affect health. It may be local, confined to a specific region, or maybe generalized. Infectious agents may enter into the host through the air, water, soil, food, etc. via inhalation, consumption, ingestion, sexual and placental transmission, animal and insect bites, etc. (Fauci 2002).

Communicable diseases are the major cause of disability, death, economic and social disruption for people internationally (Global Burden of Disease Study 2013). Infections and co-infection are more common in developing countries. One infectious disease makes the host prone to other diseases (Hotez et al. 2004). The best example of such circumstances includes HIV/AIDS co-infection with malaria, tuberculosis, and multiple other diseases (Korenromp et al. 2005). To protect against the invasion of these infectious agents, body has protective layers called skin, hair, nail as primary defense systems. The body also responds by producing different leukocytes, antibodies, antitoxins, and sometimes by increasing body temperature. These antibodies may provide short-term or lifelong immunity while increased body temperature increases metabolism which helps in easy recovery of the infectious person.

Infectious diseases cannot be avoided, but there are many existing treatment options which provide protection and cure from infection. It is still a serious problem in developing and underdeveloped countries even after the development of preventive or treatment options (Dwivedi et al. 2016). The impact of infectious diseases increased manyfold due to two factors, i.e., microbial resistance to therapeutics and lack of scientific effort to innovate novel therapeutics like new vaccines and antibiotics (Jones et al. 2008). In recent scenario, infectious bacteria had been developed resistance against different clinically used antibiotics and other therapeutics (Ioset and Chatelain 2011; Kohanski et al. 2010). HIV/AIDS, lower respiratory infections, diarrheal diseases, malaria and tuberculosis (TB) like diseases are still in the top 10 reason of death in low and middle-income countries (Dwivedi et al. 2017). According to the latest information provided by WHO, tuberculosis, AIDS, and malaria are still the major killing impacts on global health.

15.2 Classification of Infectious diseases

According to the host defense capacity, microorganisms can be classified into two groups, i.e., primary pathogens and opportunistic pathogens (Madigan et al. 2015). Primary pathogens are biological agents that cause diseases when it enters into the host body. The opportunistic pathogen is usually affecting the host when they reach specific sites and suitable condition arises mainly in immunocompromised persons. Based on epidemiology, infectious diseases are classified as occasionally occurred diseases or sporadic diseases, regular occurred diseases found in a particular region or endemic diseases, privileged diseases in a particular region or epidemic and global epidemic or pandemic (Parkhill and Wren 2011).

15.2.1 Bacterial Infectious Diseases

Bacteria are omnipresent organisms present in almost all kinds of habitats. They belong to the eubacteria domain of life. Most of the bacteria are harmless however some bacteria are pathogenic to humans (Dwivedi et al. 2017). It is estimated that most of the earth's biomass is made up of bacteria. The number of bacteria on the earth is approximately one nonillion (Saag et al. 2017). A bacterial infection means the proliferation of a harmful strain on the surface or inside of the host body. They can infect any part of the body. Although bacteria are present everywhere, they cannot cause disease all time (Contini 2008).

In the year 1884, a method was developed by Hans Christian Gram to distinguish diverse group of bacteria. The process includes a chemical stain which colorize bacterial cell and followed by visualization through microscope. The result is based on the thickness of the cell envelope. According to this staining method, bacteria can be grouped into two groups: Gram-positive and Gram-negative. Gram-positive bacteria (GPB) show purple/violet colored stain, while Gram-negative bacteria (GNB) emerge pink or red coloration (Bartholomew and Mittwer 1952).

The cell envelope of GPB contains a thick layer of peptidoglycan in the cell wall that surrounds a single membrane. The cell envelope of GNB contain three different structural components, i.e., an inner or cytoplasmic membrane, a thin peptidoglycan layer, and an outer membrane. Lysozyme, glycolipids, and lipopolysaccharide present in GNB (Konovalova and Silhavy 2015).

Both GPB and GNB are frequently found in different shapes and sizes. Due to multi-structural components of the cell wall, GNB are more dangerous than GPB ones, some GPB also cause many of the deadliest diseases. Some of them are given in Table 15.1.

Teixobactin is a new group of antibiotics that is effective against multidrug-resistant GPB, making it a potential drug for GPB infections. Most of the GPB have well existing therapeutic options (Piddock 2015).

Table 15.1 Gram-positive bacterial infections

Causative agent	Diseases	Treatment options	Reference
Bacillus anthracis	Anthrax	Penicillin Doxycycline Ciprofloxacin	Heine et al. (2017)
Corynebacterium diphtheriae	Diphtheria	Penicillin Erythromycin DPT vaccine	Edwards et al. (2011)
Listeria monocytogenes	Listeriosis	Ampicillin Gentamycin	Daneshvar (2013)
Nocardia spp.	Nocardiosis	Imipenem Cefotaxime Amikacin Ceftriaxone	Wilson (2012)
Clostridium botulinum	Botulism	Penicillin	Swenson et al. (1980)
Streptococcus spp.	Meningitis, Rheumatic fever, and Pneumococcal infection	Vaccine Levofloxacin Fluoroquinolone Penicillin Azithromycin Clindamycin	Korsgaard et al. (2005)
Staphylococcus spp.	Skin diseases and toxic shock syndrome	Penicillin Methicillin Vancomycin Dicloxacillin	Rayner and Munckhof (2005)

15.2.2 Major Infectious Diseases Caused by Gram-Negative Bacteria

According to UN National health care safety network data, more than 30% of hospital-acquired infections are caused by GNB. Similarly, in the intensive care unit (ICU) GNB are responsible for 70% of bacterial infections (Dwivedi et al. 2017). They have mainly been associated with food and water-borne diseases, wound infections, respiratory tract, and nosocomial infections, sexually transmitted infections, UTI, cystic fibrosis, meningitis, cholera, plague, listeriosis, syphilis, tuberculosis, peptic ulcer, etc. (Table 15.2).

Antimicrobial resistance (AMR) in pathogenic GNB is a global burden. Due to development of different resistance mechanisms most of the therapeutics are ineffective against bacteria. So only a few treatment options are available now (Global Burden of Disease Study 2013). Approximately 70,000 deaths in each year are occurring due to antimicrobial resistance worldwide. So if no urgent action is taken, then it is estimated that by 2050 there will be over ten million deaths (Neil et al. 2015).

Once upon a time carbapenems was supposed to be safe and reliable options for severe diseases caused by MDR-GNB, however, increased resistance against this drug was reported in the last decades. Carbapenem-resistant critical superbugs cause

Table 15.2 Gram-negative bacterial infections

Causative agent	Diseases	Treatment option	Reference
Enterobacteriaceae (E. coli, Klebsiella, Salmonella, Shigella)	Food poisoning, Urinary tract infection, Gastroenteritis, Diarrhea and Sepsis	Fluroquinolone Cephalosporin Gentamicin	Kirby (2020)
Pseudomonas aeruginosa	Lungs infection, Cystic fibrosis, UTI	Penicillin Cephalosporin Carbapenems Aminoglycosides Quinolones	Ibrahim et al. (2020)
Acinetobacter baumannii	Several types of infections in wound	Meropenem Colistin Amikacin Rifampin	Galac et al. (2020)
Neisseria gonorrhoeae	Meningitis & pelvic inflammatory diseases	Penicillin Cefotaxime Ceftriaxone	Cyr et al. (2020)
Vibrio cholera	Cholera	Cephalosporin Tetracycline Doxycycline	Das et al. (2020)
Yersinia pestis	Plague	Aminoglycosides Tetracycline Fluroquinolones	Godfred-Cato et al. (2021)
Listeria monocytogenes	Listeriosis	Ampicillin Sulfamethoxazole Vancomycin	Baquero et al. (2020)
Campylobacter jejuni	Diarrheal illness	Ciprofloxacin Azithromycin Erythromycin	Eiland and Jenkins (2008)
Chlamydia trachomatis	Pelvic inflammatory diseases	Doxycycline Azithromycin	Marrazzo and Suchland (2014)
Treponema pallidum	Syphilis	Erythromycin Azithromycin Tetracycline	Stamm (2015)
Mycobacterium tuberculosis	TB	Rifampin Tetracycline Aminoglycosides	Wipperman et al. (2017)
Helicobacter pylori	Peptic and gastric ulcer	Amoxicillin Tetracycline Clarithromycin	Ghotaslou (2015)
Borrelia sp.	Lyme relapsing fever	Cephalosporin Amoxicillin Doxycycline	Leong et al. (2017)
Mycoplasma sp.	Respiratory tract infection	Tetracycline Erythromycin Macrolides	Chernova et al. (2016)

systemic infections with high mortality due to a lack of effective treatments (File 2011).

The antibacterial susceptibility of the existing antibiotics varied from one bacteria to another which significantly affects the morbidity and mortality rate. The mortality rate is high in carbapenem-resistant infections especially by *P. aeruginosa* (Falagas et al. 2014).

In the case of infections caused by GNBs, advanced therapy methods such as surgery, transplantations, chemotherapy, and intensive care are approachable; however, situations at times disfavor (Bassetti et al. 2017).

15.2.2.1 Antibiotics Available for Gram-Negative Bacteria

Since ancient times, the most commonly used antibiotics, i.e., penicillins act by inhibiting the synthesis of peptidoglycan layer. Apart from these, glycopeptides, cephalosporins, and carbapenem group of antibiotics interfere with the synthesis of the bacterial cell wall in bacteria. The polymyxin group of antibiotics alter the membrane proteins of the bacteria. Some of the antibiotics alter the translation machinery in the bacterial ribosomes, i.e., 30S and 50S. Other antibiotics enact on the nucleic acid replication such as rifampin. In case of reduced efficiency of these, combination of the antibiotics also helps in treatment under critical situations (Rochon and Gurwitz 1995). Because of the prevalence of MBL and MDR-producing isolates in GNB, only limited treatment options are left, signaling the need for the development of new, potent therapeutic agents with novel modes of action (Dwivedi et al. 2016). Figure 15.1 shows the different mechanisms of antibiotics acting against bacteria.

15.2.3 Mechanism of Resistance

As there are many ways in which antibiotics can kill or inhibit the growth and multiplication of bacteria, there are also many mechanisms of resistance that microorganisms innately possess or have developed over time exposure to antibiotics or received from other genus via horizontal gene transfer (Fig. 15.2).

15.2.3.1 Transformation

In this bacteria transfer their necked DNA or genetic material from donor to recipient via a medium (Fig. 15.3).

15.2.3.2 Transduction

It can be defined as the transfer of genetic material or nucleic acids from donor bacterium to another recipient bacterium through a phage or virus. In other words, this can be expressed as a virus-mediated transfer of genetic material from one bacterium to another (Fig. 15.4).

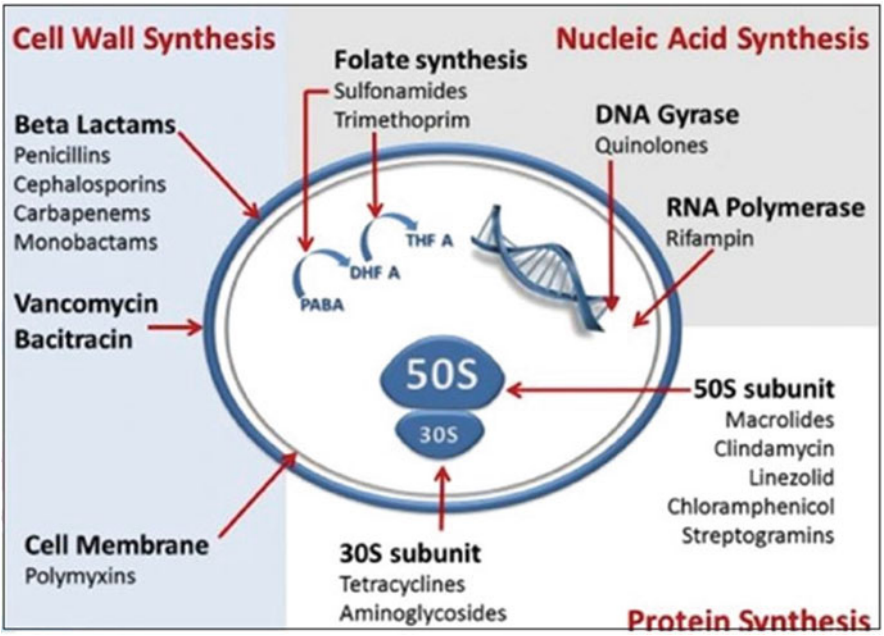

Fig. 15.1 Mechanism of action of antibiotics (Adapted from Kapoor et al. 2017) (Creative Commons Attribution-Non Commercial-ShareAlike License (CC BY-NC-SA))

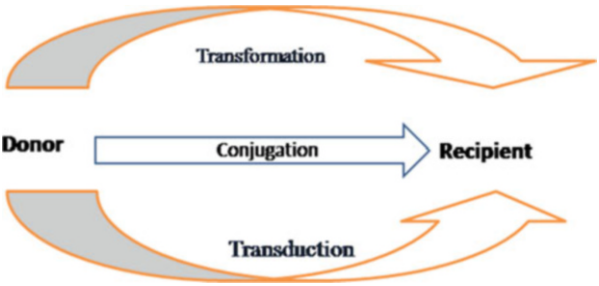

Fig. 15.2 Representing transformation, transduction, and conjugation

15.2.3.3 Conjugation

It can be defined as the relocation of genetic material from donor bacterium to receiver bacterium by cellular contact or through a conjugation tube (Fig. 15.5).

15.2.3.4 Enzymes for Antibiotic Inactivation

The bacteria are getting resistant to antibiotics day by day by producing different enzymes that make the antibiotics ineffective. It also decreases the efficacy of antibiotics. One such example is β-lactamase which can break the β-lactam ring of β-lactam antibiotics. A broken β-lactam ring stops the antibiotics from attachment to the peptidoglycan precursors (Sageman et al. 2014). β -lactamase can be divided into three groups, i.e., Serine β-lactamase, Expended spectrum β-lactamase, and Metallo

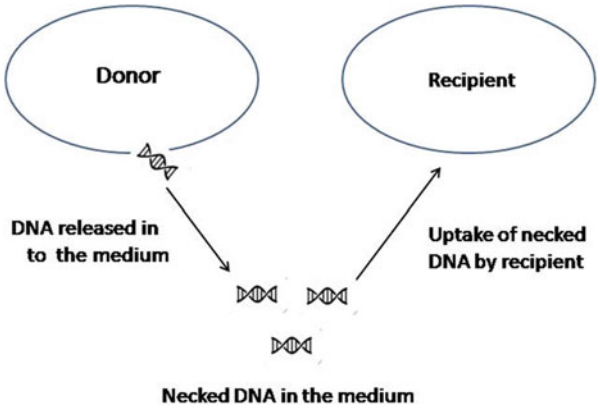

Fig 15.3 Transformation

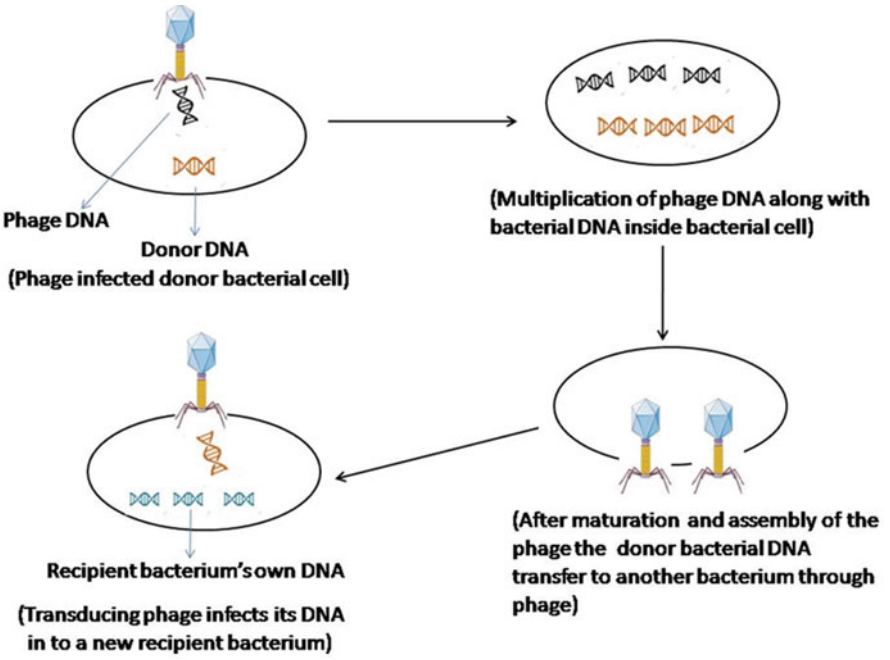

Fig. 15.4 Transduction

β-lactamase. They not only make penicillin and cephalosporins ineffective but also make carbapenem inactive which was considered as the last hope for the treatment of MDR-GNB. There are nine different variants of a large gene family that encodes for carbapenemase (IMP, VIM, SPM, GIM, AIM, SIM, KHM, NDM, and DIM). The bacteria producing this enzyme are regarded as superbugs because the infection caused by them are hard to treat (Dwivedi et al. 2016).

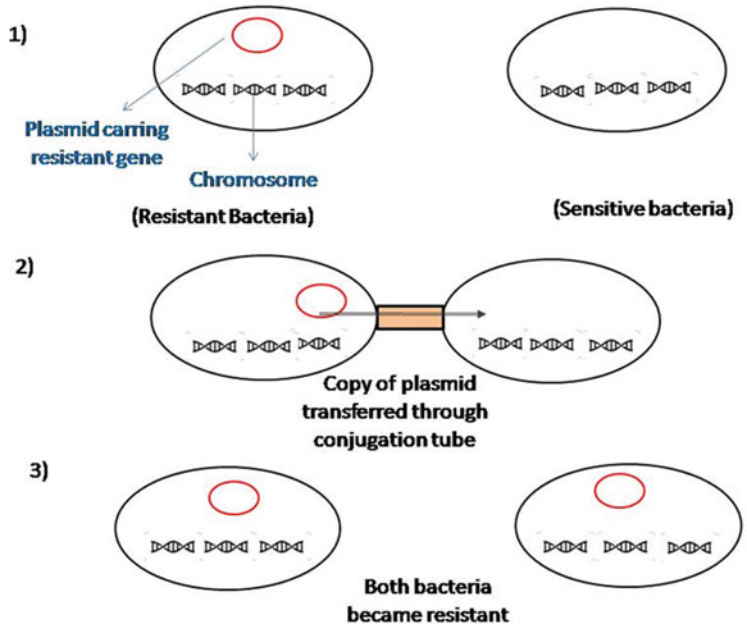

Fig. 15.5 Horizontal gene transfer through conjugation tube

15.2.3.5 Reduced Membrane Permeability

GNB have an outer and inner membrane with periplasmic space, whereas the outer membrane provides a first-line defense mechanism that regulates the passage of toxic molecules into the periplasm. Bacterial outer membrane components include outer membrane proteins called porins, which is responsible for pathogenicity and drug resistance. The cell can respond to the presence of noxious agents that can pass through the porins by reducing or down-regulating its number. There are two major porin-based mechanisms for antibiotics resistance: a) reduced number of porins, b) specific mutation of porins which possess altered functions (Dwivedi et al. 2016; Global Burden of Disease Study 2013).

15.2.3.6 Biofilm Formation

Bacterial biofilm is a survival mechanism that confers the ability to resist environmental stress and antimicrobials due to a variety of reasons, including low metabolic activity. In this mechanism bacterial cells stick to each other and attached in a living or non-living material within a framework of extracellular polymeric substances (EPS). The biofilm provides protection to the cell from environmental hassle. It is contagious in nature and results in hospital acquired infections. About 65% of the bacterial infections and 80% of the total chronic infections are due to biofilms (National Institutes of Health-NIH report). Formation of bacterial biofilm contains several steps, including attachment to a surface, formation of micro colony, which is followed by formation of a 3D structure. Then it is followed by maturation and

finally detachment from the substratum. Bacterial biofilm is less available to antibiotics so it is responsible for a variety of infectious diseases. The presence of exopolysaccharides acts as a barrier preventing the antibiotics to enter/diffuse into the deeper cell, making them antibiotic-resistant (Jamal et al. 2015).

15.2.3.7 Efflux Pump

An efflux pump is a biological pump that can expel out the noxious compounds from the cell. Five different types of efflux pump mechanisms have been reported, namely MFS, ABC, RND, SMR and MATE. In GNB, RND efflux pumps are the most effective for the MDR mechanism. This mechanism may create resistance to more than one class of antibiotics, especially against macrolides, tetracycline, and fluoroquinolones, etc. because they need to be intracellular to reach the target site (Lu et al. 2019).

15.2.3.8 Modification of Target Site

Many antibiotics act by binding to a specific target site. Bacteria can reduce the effectiveness of a drug by slightly changing its target molecule. Modification in the target sites of antibiotics is a frequently reported mechanism which is responsible for antimicrobial resistance (Fig. 15.6). The alteration of target site may be due to continuous overuse and misuse of antibiotics. Mutations in RNA polymerase and DNA gyrase enzymes develop resistance against quinolones and to the rifamycins, respectively (Lambert 2005).

According to the resistance mechanisms the prokaryotes especially bacteria can be divided into three groups. (a) Specific drug-resistant (SDR), (b) Multidrug-resistant (MDR), (c) Pan drug-resistant (PDR).

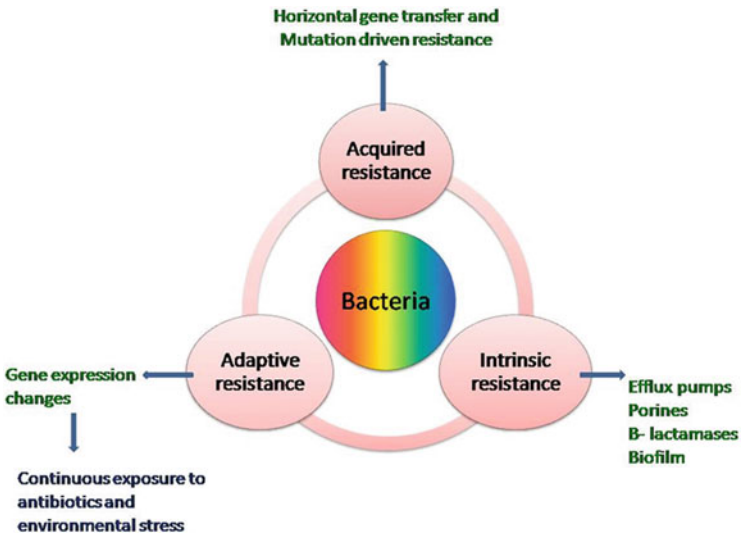

Fig. 15.6 Bacterial resistance (acquired, adaptive, and intrinsic)

15.2.4 Specific or Single-Drug Resistance

The bacterial drug resistance includes a mutation in the target sites, production of hydrolyzing enzymes like β lactamases, alteration in transporters proteins to prevent antibiotic entry, etc. Some pathogenic strains show resistance against a particular group of antibiotics but show sensitivity against another group of antibiotics. In recent era, there are many treatment options including antibiotics are still efficient to treat GPB pathogens but ineffective against GNB, it may be due to the existence of certain barriers in GNB (Dwivedi et al. 2016).

15.2.5 Multidrug Resistance

R-plasmids possess regions where the resistance genes are present. The pathogenic bacteria showing resistance against two or more structurally unrelated groups of antibiotics are termed multidrug-resistant bacteria. The resistance pattern is mediated by the same R-factor. The prevalence of MDR bacteria is a serious clinical problem. Carbapenem resistance in E.coli, Methicillin resistance in Staphylococcus have attributed attention of the clinicians to search for a novel treatment option (Harbottle et al. 2006; Levy 2002).

15.2.6 Pan Drug Resistance (Superbugs)

Bacterial strains exhibiting extreme rates of drug resistance phenomenon can be categorized as pan drug resistant (PDR). They are of epidemiological significance not because of their localized pathogenicity but also for the rapid spread nature around the globe. Apart from this in several literature, the terminology Extensively Drug Resistance (XDR) has been otherwise referred under different contexts which in due to course of time may turn into PDR (Magiorakos et al. 2012).

According to the World Health Organization Global Burden of Disease Study 2013 report, a group of antibiotic-resistant bacteria (superbugs) threaten to human health. So now researchers, hospital experts, and pharmaceutical companies are focusing on the development of novel therapeutics to overcome from the dangerous pathogenic infections. As in the list 12 bacteria are placed under critical, high, and medium priority groups that are collectively called "Dirty dozen." The WHO declared three pathogens as "critical priority" and they are *P. aeruginosa,* carbapenem-resistant *A. baumannii,* and *Enterobacteriaceae* family showing resistance to both third-generation cephalosporins and carbapenems (Global Burden of Disease Study 2013).

15.2.6.1 *Pseudomonas aeruginosa*

Pseudomonas species are Gram-negative, aerobic ubiquitous, and opportunistic pathogenic bacilli ranging from 0.5–1.0 μm long and possess large genomes of about (~5–7 Mbp). It shows incredible nutritional flexibility. Benzoate like organic

compounds can catabolized by this bacteria. Although it is one of the most abundant organisms on earth sometimes it causes serious health issues. *They produce secondary metabolites in the form of pigments, called pyocyanin* (Moradali et al. 2017). *It is an* opportunistic pathogen that affects immunologically weaker persons. This organism has reported to cause morbidity and mortality in cystic fibrosis (CF) respiratory infections. Largely associated with hospital-borne infections including ventilator related pneumonia and bloodstream infections. This organism has been associated with bacteremia and 15% of Gram-negative bacteremia cases are caused by this. On the whole, death associated with such kind of infection is about 50% (Glessner et al. 1999). It has a type III secretion system to extrude cytotoxins directly into host cells. Naturally, it is known as a host for siderophores (Fe3 + carriers) and pigments. These compounds allow the bacteria to escape from the innate immunity of the host defence mechanism. It has outer membrane porins which cause the outer membrane impermeable to antibiotics. It shows a high tendency to biofilms formation that is responsible to boost resistances to antibiotics in GNB. Some strains of the *Pseudomonas Spp.* have been adapted group of β-lactamases including ESBLs, carbapenemase, and metallo-β-lactamases (MBLs). Some pathogenic strains show up-regulation of efflux pump systems. Carbapenems and fluoroquinolones resistance is due to mutations and loss of the OprD porin gene united with up-regulation of MexEF-OprN efflux pumps. Up-regulation of MexCD-OprJ makes fluoroquinolones and some β-lactams ineffective. Similarly, MexAB-OprM makes β-lactams, sulfonamides, cephalosporins, macrolides, fluoroquinolones, tetracycline, chloramphenicol infective. DNA gyrase and topoisomerase IV mutations also give them additional resistance to antibiotics (Panda et al. 2022, Fair and Tor 2014).

15.2.6.2 *Escherichia coli*

E. coli is a Gram-negative, rod-shaped, nonspore forming, facultative anaerobic bacterium belonging to the family *Enterobacteriaceae*. Size ranges from 2.0 μm long and 0.25–1.0 μm in diameter. It is found as normal flora and colonizes in the gastrointestinal tract of animals and humans as most *of the* strains are harmless. Some strains have evolved into pathogenic *strains* by adapting virulence factors through plasmids, transposons, bacteriophages, mutations, horizontal gene transfer, and environmental stress.

ESBLs and MBL producing strains show resistance against third-generation cephalosporins. They also show resistance to fluoroquinolones and gentamicin. It has gained the New Delhi Metallo-β-lactamase-1 (NDM-1) enzyme, which is responsible to develop resistance against all β-lactam group of antibiotics including carbapenems. But resistance to monobactam and aztreonam has not been reported yet. Resistance to fluoroquinolone is also common among them by overexpressing *FomA* and *FomB* plasmid genes (Fair and Tor 2014). Pathogenic strains of a prophage can produce deadly endotoxins including verotoxins or Shiga-like toxins, which causes renal failure and severe uremic syndrome. It causes diarrhea and a variety of infections including urinary tract infections (UTI), meningitis, and septicemia. Diarrhea causing strain causes over two million deaths annually (FAO/WHO 2018).

15.2.6.3 *Acinetobacter baumannii*

It is a naturally short, round, rod-shaped, encapsulated Gram-negative bacteria that are generally non-motile generally found in the hospitals predominantly in intensive care units (ICU). It is present in nature and sometimes acts as an opportunistic pathogen in humans. It generally cause infections in immune-compromised persons and is responsible for nosocomial infections (Antunes et al. 2014). It contains a relatively thick cell wall that protects them from dryness, temperature, pH, and nutrient changes. They show resistance against different groups of antibiotics due to down-regulation of membrane transport and up-regulation of efflux pump mechanism. Overexpression of the efflux pump makes them resistant to fluoroquinolones, aminoglycoside, tigecycline, and cephalosporins. 30% of *A. baumannii* isolates can be able to produce biofilms, cytotoxinβ-lactamases like ESBLs, MBLs, oxacillinases, and carbapenemases (Fair and Tor 2014). I*t is generally* associated with severe hospital acquired infections, soft tissue and skin infections, secondary meningitis, wound infections, and urinary tract infections, etc. Bloodstream infections and pneumonia caused by the association of ventilator are lethal. *It* can easily enter the body through intravascular catheters and open wounds. It can also cause pneumonia and bacteremia.

15.2.7 Available Drugs for These Critical Superbugs

Ceftazidime-avibactam is a novel β-lactamase inhibitor blend accepted by the Food and Drug Administration (FDA) as a therapeutic for urinary tract infection and intra-abdominal infections especially caused by Enterobacteriaceae family. Ceftolozane-tazobactam has anti-pseudomonal activity. It is now used to conquer antimicrobial resistance. It has activity against the ESBL producing strains but not carbapenemase-producing isolates (Tumbarello et al. 2018). Imipenem in combination with cilastatin and relebactam has proved to have a synergistic effect against a wide spectrum of MDR Gram-negative strains like *P. aeruginosa, K. pneumoniae,* and *Enterobacter spp*. Other drugs like vaborbactam, meropenem, and aztreonam-avibactam have limited effects (Fernandes and Martens 2017). Avibactam is a broad spectrum β-lactamase inhibitor. The combination of meropenem with the vaborbactam had received approval from the FDA in August 2017. Ertapenem, colistin, rifampicin, amikacin, cinoxacin, and polymyxin B are the antibiotics that are presently used for critical superbugs (Kish 2018) (Drug bank site).

15.2.8 Drugs in the Pipeline

As these three organisms are placed at the top of most "bad bugs" list, now it is in the center of attention of the researchers. Many novel therapeutics and antibiotics are also under examination and pipelines (Table 15.3).

Table 15.3 List of antibiotics in the pipeline

Name of antibiotics	Antibiotics group	Mechanism of action	Antibacterial spectrum	Developmental stage
Ozenoxacin	Quinolone	DNA replication	Both GPB & GNB	FDA approved
Azidocillin	Penicillin	Cell wall biosynthesis	GNB	Experimental
Cefiderocol	Siderophore-cephalosporin	Cell wall biosynthesis	Both GNB & GPB	Phase-III
Arbekacin	Aminoglycoside	Protein biosynthesis	Both GNB & GPB	Experimental
Carindacillin	Penicillin	Cell wall biosynthesis	Both GNB & GPB	Pipeline
Pefloxacin	Fluoroquinolone	DNA replication	Both GNB & GPB	Pipeline
Relebactam	β-lactamase inhibitor	Cell wall biosynthesis	GNB	Phase-III
Finafloxacin	Fluoroquinolone	DNA replication	Both GNB & GPB	FDA approved

15.3 Non-antibiotics/Biosimilars Based Therapeutics

15.3.1 Phage Therapy

To defeat the antibiotic resistance and to overcome from the novel antibiotics scarcity, the use of phages has attributed scientific attention for the treatment of bacterial infections as an alternative therapeutic method. Phages used in therapy are viruses that damage bacterial cells but are safe for humans. As they are species/strain-specific they only target the pathogenic bacteria, so there are comparatively fewer side effects often associated with antibiotics (Kish 2018). They destroy the bacterial cell wall as well as the cell membrane and kill bacterial cell by enzymatic actions. Phages can assimilate bacterial biofilms (Sulakvelidze et al. 2011).

15.3.2 Phytochemicals

Traditionally plants and plant-based materials were used to cure diseases. Plant-based secondary metabolites like alkaloids, flavonoids, tannins, terpenoids, and polyphenols are used in pharmacological production. They have a wide range of antimicrobial properties. Many plant products like tomatidine, berberine, reserpine, 2-phenylethyl-isothiocyanate, ajoene, piperine, isothiocyanates, thymol, allicin, lycopene, carvacrol, kaempferol, proanthocyanidins, cinnamic acid have a wide range of antimicrobial properties and can able to destroy bacterial cell membrane, inhibit the β-lactamase and toxin production and bacterial biofilm formation and

efflux pump mechanism (Dwivedi et al. 2021a, Dwivedi et al. 2021b, Barbieri et al. 2017).

15.3.3 Antibacterial Peptides (AMPs)

These are mainly oligopeptides with a varying number of amino acids. Most of the cationic peptides hamper bacterial cell membranes and damage the lipid bilayer configuration. They have a broad spectrum of besieged viruses to bacteria. The first reported animal-originated antibacterial peptide is defensin (Park et al. 2017). Nissin, lactoferrin, LL-37, NRC-16, P9A, and P9B are some examples of antimicrobial peptides having activity against *Staphylococcus aureus, Pseudomonas, Enterobacter* spp.

15.3.4 Vaccines

Vaccines enhance the immune defense mechanism by activating the immune system of the host body which can identify and react to a pathogen. By vaccination, it helps to diseases prevention and narrows down the excessive use of antibiotic (Jansen et al. 2018). Several *P. aeruginosa* vaccines have been under trial but no vaccine has yet been approved for clinical use as they use several pathways to cause infection (Grimwood et al. 2015; Gellatly and Hancock 2013). GlycoVaxyn like vaccines is used for UTI infections caused by *E. coli*. The vaccines are based on live attenuated strains which are safe. These vaccines are mainly developed by using proteomic and genomic approaches (Rojas-Lopez et al. 2018). In *A. baumannii*, different antigens are used as vaccine candidates. Whole-cell vaccines and pure protein-based vaccines are used against this organism (Ahmad et al. 2016).

15.3.5 Nanoparticles

Nanoparticles (NPs) are ultrasmall particles that range from 1 to 100 nm and can be synthesized by using different inorganic materials like metal, metal oxides, and organic materials like carbon, etc. Due to their unique physical and physiological properties they show broad spectrum of antibacterial activity against both GPB and GNB. The NPs are used as antimicrobial agents, drug-resistant reversal agents, drug delivery systems, and direct as nano-drugs for the treatment of diseases (Paridah et al. 2016). Nanoparticles possess an efficient large surface area which provides drugs to bind. It has a spontaneous exterior which renders the consequent analogs to act on (Dwivedi et al. 2021a; Pandey et al. 2021; Dwivedi et al. 2016).

The size of the particle regulates the toxicity like the size 10 nm or less reported to have a strong bactericidal effect (Pérez-de-Luque 2017). Except for size, the ability

of NPs also depends on their shape (plane, spherical, cylindrical, tubular, conical, ring, hollow core, spiral, flat, fiber, and triangular). It can be classified into three groups that are organic NPs (Dendrimers, micelles, liposomes, and ferritin), inorganic NPs (metal and metal oxide-based NPs), and carbon-based NPs (carbon nanotubes (CNT), fullerenes, graphene, carbon nanofibers) (Ealias and Saravanakumar 2017).

15.3.6 Mechanism of Action

As the MDR mechanism is a major public health threat, to tackle this global problem an alternative treatment approach is urgently needed. Now NPs are getting attention as an alternative for bacterial infection treatment. Release of metal ions, oxidative stress induction, and non-oxidative mechanisms are responsible to kill or inhibit bacteria by the NPs (Fig. 15.7, Table 15.4). To develop resistance against NPs, the bacterial cell requires multiple gene mutations which is quite difficult (Linlin et al. 2017). The attachment of the NPs to the bacterial cell is due to electrostatic attraction, receptor–ligands and hydrophobic interactions, van der Waals forces etc (Colombo et al. 2016). After crossing the cell wall NPs then cross the bacterial cell membrane and interfere with the metabolic pathways. Then NPs interfere with different cellular components like lysosomes, ribosomes, DNA, and enzymes present in the cell (Xu et al. 2016)(Djurišić et al. 2015). Reactive nitrogen species is a common mechanism of NPs leading to programmed cell death (Prabhu and Poulose

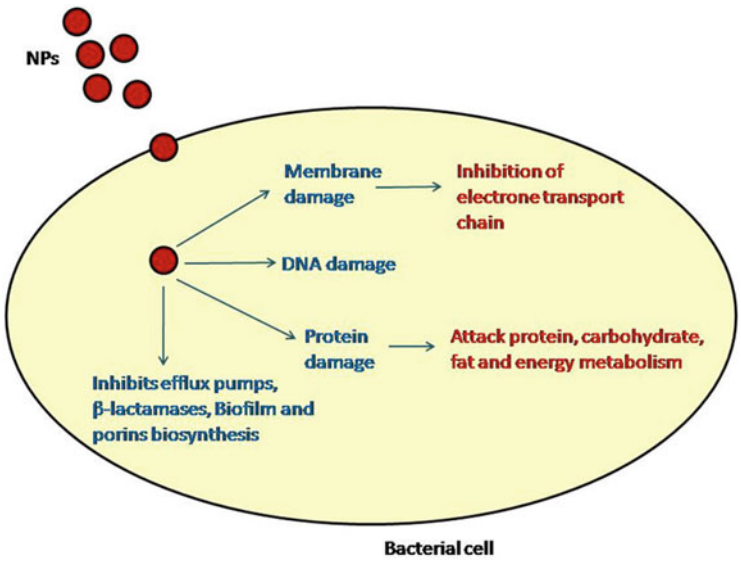

Fig. 15.7 The possible mechanism of actions of Nanoparticles

Table 15.4 Nanoparticles reported for antibacterial/drug resistance reversal activity

Sl no.	Name	Chemical property	Mode of action	Activity spectrum	Reference
1	Gold	Inorganic metal	Oxidative stress induction	Narrow	Okkeh et al. (2021)
2	Silver	Inorganic metal	Oxidative stress induction	Broad	Mohanta et al. (2020)
3	Zinc oxide	Inorganic metal	Oxidative stress induction and biofilm prevention	Broad	Mohd Yusof et al. (2021)
4	Copper oxide	Inorganic metal	Membrane disruption and oxidative stress induction	Narrow	John et al. (2021)
5	Cobalt	Inorganic metal	Membrane disruption and oxidative stress induction	Broad	Al-Fakeh and Alsaedi (2021)
6	Cadmium (quantum dots)	Inorganic metal	DNA damage and free radical production	Narrow	Abdel-Salam et al. (2020)
7	Magnesium oxide	Inorganic metal	Inhibit biofilm synthesis	Broad	Maji et al. (2020)
8	Nitric oxide	Inorganic metal	Biofilm inhibition	Broad	Urzedo et al. (2020)
9	Dendrimer	Organic	Membrane disruption	Broad	Aurelia Chis et al. (2020)
10	Liposomes	Organic	Biofilm inhibition	Broad	Pinilla et al. (2021)
11	Carbon	Organic metal	Attack metabolic pathway and membrane disruption	Broad	Rajabathar et al. (2020)
12	Titanium dioxide	Inorganic metals	Effect on efflux pumps and quorum sensing genes	Narrow	Ahmed et al. (2021)
13	Chitosan	Organic	Efflux pump	Narrow	Jesus et al. (2020)
14	Polycationic nanoparticles	Organic	Induce programmed cell death	Broad	Bhattacharjee (2019)
15	Iron oxide	Inorganic metals	Oxidative stress caused by reactive oxygen species, such as superoxide radicals, singlet oxygen, hydroxyl radicals, or hydrogen peroxide	Narrow	Ahmed et al. (2021)

2012) (Table 15.4). Many of the NPs target porin biosynthesis, efflux pumps, biofilm, and metallo β-lactamases enzyme production (Dwivedi et al. 2016; Pandey et al. 2021). NPs can be used as therapeutics, preventives, drug carriers, and synergetics with other molecules to treat bacterial infections (Paridah et al. 2016).

15.4 Conclusion and Future Prospective

Drug resistance in organism, changes the current situation of drug discovery with least hope of important antibacterials against these organisms. Bacteria acquire different categories of resistance to overcome the burden of different groups of antibiotics. In the absence of novel antibiotics/combinations to convey critical superbugs, the exploration of the newer sources of antibacterial/drug resistance reversal agents is required. NPs may have antimicrobial potency itself or rejuvenate the used antibiotics. It may help to diminish the development of drug-resistant mutant. The lower concentration of antibiotics in combination with NPs may be responsible for not acquiring resistance against antibiotics. However, NPs may also be used for targeted drug delivery.

References

Abdel-Salam M, Omran B, Whitehead K, Baek KH (2020) Superior properties and biomedical applications of microorganism-derived fluorescent quantum dots. Molecules 25(19):4486. https://doi.org/10.3390/molecules25194486

Ahmad TA, Tawfik DM, Sheweita SA, Haroun M, El-Sayed LH (2016) Development of immunization trials against Acinetobacter baumannii. Trial Vaccinol 5:53–60. https://doi.org/10.1016/j.trivac.2016.03.001

Ahmed FY, Aly UF, Abd El-Baky RM, Waly NGFM (2021) Effect of titanium dioxide nanoparticles on the expression of efflux pump and quorum-sensing genes in MDR pseudomonas aeruginosa isolates. Antibiotics 10(6):625. https://doi.org/10.3390/antibiotics10060625

Alanis AJ (2005) Resistance to antibiotics: are we in the post-antibiotic era? Arch Med Res 36(6):697–705. https://doi.org/10.1016/j.arcmed.2005.06.009

Al-Fakeh MS, Alsaedi RO (2021) Synthesis, characterization, and antimicrobial activity of CoO nanoparticles from a Co (II) complex derived from polyvinyl alcohol and Aminobenzoic acid derivative. Sci World J 2021:6625216. https://doi.org/10.1155/2021/6625216

Antunes LCS, Visca P, Towner KJ (2014) Acinetobacter baumannii: evolution of a global pathogen. Pathogen Dis 71(3):292–301. https://doi.org/10.1111/2049-632X.12125

Aurelia Chis A, Dobrea C, Morgovan C, Arseniu AM, Rus LL, Butuca A et al (2020) Applications and limitations of dendrimers in biomedicine. Molecules 25(17):3982. https://doi.org/10.3390/molecules25173982

Baquero F, Lanza VF, Duval M, Coque TM (2020) Ecogenetics of antibiotic resistance in listeria monocytogenes. Mol Microbiol 113(3):570–579. https://doi.org/10.1111/mmi.14454

Barbieri R, Coppo E, Marchese A, Daglia M, Sobarzo-Sánchez E, Nabavi SF, Nabavi SM (2017) Phytochemicals for human disease: an update on plant-derived compounds antibacterial activity. Microbiol Res 196:44–68. https://doi.org/10.1016/j.micres.2016.12.003

Bartholomew JW, Mittwer T (1952) The Gram stain. Bacteriol Rev 16(1):1–29

Bassetti M, Poulakou G, Ruppe E, Bouza E, Van Hal SJ, Brink A (2017) Antimicrobial resistance in the next 30 years, humankind, bugs and drugs: a visionary approach. Intensive Care Med 43(10):1464–1475. https://doi.org/10.1007/s00134-017-4878-x

Bhattacharjee S (2019) Polymeric nanoparticles. In: Principles of nanomedicine, pp 195–240. https://doi.org/10.1201/9780429031236-8

Chernova OA, Medvedeva ES, Mouzykantov AA, Baranova NB, Chernov VM (2016) 8(29), 24–34

Colombo E, Feyen P, Antognazza MR, Lanzani G, Benfenati F (2016) Nanoparticles: a challenging vehicle for neural stimulation. Front Neurosci 10:105. https://doi.org/10.3389/fnins.2016.00105

Contini C (2008) Clinical and diagnostic management of toxoplasmosis in the immunocompromised patient. Parassitologia 50(1–2):45–50

Cyr SS, Barbee L, Workowski KA, Bachmann LH, Pham C, Schlanger K (2020) CDC STI gonorrhea treatment guidelines 69(50), 1911–1916

Daneshvar DH (2013) Athletic exposure to repetitive brain trauma and its effect on the development of chronic traumatic encephalopathy. ProQuest Dissertations and Theses 55(3):253. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC372831/pdf/microrev00034-0152.pdf%0Ahttp://myaccess.library.utoronto.ca/login?url=http://search.proquest.com/docview/1545679090?accountid=14771%5Cnhttp://bf4dv7zn3u.search.serialssolutions.com/?ctx_ver=Z39.88-2

Das B, Verma J, Kumar P, Ghosh A, Ramamurthy T (2020) Antibiotic resistance in vibrio cholerae: understanding the ecology of resistance genes and mechanisms. Vaccine 38(Suppl 1):A83–A92. https://doi.org/10.1016/j.vaccine.2019.06.031

Djurišić AB, Leung YH, Ng AMC, Xu XY, Lee PKH, Degger N, Wu RSS (2015) Toxicity of metal oxide nanoparticles: mechanisms, characterization, and avoiding experimental artefacts. Small 11(1):26–44. https://doi.org/10.1002/smll.201303947

Dwivedi GR, Sanchita, Singh DP, Sharma A, Darokar MP, Srivastava SK (2016) Nano particles: emerging warheads against bacterial superbugs. Curr Top Med Chem 16(18):1963–1975. https://doi.org/10.2174/1568026616666160215154556

Dwivedi GR, Singh A, Upadhyay HC, Pati S, Singh DP, Prasad KN et al (2017) Determination of drug resistance mechanism (s) of clinical isolates of P. aeruginosa and Phytoextract as drug resistance reversal agent. EC Microbiol 13(November):35–41

Dwivedi GR, Rai R, Pratap R, Singh K, Pati S, Sahu SN, Kant R, Darokar MP, Yadav DK (2021a) Drug resistance reversal potential of multifunctional thieno[3,2-c]pyran via potentiation of antibiotics in MDR P. aeruginosa. Biomed Pharmacother 142:112084. https://doi.org/10.1016/j.biopha.2021.112084. Epub 2021 Aug 24. PMID: 34449308

Dwivedi GR, Khwaja S, Singh Negi A, Panda SS, Swaroop Sanket A, Pati S, Chand Gupta A, Bawankule DU, Chanda D, Kant R, Darokar MP (2021b) Design, synthesis and drug resistance reversal potential of novel curcumin mimics Van D: synergy potential of curcumin mimics. Bioorg Chem 106:104454. https://doi.org/10.1016/j.bioorg.2020.104454. Epub 2020 Nov 4. PMID: 33213895

Ealias AM, Saravanakumar MP (2017) A review on the classification, characterisation, synthesis of nanoparticles and their application. IOP Conf Ser Mater Sci Eng 263(3):032019. https://doi.org/10.1088/1757-899X/263/3/032019

Edwards B, Hunt AC, Hoskisson PA (2011) Recent cases of non-toxigenic Corynebacterium diphtheriae in Scotland: justification for continued surveillance. J Med Microbiol 60(4):561–562. https://doi.org/10.1099/jmm.0.025643-0

Eiland LS, Jenkins LS (2008) Therapeutic dilemma optimal treatment of campylobacter dysentery. J Pediatr Pharmacol Ther 13(3):170–174. Retrieved from www.jppt.org

Fair RJ, Tor Y (2014) Bacterial resistance in the 21st century. Perspect Medicin Chem 6:25–64. https://doi.org/10.4137/PMC.S14459.Received

Falagas ME, Lourida P, Poulikakos P, Rafailidis PI, Tansarli GS (2014) Antibiotic treatment of infections due to Carbapenem-resistant Enterobacteriaceae: systematic evaluation of the available evidence. Antimicrob Agents Chemother 58(2):654–663. https://doi.org/10.1128/aac.01222-13

FAO/WHO (2018) Shiga toxin-producing Escherichia coli (STEC) and food: attribution, characterization, and monitoring. https://apps.who.int/iris/bitstream/handle/10665/272871/9789241514279-eng.pdf?ua=1

Fauci AS (2002) Infectious diseases: considerations for the 21st century. Clin Infect Dis 32(5):675–685. https://doi.org/10.1086/319235

Fernandes P, Martens E (2017) Antibiotics in late clinical development. Biochem Pharmacol 133:152–163. https://doi.org/10.1016/j.bcp.2016.09.025

File TM (2011) Highlights from international clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: a 2010 update by the infectious diseases society of America and the European Society for Microbiology and Infectious. Infect Dis Clin Pract 19(4):282–283. https://doi.org/10.1097/IPC.0b013e3182231994

Galac MR, Snesrud E, Lebreton F, Stam J, Julius M, Ong AC et al (2020) A diverse panel of clinical Acinetobacter baumannii for research and development. Antimicrob Agents Chemother 64(10): e00840–e00820. https://doi.org/10.1128/AAC.00840-20

Gaurav Raj, Dwivedi Reeta, Rai Ramendra, Pratap Khusbu, Singh Sanghamitra, Pati Satya Narayan, Sahu Rajni, Kant Mahendra P., Darokar Dharmendra K., Yadav (2021) Drug resistance reversal potential of multifunctional thieno[32-c]pyran via potentiation of antibiotics in MDR P. aeruginosa. Biomedicine & Pharmacotherapy 142112084-10.1016/j.biopha.2021.112084

Gaurav, Raj Dwivedi Sadiya, Khwaja Arvind, Singh Negi Swati S., Panda A., Swaroop Sanket Sanghamitra, Pati Amit, Chand Gupta Dnyaneshwar Umrao, Bawankule Debabrata, Chanda Rajni, Kant Mahendra P., Darokar (2021) Design synthesis and drug resistance reversal potential of novel curcumin mimics Van D. Bioorganic Chemistry 106104454-10.1016/j.bioorg.2020.104454

Gellatly SL, Hancock REW (2013) Pseudomonas aeruginosa: new insights into pathogenesis and host defenses. Pathogen Dis 67(3):159–173. https://doi.org/10.1111/2049-632X.12033

Ghotaslou R (2015) Prevalence of antibiotic resistance in helicobacter pylori: a recent literature review. World J Methodol 5(3):164. https://doi.org/10.5662/wjm.v5.i3.164

Glessner A, Smith RS, Iglewski BH (1999) Systems in control of twitching motility. J Bacteriol 181(5):1623–1629

Global Burden of Disease Study (2013 Collaborators) Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990–2013: a systematic analysis for the global burden of disease study 2013. Lancet 386(9995):743–800. https://doi.org/10.1016/S0140-6736(15)60692-4. Epub 2015 Jun 7. PMID: 26063472; PMCID: PMC4561509. https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed

Godfred-Cato S, Cooley KM, Fleck-Derderian S, Beckstead HA, Russell Z, Meaney-Delman D et al (2021) Treatment of human plague: A systematic review of published aggregate data on antimicrobial efficacy, 1939–2019. Clin Infect Dis 70(Suppl 1):S11–S19. https://doi.org/10.1093/CID/CIZ1230

Grimwood K, Kyd JM, Owen SJ, Massa HM, Cripps AW (2015) Vaccination against respiratory Pseudomonas aeruginosa infection. Human Vaccines Immunotherap 11(1):14–20. https://doi.org/10.4161/hv.34296

Harbottle H, Thakur S, Zhao S, White DG (2006) Genetics of antimicrobial resistance. Anim Biotechnol 17(2):111–124. https://doi.org/10.1080/10495390600957092

Heine HS, Shadomy SV, Boyer AE, Chuvala L, Riggins R, Kesterson A et al (2017) Evaluation of combination drug therapy for treatment of antibiotic-resistant inhalation anthrax in a murine model. Antimicrob Agents Chemother 61(9):1–9. https://doi.org/10.1128/AAC.00788-17

Hotez PJ, Remme JHF, Buss P, Alleyne G, Morel C, Breman JG (2004) Combating tropical infectious diseases: report of the disease control priorities in developing countries project. Clin Infect Dis 38(6):871–878. https://doi.org/10.1086/382077

Ibrahim D, Jabbour JF, Kanj SS (2020) Current choices of antibiotic treatment for Pseudomonas aeruginosa infections. Curr Opin Infect Dis 33(6):464–473. https://doi.org/10.1097/QCO.0000000000000677

Ioset J-R, Chatelain E (2011) Drug discovery and development for neglected diseases: the DNDi model. Drug Des Devel Ther 5:175–181. https://doi.org/10.2147/dddt.s16381

Jamal M, Tasneem U, Hussein T, A. S. (2015) Bacterial biofilm: its composition, formation and role in human infections. Research & reviews. J Microbiol Biotechnol 4(3):1–14. http://www.rr

Jansen KU, Knirsch C, Anderson AS (2018) The role of vaccines in preventing bacterial antimicrobial resistance. Nat Med 24(1):10–20. https://doi.org/10.1038/nm.4465

Jesus S, Marques AP, Duarte A, Soares E, Costa JP, Colaço M et al (2020) Chitosan nanoparticles: shedding light on immunotoxicity and hemocompatibility. Front Bioeng Biotechnol 8:100. https://doi.org/10.3389/fbioe.2020.00100

John MS, Nagoth JA, Zannotti M, Giovannetti R, Mancini A, Ramasamy KP et al (2021) Biogenic synthesis of copper nanoparticles using bacterial strains isolated from an Antarctic consortium associated to a psychrophilic marine ciliate: characterization and potential application as antimicrobial agents. Mar Drugs 19(5):263. https://doi.org/10.3390/md19050263

Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, Daszak P (2008) Global trends in emerging infectious diseases. Nature 451(7181):990–993. https://doi.org/10.1038/nature06536

Kapoor G, Saigal S, Elongavan A (2017) Action and resistance mechanisms of antibiotics: a guide for clinicians. J Anaesthesiol Clin Pharmacol 33(3):300. https://doi.org/10.4103/joacp.JOACP_349_15

Kirby JE (2020) Roman Manetsch, pp 617–667

Kish T (2018) New antibiotics in development target highly resistant gram-negative organisms. P & T 43(2):116–120

Kohanski MA, Dwyer DJ, Collins JJ (2010) How antibiotics kill bacteria: from targets to networks. Nat Rev Microbiol 8(6):423–435. https://doi.org/10.1038/nrmicro2333

Konovalova A, Silhavy TJ (2015) Outer membrane lipoprotein biogenesis: lol is not the end. Philos Trans R Soc B Biol Sci 370(1679):20150030. https://doi.org/10.1098/rstb.2015.0030

Korenromp MJ, Christiaens GCML, Van Den Bout J, Mulder EJH, Hunfeld JAM, Bilardo CM et al (2005) Long-term psychological consequences of pregnancy termination for fetal abnormality: a cross-sectional study. Prenat Diagn 25(3):253–260. https://doi.org/10.1002/pd.1127

Korsgaard J, Møller JK, Kilian M (2005) Antibiotic treatment and the diagnosis of Streptococcus pneumoniae in lower respiratory tract infections in adults. Int J Infect Dis 9(5):274–279. https://doi.org/10.1016/j.ijid.2004.07.013

Lambert PA (2005) Bacterial resistance to antibiotics: modified target sites. Adv Drug Deliv Rev 57(10):1471–1485. https://doi.org/10.1016/j.addr.2005.04.003

Leong JY, Patel AS, RR (2017) 乳鼠心肌提取 HHS public access. Physiol Behav 176(5), 139–148. https://doi.org/10.1097/MOP.0000000000000167.Update

Levy SB (2002) Factors impacting on the problem of antibiotic resistance. J Antimicrob Chemother 49:25–30

Linlin W, Chen H, Longquan S (2017) The antimicrobial activity of nanoparticles: present situation and prospects for the future. Int J Nanomedicine 12:1227–1249. https://doi.org/10.2147/IJN.S121956

Lowy F (1884) Bacterial classification, structure and function. In: Columbia University, pp 1–6

Lu W-J, Lin H-J, Hsu P-H, Lai M, Chiu J-Y, Lin H-TV (2019) Brown and red seaweeds serve as potential efflux pump inhibitors for drug-resistant Escherichia coli. Evid Based Complement Alternat Med 2019:1836982. https://doi.org/10.1155/2019/1836982

Madigan MT, Martinko JM, Parker J (2015) Brock biology of micro-organisms, p 1041

Magiorakos A, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG et al (2012) Bacteria: an international expert proposal for interim standard definitions for acquired resistance. Microbiology 18(3):268–281. http://onlinelibrary.wiley.com/doi/10.1111/j.1469-0691.2011.03570.x/full

Maji J, Pandey S, Basu S (2020) Synthesis and evaluation of antibacterial properties of magnesium oxide nanoparticles. Bull Mater Sci 43(1):1–10. https://doi.org/10.1007/s12034-019-1963-5

Marrazzo J, Suchland R (2014) Recent advances in understanding and managing chlamydia trachomatis infections. F1000Prime Rep 6:120. https://doi.org/10.12703/P6-120

Mohanta YK, Biswas K, Jena SK, Hashem A, Abd-Allah EF, Mohanta TK (2020) Anti-biofilm and antibacterial activities of silver nanoparticles synthesized by the reducing activity of phytoconstituents present in the Indian medicinal plants. Front Microbiol 11:1143. https://doi.org/10.3389/fmicb.2020.01143

Mohd Yusof H, Abdul Rahman N, Mohamad R, Hasanah Zaidan U, Samsudin AA (2021) Antibacterial potential of biosynthesized zinc oxide nanoparticles against poultry-associated foodborne pathogens: an in vitro study. Animals 11(7):2093. https://doi.org/10.3390/ani11072093

Moradali MF, Ghods S, Rehm BHA (2017) Pseudomonas aeruginosa lifestyle: a paradigm for adaptation, survival, and persistence. Front Cell Infect Microbiol 7:39. https://doi.org/10.3389/fcimb.2017.00039

Neil A, Carr V, MacKinnon A, Morgan VA (2015) Evidence or ideology? Let the debate continue ... More evaluation please! Aust N Z J Psychiatry 50(5):495–496. https://doi.org/10.1177/0004867415623861

Okkeh M, Bloise N, Restivo E, De Vita L, Pallavicini P, Visai L (2021) Gold nanoparticles: can they be the next magic bullet for multidrug-resistant bacteria? Nano 11(2):1–30. https://doi.org/10.3390/nano11020312

Panda SS, Singh K, Pati S, Kant R, Singh R, Dwivedi GR (2022) *Pseudomonas aeruginosa:* pathogenic adapter bacteria. In: Kumar V, Shriram V, Paul A, Thakur M (eds) Antimicrobial resistance – underlying mechanisms and therapeutic approaches. Spriger Nature Publishing, Singapore. https://doi.org/10.1007/978-981-16-3120-7_4. ISBN 978-981-16-3119-1

Paridah M, Moradbak A, Mohamed A, Owolabi F Abdulwahab Taiwo, Asniza, M., & Abdul Khalid, S. H. (2016). We are IntechOpen, the world' s leading publisher of open access books Built by scientists, for scientists TOP 1%. Intech, i(tourism), 13. https://doi.org/10.5772/57353

Park AJ, Okhovat JP, Kim J (2017) Antimicrobial peptides. In: Clinical and basic immunodermatology, 2nd edn., pp 81–95. https://doi.org/10.1007/978-3-319-29785-9_6

Parkhill J, Wren BW (2011) Bacterial epidemiology and biology—lessons from genome sequencing. Genome Biol 12(10):1–7. https://doi.org/10.1186/gb-2011-12-10-230

Pérez-de-Luque A (2017) Interaction of nanomaterials with plants: what do we need for real applications in agriculture? Front Environ Sci 5:1–7. https://doi.org/10.3389/fenvs.2017.00012

Piddock LJV (2015) Teixobactin, the first of a new class of antibiotics discovered by ichip technology? J Antimicrob Chemother 70(10):2679–2680. https://doi.org/10.1093/jac/dkv175

Pinilla CMB, Lopes NA, Brandelli A (2021) Lipid-based nanostructures for the delivery of natural antimicrobials. Molecules 26(12):1–22. https://doi.org/10.3390/molecules26123587

Prabhu S, Poulose EK (2012) Silver nanoparticles: mechanism of antimicrobial, pp 1–10. http://www.inl-journal.com/content/pdf/2228-5326-2-32.pdf

Pandey P, Sahoo R, Singh K, Pati S, Mathew J, Pandey AC, Kant R, Han I, Choi EH, Dwivedi GR, Yadav DK (2021) Drug resistance reversal potential of nanoparticles/nanocomposites via antibiotic's potentiation in multi drug resistant P. aeruginosa. Nanomaterials (Basel) 12(1):117. https://doi.org/10.3390/nano12010117

Rajabathar JR, Periyasamy G, Alanazi AM, Govindasamy M, Arunachalam P (2020) Review on carbon nanotube varieties for healthcare application: effect of preparation methods and mechanism insight. Processes 8(12):1–16. https://doi.org/10.3390/pr8121654

Rayner C, Munckhof WJ (2005) Staphaur-current-antibiotitreat. Intern Med J 35(3):1–16

Rochon PA, Gurwitz JH (1995) Drug therapy: aztreonam. Lancet 346(8966):32–36

Rojas-Lopez M, Monterio R, Pizza M, Desvaux M, Rosini R (2018) Intestinal pathogenic Escherichia coli: insights for vaccine development. Front Microbiol 9:440. https://doi.org/10.3389/fmicb.2018.00440

Rudan I, Boschi-Pinto C, Biloglav Z, Mulholland K, Campbell H (2008) Epidemiology and etiology of childhood pneumonia. Bull World Health Organ 86(5):408–416. https://doi.org/10.2471/BLT.07.048769

Saag L, Varul L, Scheib CL, Stenderup J, Allentoft ME, Saag L et al (2017) Extensive farming in Estonia started through a sex-biased migration from the steppe. Curr Biol 27(14):2185–2193.e6. https://doi.org/10.1016/j.cub.2017.06.022

Sageman BB, Singer BS, Meyers SR, Siewert SE, Walaszczyk I, Condon DJ et al (2014) Integrating 40Ar/39Ar, U-Pb, and astronomical clocks in the cretaceous Niobrara formation, Western Interior Basin, USA. Bull Geol Soc Am 126(7–8):956–973. https://doi.org/10.1130/B30929.1

Stamm LV (2015) Syphilis: antibiotic treatment and resistance. Epidemiol Infect 143(8): 1567–1574. https://doi.org/10.1017/S0950268814002830

Sulakvelidze A, Alavidze Z, Morris JG (2011) The challenges of bacteriophage therapy. Indus Pharm 45(31):14–18. https://doi.org/10.1128/AAC.45.3.649

Swenson JM, Thornsberry C, McCroskey LM (1980) Susceptibility of Clostridium botulinum to thirteen antimicrobial agents. Antimicrob Agents Chemother 18(1):13–19. https://doi.org/10.1128/AAC.18.1.13

Tumbarello M, Losito AR, Helen G (2018) Optimizing therapy in CRE infections. Curr Opin Infect Dis 31:566–577. https://doi.org/10.1097/qco.0000000000000493

Urzedo AL, Gonçalves MC, Nascimento MHM, Lombello CB, Nakazato G, Seabra AB (2020) Multifunctional alginate nanoparticles containing nitric oxide donor and silver nanoparticles for biomedical applications. Mater Sci Eng C 112:110933. https://doi.org/10.1016/j.msec.2020.110933

Wilson JW (2012) Nocardiosis: updates and clinical overview. Mayo Clin Proc 87(4):403–407. https://doi.org/10.1016/j.mayocp.2011.11.016

Wipperman MF, Fitzgerald DW, Juste MAJ, Taur Y, Namasivayam S, Sher A et al (2017) Antibiotic treatment for tuberculosis induces a profound dysbiosis of the microbiome that persists long after therapy is completed. Sci Rep 7(1):1–11. https://doi.org/10.1038/s41598-017-10346-6

Xu F, Reiser M, Yu X, Gummuluru S, Wetzler L, Reinhard BM (2016) Lipid-mediated targeting with membrane-wrapped nanoparticles in the presence of corona formation. ACS Nano 10(1): 1189–1200. https://doi.org/10.1021/acsnano.5b06501

Nanotechnology: A Recent Breakthrough Against Resistant Biofilm Infection

16

Hammad Alam, Vartika Srivastava, and Aijaz Ahmad

Abstract

Microbial biofilms are involved in most medical device-related illnesses, and they continue to be a problem for modern medicine. The multidrug drug-resistant characteristics of biofilm associated infections are the major concern as they cause more than 60% of chronic infections and therefore have wreaked havoc on health-care systems across the world. As revealed by health organizations, biomedical nanotechnology is dedicated to investigating nanoscience and nanotechnology for health wellbeing, with the goal of individualized health management. Nanotechnology has the potential to be used in medical diagnostics and therapies. Although nanomaterials have been extensively studied for preventing biofilm formation on medical devices, their applications in clinical settings have yet to be explored. Proteomic and genomic investigations, illness diagnostics, pharmacological screening, drug administration, protein purification, cancer therapy, and bio-imaging are just a few of the applications for nanomaterials in biology and medicine. Nanotechnology advancements have resulted in rapid advancements in medicine delivery and targeted therapeutic therapy. The invention, characterization, and assessment of nanotechnology for the prevention or treatment of biofilm infections are elaborated of this chapter.

H. Alam · V. Srivastava
Department of Clinical Microbiology, and Infectious Diseases, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

A. Ahmad (✉)
Department of Clinical Microbiology, and Infectious Diseases, School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa

Division of Infection Control, Charlotte Maxeke, Johannesburg Academic Hospital, National Health Laboratory Service, Johannesburg, South Africa
e-mail: Aijaz.Ahmad@wits.ac.za; Aijaz.Ahmad@nhls.ac.za

S. Hameed, S. Rehman (eds.), *Nanotechnology for Infectious Diseases*,
https://doi.org/10.1007/978-981-16-9190-4_16

Keywords

Nanotechnology · Microbial biofilms · Nanoparticles · Multidrug resistant

16.1 Introduction

Biofilms are multi-component structures formed by bacterial and fungal species. The biofilm formation starts when free-floating microorganisms adhere to one another on living or non-living surfaces (Gu et al. 2016; Srivastava et al. 2015; Jadaun et al. 2015, and Singh et al. 2015). Biofilms formed by a wide range of microbial pathogens may cause major health risk that is difficult to combat with available therapeutic regime (Frieri et al. 2017). A larger no of microorganisms including bacteria and fungus are reported which have the ability of biofilm formation. Gram-positive and Gram-negative bacteria can form biofilms, with *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Staphylococcus aureus*, *S. epidermidis*, *Streptococcus viridans*, *Proteus mirabilis*, and *Pseudomonas aeruginosa* among the most common (Konduri et al. 2021; Khatoon et al. 2018, and Hassan et al. 2011). Biofilm formation is also prevalent among fungal species, for instance, *Candida* species*., Aspergillus* species*, Fusarium* species*, Cryptococcus, Pneumocystis* species*, Blastoschizomyces capitatus, Malassezia pachydermatis, Trichosporon asahii, Rhizopus* species*.,* and *Rhizomucor* species. (Peiqian et al. 2014; Sun et al. 2012). Antibiotic resistance is one of the most serious issues related with biofilms, and it necessitates increasing the antimicrobial dose which can raise the risk of systemic toxicity when using a traditional antibiotic (Gebreyohannes et al. 2019a, b).

Nanotechnology represents a novel strategy for combating and eliminating biofilm-forming microbes (Khan et al. 2020a, b, and Sadekuzzaman et al. 2015). It is becoming increasingly significant in overcoming these barriers by employing various strategies for drug delivery to biofilm, which is considered an effective technique for combating bacterial as well as fungal biofilm. (Khan et al. 2019). As our understanding of diseases at the molecular level increases, or as a nanomaterial-subcellular scale comparable marker identification offers up new diagnostic and treatment alternatives, nanomedicine will become more widely used (Patra et al. 2018a, b). Understanding disease molecular fingerprints will lead to future advances in nanomedicine applications (Patra et al. 2018a, b). Biofilms are surface-attached bacterial colonies embedded in an extracellular matrix that form microenvironments that are isolated and protected (Naha et al. 2019).

16.2 What are Biofilms?

The term biofilm refers to a group of one or more than one types of microbes that can proliferate on different types of surfaces, ranging from abiotic to biotic surfaces. Microorganisms having the capability to form biofilm are bacteria, fungi, and protists.

16.3 Biofilm Formation

The microorganisms mainly survive by adhering to and growing upon suitable biotic or abiotic surfaces. These surfaces may range from aquatic and soil systems, medical implants and devices, and animal tissues, for instance, tooth enamel, middle ear, lungs, and heart valves. For better understanding, biofilm formation can be split into four steps: early reversible contact with substratum (1), irreversible attachment and matrix formation (2), ripening of biofilm (3), and detachment and spreading of microbial cells (4) as depicted in Fig. 16.1. The biofilm formation begins when actively moving planktonic microbes establishes contact with the suitable substratum (biotic/abiotic) and the attachment remains reversible. Thereafter, the microbe begins formation of a monolayer and production of extracellular matrix for protection against outer environment. Extracellular polymeric substances (EPSs), which include extracellular polysaccharides, cell debris, structural proteins, and nucleic acids, are the major components of the matrix. The beginning of the extracellular matrix is mainly dominated by extracellular DNA (eDNA) and later other components such as polysaccharides and structural proteins are formed. Later, microcolonies with significant growth potential and strong cell-cell communication such as quorum sensing (QS) are formed resulting in a three-dimensional growth of biofilm and the attachment to substratum becomes irreversible. The last stage is defined when the microbial cells start detaching from the mature biofilm and spread into the environment and give rise to a new cycle of biofilm formation.

An EPS enclosing the microbial population within the biofilm contains large amount of water molecules and thus helps formation of hydrogen bonds among the rooted microorganisms. The basic configuration of a biofilm depends upon the structure and composition of the polysaccharides. Mostly, the backbone of EPS is

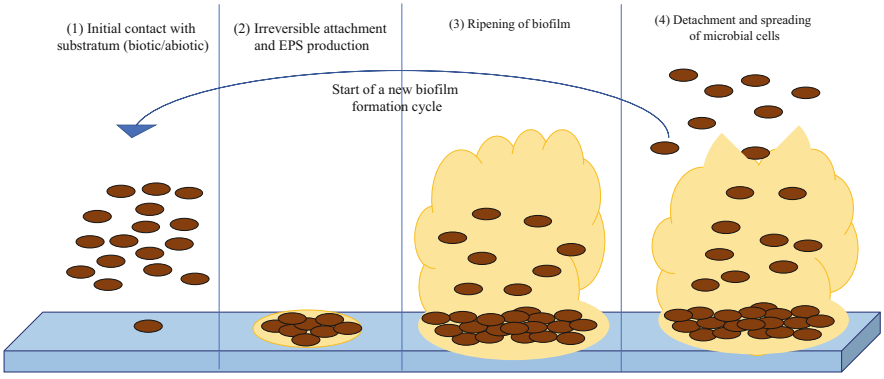

Fig. 16.1 Stages of formation and development of biofilm. The process of biofilm formation starts with the attachment of actively moving planktonic microbes (brown ovals) in a reversible manner to a suitable biotic/abiotic surface (blue) (1). Next, a monolayer is formed that represents to irreversible attachment and extracellular matrix is produced and resulting to multilayer appearance (2). Later, the maturation of biofilm starts which shows a typical "mushroom" shape (3). At last, detachment of cells starts resulting in the establishment of a new cycle

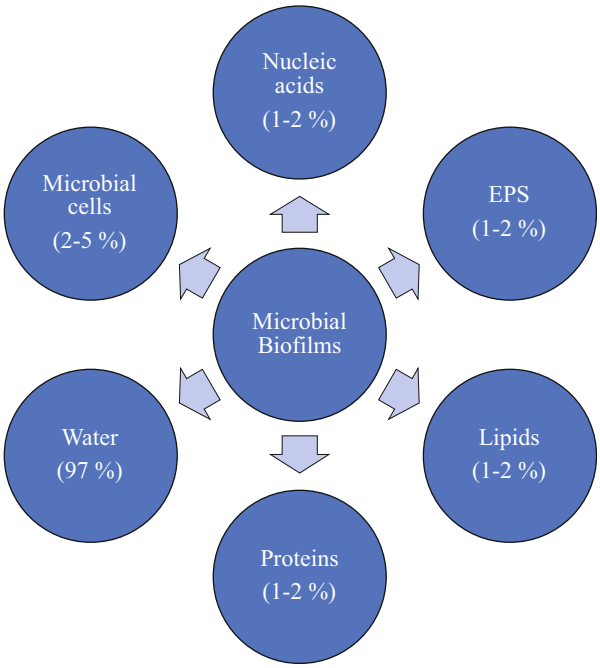

Fig. 16.2 Description of various components of microbial biofilms

composed of hexose sugar units giving it rigidity and low solubility (Sutherland 2001). However, the quantity of EPS produced differs significantly among various microorganisms and the amount increases with the maturation age (Leriche et al. 2000). Furthermore, besides polysaccharides, the biofilms are composed of other biomolecules such as nucleic acids, protein, and lipids (Fig. 16.2). The biofilms are significantly intrinsic as well as heterogeneous in nature comprising of cellular deposition which may range from monolayer to several layers, but the structure of biofilm is also dependent on the type of microbe. It has been reported that pure bacterial cultures such as *K. pneumoniae* and *P. aeruginosa* have tendency to form biofilms and the thickness may vary between 15 and 30 μm, whereas the thickness of biofilm formed by mixed microbial culture is much higher as one species augments the stability of other species (Ica et al. 2012).

The EPS provides barrier against a wide range of antimicrobial drugs, whereas the other components such as proteins, lipids, and nucleic acids increase the mechanical stability of the biofilm. It also enables the attachment of microbial communities to various biotic and abiotic surfaces such as catheters, dentures, artificial valves, etc. It has been reported that biofilm forms three-dimensional structure which is well organized and associated with specialized functions (Limoli et al. 2015).

16.4 Biofilm Resistance to Host Immune System

Microorganisms exist as microflora in every part of human body such as skin, gut, and up the nose and most of the time they live as a commensal but sometimes they turn into pathogen and serve as a reason to human sickness. Therefore, existence of microbes in all types of environmental conditions is alarming to human. Though, microbial community has various plus over individual microbial cells and a complex interaction between human host immune system and microbes forming biofilms has been reported by researchers which in turn make biofilm-forming microbes resistant to human immune system (Moser et al. 2017). The infections caused by morbific microbial communities can be life threatening as they can be resistant to standard treatments available. Biofilm provides a highly protected environment for microbial communities, devoid them from various adverse factors, and allow them to survive external stresses of host immune system and antimicrobials agents (Gebreyohannes et al. 2019a, b). The EPS provides first level of defense and the exopolysaccharide alginate prevents cells from host leukocyte phagocytosis and as a result, biofilm associated infections are difficult to resolve by individuals' own immune system (Leid et al. 2005). The biofilm related infections on host tissue are usually chronic such as endocarditis, chronic lung infections in cystic fibrosis patients, chronic rhinosinusitis, recurrent urinary tract infection, periodontitis, and dental caries (Burmølle et al. 2010; Sharma et al. 2019). The most important biofilm related infections in human are mentioned in Table 16.1.

16.5 Quorum Sensing: A Microbial Crosstalk

Biofilms and quorum sensing (QS) are very closely related and are regulated by expression of a panel of genes. QS can be described as cell-to-cell communication and plays a critical role in biofilm formation. It monitors the microbial population and when the desired cellular density is achieved it triggers the signaling pathway that produces autoinducers and supports the process of QS (Federle and Bassler 2003). On the other hand, high cellular density enables the microorganisms to secrete autoinducers into the outer environment and this in turn elicits QS mechanism. Studies have established the significance of QS in raising the prospects of nutrition availability and allow the bacterial community to compete with other group of microbes (Thoendel et al. 2011). The autoinducing peptides play a critical role in inducing QS among Gram-positive bacteria, these peptides are found to be species and, strain-specific and, specifically reported in *Clostridium*, *Staphylococci*, and *Enterococci* species. Another class of autoinducer, acyl-homoserine lactones (AHLs) is commonly found associated with Gram-negative bacteria mainly, *Acinetobacter*, *Pseudomonas*, and *Burkholderia*, species. Other widely reported types of signaling molecules in bacterial species include norepinephrine, fatty acids, epinephrine, quinolones, and ketones (Rémy et al. 2018).

Table 16.1 Common biofilm associated infections in human

S. no.	Microorganisms	Infection/Diseases	Surface	References
1.	*S. mutans*	Dental caries and endocarditis	Tooth surface and vascular grafts	Abranches et al. (2011), Metwalli et al. (2013)
2.	*E. faecalis*	Dental infections and endocarditis	Urinary catheters, tooth central, heart valves, and venous catheters	Minardi et al. (2012)
3.	*K. pneumonia*	Pneumonia, pyogenic liver abscess, and respiratory and urinary tract infections	Lungs and liver	Chung (2016)
4.	*P. aeruginosa*	Nosocomial infection, otitis media, and cystic fibrosis	Lungs, central venous, catheters, middle ear, prostheses, and contact lenses	Werner et al. (2004), Wiley et al. (2012), Huse et al. (2013)
5.	*Staphylococcus sp.* (*S. aureus,* and *S. epidermidis*)	Nosocomial infections, chronic wounds, endocarditis, musculoskeletal, and infections otitis media	Sutures, central venous catheters, arteriovenous shunts, prostheses surfaces/ deep skin prostheses, heart valves bones, and middle ear	Qu et al. (2010), Arciola et al. (2012)
6.	*E. coli*	Bacterial prostatitis, Urinary tract infection, and Otitis media	Prostheses, urinary tract, urinary catheters, and middle ear	Jackson et al. (2002)
7.	*Haemophilus influenzae*	Otitis media	Middle ear	Romero Diaz et al. (2011), Takei et al. (2013)
8.	*Burkholderia cepacia*	Cystic fibrosis	Lungs	Zlosnik et al. (2011)
9.	*Mycobacterium tuberculosis*	Tuberculosis	Lungs	Qvist et al. (2014)
10.	*Candida* sp.	Candidiasis	Vascular catheters Urinary catheters dentures Subcutaneous implant	Nett (2016)

16.6 Biofilms and Antimicrobial Resistance

In addition to critical host characteristics, the biofilms are resistant to a variety of antimicrobial treatments. The expression of various essential genes in microbial communities is the most important factor for increased antimicrobial resistance in biofilm. Along with the genetic makeup which triggers the expression of critical

genes in support of the microbes, the EPS checks the entry of antimicrobial drugs into the matrix and another crucial factor is quorum sensing that gives extra leverage to the microbial communities embedded in the biofilm and therefore, the biofilm enables the pathogen to withstand the antimicrobial agent (Brackman et al. 2011). The reduced capacity of administered medicines to pass through bacteria biofilms is thought to be the primary cause of increased antimicrobial resistance. The reason may include various chemical interactions happening within the biofilm or due to the presence of anionic polysaccharides. Previous studies in *P. aeruginosa* have demonstrated the importance of alginates in preventing the entry of positively charged amino glycosides into the biofilms (Walters III et al. 2003).

Mechanisms of drug resistance in biofilm communities are not analogous to planktonic cells, for instance, mutations in target site, reduced cell permeability, overexpression of efflux pumps, involvement of enzymes responsible for drug modification, and presence of drug neutralizing proteins (Sharma et al. 2019). However, the contribution of conventional antimicrobial resistance mechanisms in biofilm communities which promote drug resistance cannot be overlooked. The antimicrobial resistance in biofilms is an outcome to various other strategies such as partial or slow entry of antibiotics into the biofilm, modification of biofilm microenvironment, presence of a subpopulation of microbes or persisted cells in a biofilm (Sharma et al. 2019). These phenomena result in multicellular behavior of biofilms and results to the antibiotic resistance in addition to the established resistance mechanisms and complicate the treatment scheme. Development of biofilm is well coordinated and supported by intercellular and intracellular signaling processes. Upregulation and downregulation of certain vital genes/proteins responsible for adherence onto the substrate and pathways differentiation have been reported by researchers (Costerton et al. 1999; O'Toole et al. 2000; Whiteley et al. 2001; Beloin and Ghigo 2005). Quorum sensing (QS) controls the ripening of the biofilm into a complex three-dimensional structure suggesting that multicellular nature of biofilm community is mainly responsible for development of antimicrobial resistance (Davies et al. 1998).

The antimicrobial resistance nature of biofilm community results in failure of treatment strategies. The biofilm associated infection can be easily spotted on biological implants such as replacement of heart valves, catheters, dentures, and joint implants. Biofilms related infections are critical to treat and pose a menace to human health. Studies revealed that EPS has been deliberating tolerance to aminoglycosides, as it may reduce the efficiency of the drug diffusing across the biofilm by reaction inhibition phenomenon and therefore chelate the drug by forming a complex or degrade via enzymatic reactions (Billings et al. 2015; Daddi Oubekka et al. 2012). Onset of unusual growth stages such as stationary phase/viable but nonculturable state is the main strategy used by biofilms communities for persistence during antimicrobial stress conditions. With increasing time, the microbial cells embedded within the biofilm enter stationary phase and show reduced susceptibility to various commonly used drug, therefore, mature, and older biofilms display greater tolerance to drugs (Brown et al. 1988; Li et al. 2014). Persisted cells are another state of microbial subpopulation present in biofilms; they do not possess

genetic variations instead they are tagged with multidrug tolerance phenotype. These cells are found dominating when cells of biofilm communities enter stationary state (Keren et al. 2011). The absorption of drug resistance genes by horizontal gene transfer is another often documented mechanism of drug resistance in biofilm. The process of horizontal gene transfer is favored by conditions such as high cell density, elevated genetic competence, and buildup of genetic elements (Fux et al. 2005; Mah 2012). Some research studies have demonstrated the potency of antimicrobials in different states of biofilm development and suggested that early stage of biofilms is less resistant to the treatment as compared to the mature biofilms (Muñoz-Egea et al. 2015, 2016).

16.7 Nanotechnology: A Recent Breakthrough in Therapeutics

Nanotechnology is a coming revolution with enormous potential in a variety of fields, including mechanics, medicine, and the food business (Fig. 16.3). It is the study of manipulating and controlling matter on an atomic and molecular scale with at least one characteristic dimension in nanometers, which is usually between 1 and 100 nm (Kapp Jr 2021). It has emerged as one of the most promising science-related technologies. Nanotechnology-produced metal nanoparticles have received a lot of attention because of their wide range of applications in biomedical, food and agriculture, electrical and electronics, optical, communication and physiochemical disciplines (Singh et al. 2016; Ray et al. 2009; Thiruvengadam et al. 2018).

Fig. 16.3 Application of nanotechnology in different fields

Nanotechnology allows for investigation down to the level of altering atoms, molecules, and chemical bonds. Nanopores, nanotubes, quantum dots, nano shells, nanospheres, nanowires, nano capsules, dendrimers, nanorods, liposomes, and other nanoparticles are among them (Dahman et al. 2017; Dahman 2017). It can be used in pharmacological research, clinical diagnosis, immune system supplementation, cryogenic storage of biological tissues, protein detection, DNA structure probing, tissue engineering, tumor destruction, and other medical fields (Shahana and Ganapathy 2020; Abiodun-Solanke et al. 2014). Smart and active packaging, nano-sensors, nano-pesticides, and nano-fertilizers, as well as the rapid advancement of nanotechnology, have all contributed in the transformation of traditional food and agriculture businesses. A variety of new nanomaterials have been produced to improve food quality and safety, crop development, and environmental monitoring (Ashraf et al. 2021; Hamad et al. 2020; He et al. 2019).

Nanotechnology is a big step forward for medicine's future. Nanoparticles have already established themselves in a variety of biomedical applications in drug delivery systems, including in vivo site-specific imaging, cancer detection, anticancer, neurodegenerative disease therapy, ocular disease therapy, respiratory disease therapy, antifungal, antiviral, and antimicrobial therapy (Bhatia 2016; Tekade et al. 2017). Nanoparticles are used in targeted drug and gene delivery, bio-imaging, tissue engineering, hyperthermia, separation and purification of biological molecules and cells, cancer therapy, antibacterial and antifungal gel and powder, bio-labeling, osteoporosis, and dental amalgams, among other biomedical applications (Sur et al. 2019; Boroumand Moghaddam et al. 2015). Nanotechnology has aided huge advancements in computing and electronics, resulting in quicker, smaller, and more portable systems capable of managing and storing ever-increasing volumes of data (Aithal and Aithal 2015). Nanotechnology for Catalysis and Solar Energy Conversion addresses today's energy conversion issues, including high efficiency, stability, safety, and low-cost alternatives. (Banin et al. 2020).

The top-down and bottom-up techniques to nano-formulation can be divided into two categories (Fig. 16.4). Nano-formulation via etching away bulk material to generate the requisite tiny structural designs is key to the top-down method, which is typically accomplished through lithographic procedures (Birajdar et al. 2013). At the heart of such developments are top-down processes, which rely on dimensional reduction by selective etching and various nanoimprinting techniques, and bottom-up approaches, which assemble atoms or molecules into nanostructured materials, sometimes using supramolecular chemistry. (Ealia and Saravanakumar 2017).

16.8 Combating Biofilm Associated Infections with Nanomaterials

Nanotechnology involves several nanomaterials, viz. metal nanoparticles, nanoliposomes, nanopores, nanotubes, nanowire, nanoshèll, nanosphere, nano capsule, nanoroads, quantum dots, dendrimers, etc. Nanoparticles can be synthesized physically, chemically, and biologically. Each method for the synthesis of

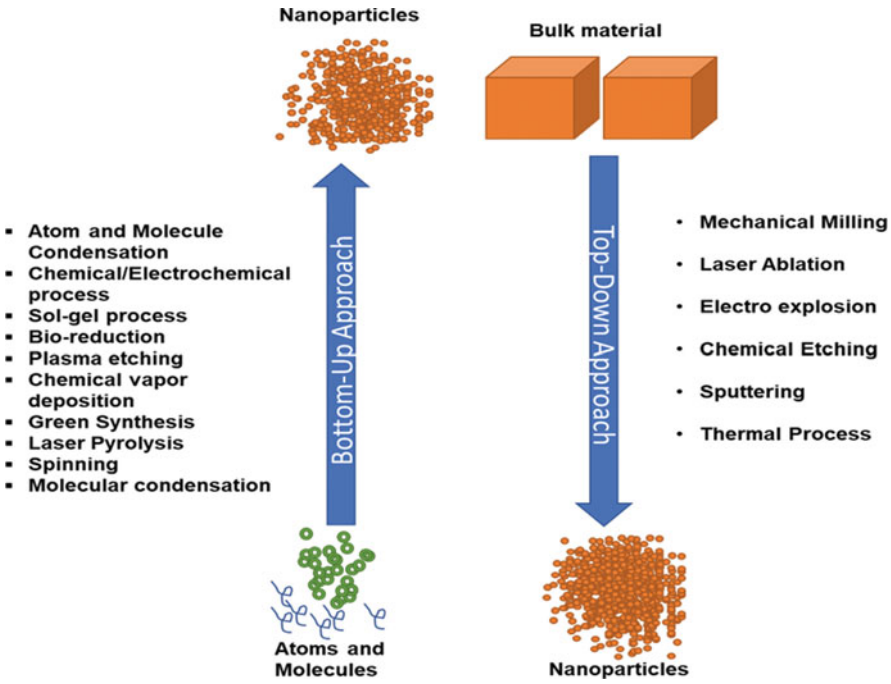

Fig. 16.4 To produce nanoparticles, there are two approaches: bottom-up and top-down

nanoparticles has different activity, which depends on the active molecules present on the surface of nanoparticles (Khan et al. 2019). In the oral environment, biofilm grows quickly, preventing the flow of silver ions and nanoparticles, protecting bacteria. The concentration of silver nanoparticles which can kill all planktonic bacteria, bacteria in biofilms does not lose 100% of their viability (Yin et al. 2020). Nanoparticles can interact in a variety of ways with microbial cell walls, such as by disrupting metabolic pathways, crossing microbial membranes, change membrane morphology and functions, inhibit enzymes, deactivate proteins, cause oxidative stress and electrolyte imbalance. (Estevez et al. 2020). Due to its eco-friendly nature, biocompatible qualities, and low cost, biological production of metal nanoparticles (NPs) has several benefits over physical and chemical synthesis. Below we have described different nanomaterials along with their importance in combating biofilm associated infectious diseases.

16.9 Physically Synthesized NPs

Nanomaterials created by plasma can be employed in a variety of biological applications and to improve human health. The physical production of nanoparticles can be done using both thermal and non-thermal plasma techniques. Sang Rye Park

et al. (2014) gave the combination treatment of low-temperature plasma with gold nanoparticles to *S. mutans* and found the enhance activity of the treatment (Park et al. 2014). The role of plasma in tackling bacteria like *E. coli, P. aeruginosa, L. casei,* and fungus *C. albicans* has been well reported because it produces a lot of reactive oxygen species (ROS) and hydroxyl radical (·OH) (Makvandi et al. 2020). Superoxide, hydrogen peroxide, singlet oxygen, hydroxyl radical, nitric oxide, ozone, and other reactive oxygen and nitrogen species are generated during plasma liquid interactions (Chauvin et al. 2017; Jha et al. 2017; Prasad 2009). The nanoparticals display antibacterial activity by following three mechanisms; (1) physical damage of cell wall, (2) produce oxidative stress by ROS, and (3) inactivate proteins and cell structures due to release of metal cations (Chauvin et al. 2017, and Prasad 2009).

A variety of irradiation processes can be used to make silver nanoparticles. Researchers like Abid et al. (2002) created silver nanoparticles (AgNPs) with defined size and shape distribution by laser irradiating an aqueous solution of silver salt and surfactant (Abid et al. 2002). Similarly, Walter et al. (2010) discovered that gold nanoparticles (AuNP) produced by laser ablation in tris buffer combined with conjugated aptamers were successful in detecting human prostate tumors (Walter et al. 2010). Microwave synthesis has been used to generate nanoparticles since it has the advantages of homogeneous heating and speed of the precursor materials, as well as a penetration property that allows for uniform heating of the reaction solution. Microwave aided AgNPs with diameters of 1–3 nm were reported by Humberto H. Lara et al. (2020) and tested against *C. auris*. Their findings revealed a significant inhibitory action against both biofilm formation and preexisting biofilms, with an IC_{50} of 0.06 ppm and 0.48 ppm, respectively. (Lara et al. 2020). Laser-ablated nanomaterials have recently been discovered to be useful in the treatment of a variety of disorders, including cancer (Qiu et al. 2019). The lead sulfide (PbS) nanoparticles were made utilizing the microwave irradiation approach with lead nitrate ($Pb(NO_3)_{2)}$ and lead acetate ($Pb(Ac)_2$) as central sources along with sulfur powder, thioacetamide, and thiourea (Sabet et al. 2019). Selenium nanoparticles (SeNPs) have recently been discovered to offer enormous promise in inhibiting biofilm formation in *C. albicans*. The study used laser ablation to produce selenium nanoparticles, which were then used to test biofilm inhibition. SeNPs alter the fungus' cellular shape by substituting sulfur groups in amino acids. As a result, the protein structure was disrupted, and *Candida* morphology was harmed. Biofilm inhibition was significantly influenced by particle size and crystallinity (Guisbiers et al. 2017).

16.10 Chemically Synthesized Nanoparticles

The uncontrolled deposition of metal-based nanoparticles in terrestrial ecosystems, particularly in biomedical systems, has posed a danger to the environment's long-term viability and the diversity of beneficial microbial communities like bacteria and fungus (Ameen et al. 2021). Antimicrobial properties of nanoparticles have been proven by metal and their oxides nanocomposites. Antibacterial, antifungal,

anticancer, and antiviral activities are all present in metal nanoparticles (Wang et al. 2017; Yaqoob et al. 2020). There are various metal and metal oxide nanoparticles (NPs) such as silver (Ag) and silver oxide (AgO), gold (Au), zinc (Zn), zinc oxide (ZnO), copper (Cu), copper oxide (CuO), nickel (Ni), magnesium (Mg), magnesium oxide (MgO), titanium (Ti), titanium oxide (TiO_2), aluminum (Al), iron (Fe), iron oxide (Fe_2O_4), silicon (Si), silicon oxide (SiO_2), calcium (Ca), calcium oxide (CaO) and aluminum oxide (Al_2O_3)NPs, as well as their mechanisms of action is well documented (Naseem and Durrani 2021; Dikshit et al. 2021). The AgNPs can permeate bacterial cell walls, altering cell membrane structure and perhaps causing cell death. Chemical reduction by organic, inorganic, and biological reducing agents is the most frequent method for producing silver nanoparticles (AgNPs). Various reducing agents, such as sodium citrate, ascorbic acid, hydrogen, sodium borohydride, polyol process, tollens reagent, N, N-dimethylformamide, and poly(ethylene glycol)-block copolymers, are used to reduce silver ions (Ag+) (Iravani et al. 2014; Lee et al. 2010; Gour and Jain 2019).

AgNPs offer unique physical, chemical, and biological features, as well as a wide spectrum of applications such as antibacterial, antiviral, antifungal, and anti-inflammatory, which has piqued interest in the therapeutic field (Zhang et al. 2016a, b). Most notably, AgNPs have a large contact area with the microorganisms due to their smaller in size and higher surface to volume proportions. The biological and chemical qualities of AgNPs greatly improve their biological and chemical capabilities and give them a strong bactericidal potential (Yun'an Qing et al. 2018). These nanoparticles can also interfere with biological functions like cell permeability and respiration (Dakal et al. 2016). Therefore, AgNPs come up as a promising antibiotics stem from their numerous modes of action, which attack germs in multiple structures at once, allowing them to kill a wide range of bacteria.

At normal temperature, AgNPs can be made by combining the appropriate metal ions with reduced poly-oxo-metalates, which act as reducing and stabilizing agents (Iravani et al. 2014). Dong et al. (2019)* investigate the bactericidal effect of different size AgNPs solutions with dimensions of 10 nm, 30 nm, 60 nm, 90 nm against, a single colony of *V. natriegens* was inoculated in 2216E medium and determined the MIC and MBC of all particle sizes and, the MIC and MBC for 10 nm AgNPs were the lowest (1 μg/m and 1.1 μg/mL) and that of 90 nm AgNPs was the highest (11.5 and 11.7 μg/mL (Dong et al. 2019). Ampicillin coated silver nanoparticles (Amp-AgNPs) susceptibility was also tested against multidrug-resistant bacteria *P. aeruginosa* and *K. pneumonia*, with MICs of 20 gμ/mL and 28.12 gμ/mL, respectively (Khatoon et al. 2019). The efficacy of Amp-AgNPs after repeated exposure to bacterial strains was also investigated (Khatoon et al. 2019).

The gold nanoparticles (AuNPs) are considered as a novel form of metal nanomaterial used in biomedicine. Because of antibiotic overuse and the production of biofilms, bacterial drug resistance is a serious problem in antibacterial therapy. Giri et al. (2015) explain how the surface charge of AuNPs affects biofilm disruption and bactericidal action against pathogens including *S. aureus* and *P. aeruginosa*, which are common causes of ventilator-associated pneumonia (Giri et al. 2015). In a study, pyrimidine-capped gold NPs (AuDAPT, AuAPT, and AuiDAPT) were

created by reducing tetra chloroauric acid with $NaBH_4$ in the presence of the respective pyrimidine ligand in methanol. The MICs for *E. coli* were 6 μg/mL, 8 μg/mL, and 6 μg/mL, respectively, while for *P. aeruginosa* was 16 μg/mL, 18 μg/mL, and 24 μg/mL, respectively (Zhao et al. 2010). In another study, Ahmad et al. (2013)* described a sono-chemical approach for producing anisotropic gold nanoparticles and investigated their size and shape-dependent fungicidal efficacy against *Candida* strains (Ahmad et al. 2013). It has been reported that AuNPs–indolicidin complex can easily permeate the biofilm matrix and cells, affecting the permeability of the fungal membrane and lowering the number of cells. AuNPs combined with the peptide indolicidin and create a very efficient molecule capable of inhibiting early biofilm formation, eradicating mature *Candida* biofilms, and even affecting viability of clinical isolates (de Alteriis et al. 2018). The AuNPs and ZnONPs were synthesised from the stem of *Tinospora cordifolia* and screened against various bacterial pathogens such as, *P. aeruginosa*, *S. mutans*, *S. pyogenes*, *V. cholerae*, *S. flexneri*, and *S. typhi*. The AuNPs were found to have remarkable anti-biofilm activity whereas, ZnONPs displayed strong antibacterial against all the test bacterial pathogens (Aditya et al. 2018). The combined effects of zinc oxide nanoparticles (ZnO-NPs) and the conventional antibiotics ciprofloxacin and ceftazidime, as well as their modes of action against resistant *A. baumannii,* were studied and it was discovered that both antibiotics and antibacterial activities were increased in the presence of a subinhibitory concentration of ZnO-NPs (Ghasemi and Jalal 2016). Recently, the interaction of metallic nanoparticles (MtNPs) with various cell models and their cellular effects was studied, and it was discovered that ROS was involved during the interaction of MtNPs with various cell lines (Zhang et al. 2016a, b; Talarska et al. 2021). Chemically produced ZnO-NP could be used as a replacement for carbapenem (beta-lactam), which inhibits carbapenem-resistant *A. baumannii* development by creating reactive oxygen species (ROS) and causing membrane damage (Tiwari et al. 2018).

16.11 Biological Synthesis of NPs

Synthesis of nanoparticles using biological sources has been well explored by researchers. On this platform Gurunathan et al. (2014) synthesized AgNPs using *Allophylus cobbe* (Tit-berry) leaf extract, and tested for anti-biofilm and antibacterial properties of AgNPs alone and with combination to other antibiotics against a variety of Gram-negative and Gram-positive bacteria such as *P. aeruginosa, Streptococcus aureus, Shigella flexneri,* and *Streptococcus pneumonia* (Gurunathan et al. 2014). AgNPs have been derived from the extracts of the plants *Semecarpus anacardium, Glochidion lanceolarium, and Breynia retusa*, were found to have excellent potential against the biofilm-forming bacteria such as *P. aeruginosa, E. coli*, and *S. aureus* (Mohanta et al. 2020). Martinez-Gutierrez et al. (2013) investigated the anti-biofilm activity of AgNPs against *A. baumannii*, *P. aeruginosa, S. aureus* (MRSA), *S. mutans,* and *C. albicans* (Martinez-Gutierrez et al. 2013). Previous reporting suggests that AgNPs have good antibacterial

characteristics and can fight a wide spectrum of pathogens, including various *Candida* species. They also have a potent inhibitory effect on the biofilms formed by various *C. auris* strains, regardless of clade, and these values are only slightly higher than the MIC values found during planktonic growth (Vazquez-Muñoz et al. 2020).

Khatoon et al. (2015) described the manufacture of silver nanoparticles utilizing Tulsi (*Ocimum sanctum*) leaf extract from a medicinal plant (Khatoon et al. 2015). The antifungal effect of silver nanoparticles made from Tulsi leaf extract was found significant, inhibiting the growth and pathogenicity of *Candida* species (Khatoon et al. 2015). Similarly, *Clerodendrum inerme* leaves extract was used for the synthesis of gold (CI-Au) and silver (CI-Ag) nanoparticles (Khan et al. 2020a, b). The antibacterial and antimycotic properties of the synthesized NPs were tested against various pathogenic pathogens like *Bacillus. subtilis, Streptococcus. aureus, K. pneumoniae* and *E. coli*, *Aspergillus niger, Trichoderma harzianum,* and *A. flavus* (Khan et al. 2020a, b). To compare the antibacterial and biofilm inhibitory effect of plant-mediated AgNPs (P-AgNPs) to commercial AgNPs (C-AgNPs) against *S. aureus* ATCC 25923, researchers used *Zataria multiflora* derived silver nanoparticles (AgNPs) and found that the P-AgNPs showed higher ability to inhibit *S. aureus* biofilm formation than C-AgNPs (Barabadi et al. 2021).

Zinc oxide nanoparticles (ZnO-NPs) have exhibited antibacterial potential against foodborne pathogens *Salmonella* species, *E. coli*, and *S. aureus* (Mohd Yusof et al. 2021). *P. aeruginosa* virulence factors and biofilm development were inhibited by Zn^{2+} and ZnO nanoparticles without limiting planktonic growth. In *P. aeruginosa*, Zn^{2+} and ZnO nanoparticles lowered down the multiple virulence determinants, including pyocyanin toxin, *Pseudomonas* quinolone signal (PQS) a quorum sensing signaling molecule, pyochelin, biofilm formation, and hemolytic activity (Lee et al. 2014). Using microorganisms such as *E. coli, P. aeruginosa, B. subtilis,* and *K. pneumoniae,* diverse morphological properties of gold, silver, cadmium sulfide, and titanium dioxide nanoparticles have been produced (Iravani 2014; Patra et al. 2018a, b). These nanoparticles, particularly silver nanoparticles, have been widely explored in vitro for their antibacterial, antifungal, and cytotoxicity potential due to their higher potential among all metal nanoparticles (Franci et al. 2015; Pajardi et al. 2016). Another metal selenium nanoparticles (SeNPs) were synthesized by incubating aqueous sodium selenite (Na_2SeO_3) with an alcoholic extract of *Psidium guajava*(guava) leaf, and the synthesized nanoparticles have antibacterial activity on both Gram-positive and Gram-negative bacteria (Alam et al. 2019). The cell free extract of probiotic bacteria *Lactobacillus acidophilus* can reduce selenium (Se^{+4}) into nano selenium (Se^0) and the synthesized LA-SeNPs have potential to degrade the biofilm of *E. coli, S. aureus, P. aeruginosa, B. subtilis,* and *K. pneumonia* (Alam et al. 2020). Nanozymes (Dex-NZM) are dextran coated iron oxide nanoparticles (FeO-NPs) that have strong catalytic activity at acidic pH values, target biofilms with high selectivity, and prevent severe caries in vivo without affecting surrounding oral tissues (Naha et al. 2019).

16.12 Role of Other Nano Carriers in Biofilm Degradation

16.12.1 Quantum Dots

Quantum dots (QDs) are tiny crystals that can transport electrons and can be synthesized in laboratory. When ultraviolet light strikes these semiconducting nanoparticles, they emit a rainbow of colors. Composites, solar cells, and fluorescent biological labels have all benefited from these artificial semiconductor nanoparticles. Duncan et al. 2015, developed a multimodal antimicrobial delivery vehicle, the nanoparticle stabilized capsules were found to be particularly effective in treating pathogenic biofilms formed by clinical isolates (Duncan et al. 2015). When compared to typical organic fluorophores, quantum dots (QDs) are a category of semiconductor nanoparticles with strong photoluminescent characteristics. As a result, drug-conjugated QDs could be a viable treatment option for biofilms, as well as a preventative measure against refractory biomaterial-associated infections caused by resistant strains (Priyadarshini et al. 2018).

Curcumin quantum dots were studied for antibacterial and anti-biofilm action to attain increased solubility and stability (Singh et al. 2017). To thwart the biofilm formation or even pathogenic invasion on implants and catheters, QDs-based nanocomposites can be used. For biofilm inhibition and other targeted treatments, QDs could be engineered by using different coating agents and bioactive ligands or biorecognition elements (Priyadarshini et al. 2018). Singh et al. (2017) discovered that Curcumin Quantum Dots (CurQDs) reduce biofilm development against *E. coli* (ATCC 25922), but not in *P. aeruginosa* (ATCC 25619) (Singh et al. 2017). When the drug was tested on high and medium biofilm generating reference strains of *S. epidermidis* (ATCC35984 and ATCC35983), it was discovered that more than half of the biofilm biomass formed by them was inhibited at concentrations of 0.25 and 0.0156 μg/mL, respectively (Singh et al. 2017). Because of the QDs assault mechanism, the creation of superoxide radicals is known to promote biofilm inhibition. It has been shown that a CdTe-2.4 eV QD-antibiotic combination therapy may nearly eradicate biofilms of *E. coli*, methicillin-resistant *S. aureus* and *P. aeruginosa* (Stamo et al. 2021). The evolution of QD partitioning and physicochemical modifications over the next 24 h were used to assess QD interactions with the biofilm/mineral interface. The stability of the QDs and the speciation of the constituent elements are expected to evolve due to the high reactivity of the biofilm/mineral interface, high site densities, and the presence of microenvironments inside the biofilm (Desmau et al. 2020; Mir et al. 2018).

16.12.2 Hydrogels

In vitro, bacteriophage-encapsulating hydrogels destroyed their host bacteria in planktonic and biofilm topologies while leaving human mesenchymal stromal cells metabolically unaffected (Wroe et al. 2020). The *P. aeruginosa* infected mouse radial segmental deficits were treated using bacteriophage-encapsulated hydrogels.

At 7 days after implantation, the hydrogels reduced live *P. aeruginosa* levels at the infection site by 4.7 times compared to bacteriophage-free hydrogels, indicating that bacteriophage-delivering hydrogels could be used to treat local bone infections (Wroe et al. 2020). Likewise, Yeo et al. (2018) described a novel hydrogel that killed bacterial biofilm through non-leaching debridement and ex situ contact-killing (DESCK) distant from the infection area (Yeo et al. 2018). In a mouse excisional wound infection model, DESCK hydrogel successfully destroys (>99.9% decrease) biofilm of methicillin-resistant *S. aureus* (MRSA), carbapenem-resistant *P. aeruginosa* (CR-PA), and *A. baumannii* (Yeo et al. 2018).

E. coli O157:H7 (ECOH) pathogenicity and biofilm development were suppressed by a persistent nano-emulsion (NE) hydrogel covering containing *Gaultheria fragrantissima* essential oil (EO) bioactive components, particularly eugenol (E-NE) and methyl salicylate (MS-NE), without impacting ECOH planktonic cell growth (Barik and Singh 2019). Hydrogels having three-dimensional crosslinked hydrophilic networks, variable mechanical properties, and substantial drug-loading capacity are suitable coating materials for killing bacteria and/or preventing bacterial adhesion on the surface, preventing biofilm development (Fu et al. 2021).

16.12.3 Liposomes

Antimicrobials and antimicrobial delivery systems based on nanotechnology have been developed in recent decades with the goal of allowing antibiotic-free treatment of pathogenic biofilms throughout the human body (Yang et al. 2021; Wang et al. 2020). Strong antibiotic dosages can be administered directly to bacteria using liposomal phospholipid bilayers that easily merge with bacterial cell membranes (Wang et al. 2020). Liposomes (small lipid vesicles) are phospholipid bilayer-coated closed vesicles in which water-soluble drugs can be introduced into the aqueous phase and lipid-soluble pharmaceuticals can be incorporated into the lipid phase (Wang 2021; Camilo et al. 2020). Liposomes' unique qualities, including as cargo variety, target selectivity, non-immunogenicity, low toxicity, and biofilm matrix/cell membrane permeability, help antimicrobial agents work more effectively and prevent infection recurrence (Akbarzadeh et al. 2013). The discovery of an antibiotic or antimicrobial delivery mechanism that permits the antimicrobial to penetrate deeply into a biofilm and kill biofilm bacteria across the entire thickness of the biofilm is a first obstacle in the development of novel infection-control techniques. (Liu et al., 2019 and Frolova et al. 2013).

16.13 Strategies Adopted by NPs to Combat Microbial Biofilms

The microbial biofilms are highly resistant to antimicrobial drugs however, earlier reports have displayed the anti-biofilm potency of NPs. The strategies involved in combating microbial biofilms can be compiled into three-stage process: (1) entry of

NPs in the biofilm environment; (2) adherence of NPs to the biofilm surface; and (3) movement of NPs in microbial biofilms (Shkodenko et al. 2020). The execution of above steps is guided by several attributes such as the physicochemical properties of the NPs, EPS, and the environment.

When NPs reach the biofilm vicinity, the initial attachments as well as movement of NPs in the biofilm matrix are guided by the EPS. The primary adherence of NPs to the boundary of biofilms can be determined by various crucial factors. Mainly, the interaction is governed by their electrostatic properties of the NPs (zeta potential) and the charge associated with the biofilm matrix (Shkodenko et al. 2020; Selvakumar et al. 2014). It has been reported that most commonly microbial biofilm matrix is polyionic due to the existence of sulfate (rarely) or uronic acid or metal-bound pyruvate with the functions of carboxylic acid and residual phosphate (Hajipour et al. 2012). Therefore, the +ve charged NPs can interact with −ve charged biofilm matrix through electrostatic forces.

After successful attachment of NPs to the biofilm matrix the next step is the entry of associated NPs into the microbial biofilm. The most common way of penetration and traveling of NPs within the biofilm is through diffusion (Peulen and Wilkinson 2011). The diffusion of NPs inside the microbial biofilm is determined by the pore size, presence of water, electrostatic interaction between NPs and EPM channels, hydrophobicity of the surrounding and the chemical gradient of the biofilm matrix (Sahle-Demessie and Tadesse 2011; Stewart 2003; Peulen and Wilkinson 2011, and Habimana et al. 2011). The pores present on the biofilm matrix can have different ionic concentrations. Various ions and molecules penetrate and diffuse the biofilm as well as get distributed with the help of these pores. Therefore, the infiltration and movement of NPs inside the microbial biofilm are mainly defined by the size and electrostatic charge of the NPs along with the structure and make-up of biofilm matrix (Ikuma et al. 2015).

Microbial biofilm is a three-dimensional structure, and the EPS makes it highly resistant to foreign substances. Previous reports have mentioned that NPs inhibit biofilm formation in pathogenic microbes by interacting with EPS of biofilm. The silver NPs are reported to inhibit biofilm formation in drug-resistant strains of *K. pneumoniae* and *E. coli* by interfering with the production of EPS (Ansari et al. 2012). Researchers have demonstrated that NPs can impede the rate of biofilm formation as well as attachment to substrate, however, the mechanism is not fully known (Peng et al. 2013; Su et al. 2009). The AgNPs synthesized by Mohanty and co-workers showed anti-biofilm activity against pathogenic bacterial strains (Mohanty et al. 2012). Similarly, Pan and co-workers showed that bacterial metabolic activity can be altered by NPs, and it is important to note that bacterial metabolism is a vital phenomenon of microbial biofilms (Qiu et al. 2016). Additionally, researchers have confirmed that potassium ion channels are responsible for carrying long-distance electrical signal among bacterial communities embedded in the biofilms. Also, potassium ions play an important role in coordinating bacterial metabolic processes both inside and outside the biofilm (Lundberg et al. 2013). Magnesium nanoparticles (MgNPs) are reported to inhibit biofilm formation in

bacterial isolates by disrupting membrane potential, augmenting lipid peroxidation, and binding to nucleic acids (Lellouche et al. 2012).

Studies have confirmed that ion channels in microbial biofilms are primary target of NPs and therefore they can act on the microbial metabolic activity. Hence, the mode of action of NPs against microbial biofilms is associated with the regulation of microbial metabolism.

16.14 Summary

With growing multidrug resistance (MDR), in which pathogens are becoming resistant to commonly used antimicrobials, it is becoming a very challenging to combat infectious diseases that are associated with high morbidity and mortality. Emerging resistance towards antibiotic is also one of the outcomes of the ability of pathogens to form biofilms which therefore results in development of chronic infections. However, the advancements in the field of medical science have improved the therapeutic regimen against biofilm associated infections as well as our understanding of the etiology at the molecular level.

Nanobiotechnology is a breakthrough in the field of research and development and offers several possibilities for improving conventional way of biofilm treatment. The NPs are considered as a conceivable substitute to antimicrobials and have a greater potential to tackle the issues related to the emergence of MDR pathogens along with biofilm associated infections. Liposomes appear to be a promising drug delivery method for antimicrobial medicines. However, the mode of anti-biofilm action of NPs is poorly studied. Surface modification of nanocarriers may improve drug targeting and biofilm treatment performance. Therefore, in-depth study related to mode of action of NPs against both plankton cells and biofilms will provide a better understanding and will allow more focused use of NPs.

Nanobiotechnology may thus aid in the development of smart, well-tolerated, cost-effective, and therapeutic drug delivery systems for the eradication of biofilm associated infections.

References

Abid JP, Wark AW, Brevet PF, Girault HH (2002) Preparation of silver nanoparticles in solution from a silver salt by laser irradiation. Chem Commun 7:792–793

Abiodun-Solanke IMF, Ajayi DM, Arigbede AO (2014) Nanotechnology and its application in dentistry. Ann Med Health Sci Res 4(3):171–177

Abranches J, Miller JH, Martinez AR, Simpson-Haidaris PJ, Burne RA, Lemos JA (2011) The collagen-binding protein Cnm is required for *Streptococcus mutans* adherence to and intracellular invasion of human coronary artery endothelial cells. Infect Immun 79(6):2277–2284

Aditya A, Chattopadhyay S, Jha D, Gautam HK, Maiti S, Ganguli M (2018) Zinc oxide nanoparticles dispersed in ionic liquids show high antimicrobial efficacy to skin-specific bacteria. ACS Appl Mater Interfaces 10:15401–15411

Aithal PS, Aithal S (2015) Ideal technology concept & its realization opportunity using nanotechnology. Int J Appl Innov Eng Manag 4(2):153–164

Akbarzadeh A, Rezaei-Sadabady R, Davaran S, Joo SW, Zarghami N, Hanifehpour Y et al (2013) Liposome: classification, preparation, and applications. Nanoscale Res Lett 8(1):1–9

Alam H, Khatoon N, Raza M, Ghosh PC, Sardar M (2019) Synthesis and characterization of nano selenium using plant biomolecules and their potential applications. BioNanoScience 9(1): 96–104

Alam H, Khatoon N, Khan MA, Husain SA, Saravanan M, Sardar M (2020) Synthesis of selenium nanoparticles using probiotic bacteria *Lactobacillus acidophilus* and their enhanced antimicrobial activity against resistant bacteria. J Clust Sci 31(5):1003–1011

Ameen F, Alsamhary K, Alabdullatif JA, ALNadhari, S. (2021) A review on metal-based nanoparticles and their toxicity to beneficial soil bacteria and fungi. Ecotoxicol Environ Saf 213:112027

Ansari MA, Khan HM, Khan AA, Sultan A, Azam A (2012) Synthesis and characterization of the antibacterial potential of ZnO nanoparticles against extended-spectrum β-lactamases-producing Escherichia coli and Klebsiella pneumoniae isolated from a tertiary care hospital of North India. Appl Microbiol Biotechnol 94(2):467–477

Arciola CR, Campoccia D, Speziale P, Montanaro L, Costerton JW (2012) Biofilm formation in *Staphylococcus* implant infections. A review of molecular mechanisms and implications for biofilm-resistant materials. Biomaterials 33:5967–5982

Ashraf SA, Siddiqui AJ, Abd Elmoneim OE, Khan MI, Patel M, Alreshidi M et al (2021) Innovations in nanoscience for the sustainable development of food and agriculture with implications on health and environment. Sci Total Environ 768:144990

Banin U, Waiskopf N, Hammarström L, Boschloo G, Freitag M, Johansson EM et al (2020) Nanotechnology for catalysis and solar energy conversion. Nanotechnology 32(4):042003

Barabadi H, Mojab F, Vahidi H, Marashi B, Talank N, Hosseini O, Saravanan M (2021) Green synthesis, characterization, antibacterial and biofilm inhibitory activity of silver nanoparticles compared to commercial silver nanoparticles. Inorg Chem Commun 129:108647

Barik SK, Singh BN (2019) Nanoemulsion-loaded hydrogel coatings for inhibition of bacterial virulence and biofilm formation on solid surfaces. Sci Rep 9(1):1–13

Beloin C, Ghigo JM (2005) Finding gene-expression patterns in bacterial biofilms. Trends Microbiol 13(1):16–19

Bhatia S (2016) Nanoparticle types, classification, characterization, fabrication methods and drug delivery applications. In: Natural polymer drug delivery systems. Springer, Cham, pp 33–93

Billings N, Birjiniuk A, Samad TS, Doyle PS, Ribbeck K (2015) Material properties of biofilms—a review of methods for understanding permeability and mechanics. Rep Prog Phys 78(3):036601

Birajdar GO, Kadam VS, Chintale AG, Halle PD, Nabde MK, Maske KS (2013) A comprehensive review on nanotechnology. Res J Pharm Technol 6(5):486–495

Boroumand Moghaddam A, Namvar F, Moniri M, Azizi S, Mohamad R (2015) Nanoparticles biosynthesized by fungi and yeast: a review of their preparation, properties, and medical applications. Molecules 20(9):16540–16565

Brackman G, Cos P, Maes L, Nelis HJ, Coenye T (2011) Quorum sensing inhibitors increase the susceptibility of bacterial biofilms to antibiotics in vitro and in vivo. Antimicrob Agents Chemother 55(6):2655–2661

Brown MR, Allison DG, Gilbert P (1988) Resistance of bacterial biofilms to antibiotics a growth-rate related effect? J Antimicrob Chemother 22(6):777–780

Burmølle M, Thomsen TR, Fazli M, Dige I, Christensen L, Homøe P et al (2010) Biofilms in chronic infections–a matter of opportunity–monospecies biofilms in multispecies infections. FEMS Immunol Med Microbiol 59(3):324–336

Camilo CJ, Leite DO, Silva AR, Menezes IR, Coutinho HD, Da Costa JG (2020) Lipid vesicles: applications, principal components and methods used in their formulations. A review. Acta Biol Colombiana 25(2):339–352

Chauvin J, Judée F, Yousfi M, Vicendo P, Merbahi N (2017) Analysis of reactive oxygen and nitrogen species generated in three liquid media by low temperature helium plasma jet. Sci Rep 7(1):1–15

Chung PY (2016) The emerging problems of *Klebsiella pneumoniae* infections: carbapenem resistance and biofilm formation. FEMS Microbiol Lett 363:219
Costerton JW, Stewart PS, Greenberg EP (1999) Bacterial biofilms: a common cause of persistent infections. Science 284(5418):1318–1322
Daddi Oubekka S, Briandet R, Fontaine-Aupart MP, Steenkeste K (2012) Correlative time-resolved fluorescence microscopy to assess antibiotic diffusion-reaction in biofilms. Antimicrob Agents Chemother 56(6):3349–3358
Dahman Y (2017) Nanotechnology and functional materials for engineers. Elsevier
Dahman Y, Deonanan K, Dontsos T, Iammatteo A (2017) Nanopolymers. In: Nanotechnology and functional materials for engineers, pp 121–144
Dakal TC, Kumar A, Majumdar RS, Yadav V (2016) Mechanistic basis of antimicrobial actions of silver nanoparticles. Front Microbiol 7:1831
Davies DG, Parsek MR, Pearson JP, Iglewski BH, Costerton JW, Greenberg EP (1998) The involvement of cell-to-cell signals in the development of a bacterial biofilm. Science 280(5361):295–298
de Alteriis E, Maselli V, Falanga A, Galdiero S, Di Lella FM, Gesuele R et al (2018) Efficiency of gold nanoparticles coated with the antimicrobial peptide indolicidin against biofilm formation and development of *Candida* spp. clinical isolates. Infect Drug Resist 11:915
Desmau M, Levard C, Vidal V, Ona-Nguema G, Charron G, Benedetti MF, Gélabert A (2020) How microbial biofilms impact the interactions of quantum dots with mineral surfaces? NanoImpact 19:100247
Dikshit PK, Kumar J, Das AK, Sadhu S, Sharma S, Singh S et al (2021) Green synthesis of metallic nanoparticles: applications and limitations. Catalysts 11(8):902
Duncan B, Li X, Landis RF, Kim ST, Gupta A, Wang LS et al (2015) Nanoparticle-stabilized capsules for the treatment of bacterial biofilms. ACS Nano 9(8):7775–7782
Ealia SAM, Saravanakumar MP (2017) A review on the classification, characterisation, synthesis of nanoparticles and their application. In: IOP conference series: materials science and engineering, vol 263, no 3. IOP Publishing, pp 032019
Estevez MB, Raffaelli S, Mitchell SG, Faccio R, Alborés S (2020) Biofilm eradication using biogenic silver nanoparticles. Molecules 25(9):2023
Federle MJ, Bassler BL (2003) Interspecies communication in bacteria. J Clin Invest 112(9): 1291–1299
Franci G, Falanga A, Galdiero S, Palomba L, Rai M, Morelli G, Galdiero M (2015) Silver nanoparticles as potential antibacterial agents. Molecules 20:8856–8874
Frieri M, Kumar K, Boutin A (2017) Antibiotic resistance. J Infect Public Health 10(4):369–378
Frolova J, Chomoucka J, Adam V, Ryvolova M, Eckschlager T, Hubalek J, Kizek R (2013) Nanocarriers for anticancer drugs-new trends in nanomedicine. Curr Drug Metab 14(5): 547–564
Fu M, Liang Y, Lv X, Li C, Yang YY, Yuan P, Ding X (2021) J Mater Sci Technol 85:169–183
Fux CA, Costerton JW, Stewart PS, Stoodley P (2005) Survival strategies of infectious biofilms. Trends Microbiol 13(1):34–40
Gebreyohannes G, Nyerere A, Bii C, Sbhatu DB (2019a) Challenges of intervention, treatment, and antibiotic resistance of biofilm-forming microorganisms. Heliyon 5(8):e02192
Gebreyohannes G, Nyerere A, Bii C, Sbhatu DB (2019b) Challenges of intervention, treatment, and antibiotic resistance of biofilm-forming microorganisms. Heliyon 5(8):1–7
Ghasemi F, Jalal R (2016) Antimicrobial action of zinc oxide nanoparticles in combination with ciprofloxacin and ceftazidime against multidrug-resistant *Acinetobacter baumannii*. J Glob Antimicrob Resist 6:118–122
Giri K, Yepes LR, Duncan B, Parameswaran PK, Yan B, Jiang Y et al (2015) Targeting bacterial biofilms via surface engineering of gold nanoparticles. RSC Adv 5(128):105551–105559
Gour A, Jain NK (2019) Advances in green synthesis of nanoparticles. Artif Cells Nanomed Biotechnol 47(1):844–851

Gu H, Chen A, Song X, Brasch ME, Henderson JH, Ren D (2016) How *Escherichia coli* lands and forms cell clusters on a surface: a new role of surface topography. Sci Rep 6(1):1–14

Guisbiers G, Lara HH, Mendoza-Cruz R, Naranjo G, Vincent BA, Peralta XG, Nash KL (2017) Inhibition of Candida albicans biofilm by pure selenium nanoparticles synthesized by pulsed laser ablation in liquids. Nanomedicine 13(3):1095–1103

Gurunathan S, Han JW, Kwon DN, Kim JH (2014) Enhanced antibacterial and anti-biofilm activities of silver nanoparticles against gram-negative and gram-positive bacteria. Nanoscale Res Lett 9(1):1–17

Habimana O, Steenkeste K, Fontaine-Aupart MP, Bellon-Fontaine MN, Kulakauskas S, Briandet R (2011) Diffusion of nanoparticles in biofilms is altered by bacterial cell wall hydrophobicity. Appl Environ Microbiol 77(1):367–368

Hajipour MJ, Fromm KM, Ashkarran AA, de Aberasturi DJ, de Larramendi IR, Rojo T et al (2012) Antibacterial properties of nanoparticles. Trends Biotechnol 30(10):499–511

Hamad HT, Al-Sharify ZT, Al-Najjar SZ, Gadooa ZA (2020) A review on nanotechnology and its applications on fluid flow in agriculture and water recourses. In: IOP conference series: materials science and engineering, vol 870, no 1. IOP Publishing, pp 012038

Hassan A, Usman J, Kaleem F, Omair M, Khalid A, Iqbal M (2011) Evaluation of different detection methods of biofilm formation in the clinical isolates. Braz J Infect Dis 15(4):305–311

He X, Deng H, Hwang HM (2019) The current application of nanotechnology in food and agriculture. J Food Drug Anal 27(1):1–21

Huse HK, Kwon T, Zlosnik JE, Speert DP, Marcotte EM, Whiteley M (2013) Pseudomonas aeruginosa enhances production of a non-alginate exopolysaccharide during long-term colonization of the cystic fibrosis lung. PLoS One 8:e82621

Ica T, Caner V, Istanbullu O, Nguyen HD, Ahmed B, Call DR, Beyenal H (2012) Characterization of mono-and mixed-culture Campylobacter jejuni biofilms. Appl Environ Microbiol 78(4): 1033–1038

Ikuma K, Decho AW, Lau BL (2015) When nanoparticles meet biofilms—interactions guiding the environmental fate and accumulation of nanoparticles. Front Microbiol 6:1–6

Iravani S (2014) Bacteria in nanoparticle synthesis: current status and future prospects. Int Sch Res Notices 2014:359316

Iravani S, Korbekandi H, Mirmohammadi SV, Zolfaghari B (2014) Synthesis of silver nanoparticles: chemical, physical, and biological methods. Res Pharm Sci 9(6):385

Jackson DW, Suzuki K, Oakford L, Simecka JW, Hart ME, Romeo T (2002) Biofilm formation and dispersal under the influence of the global regulator CsrA of *Escherichia coli*. J Bacteriol 184: 290–301

Jadaun V, Singh BR, Paliya BS, Upreti DK, Rao CV, Rawat AKS, Singh BN (2015) Honey enhances the anti-quorum sensing activity and anti-biofilm potential of curcumin. RSC Adv 5(87):71060–71070

Jha N, Ryu JJ, Choi EH, Kaushik NK (2017) Generation and role of reactive oxygen and nitrogen species induced by plasma, lasers, chemical agents, and other systems in dentistry. Oxidative Med Cell Longev 2017:1–14

Kapp RW Jr (2021) Book review: information resources in toxicology

Keren I, Minami S, Rubin E, Lewis K (2011) Characterization and transcriptome analysis of *Mycobacterium tuberculosis* persisters. mBio 2(3):e00100–e00111

Khan I, Saeed K, Khan I (2019) Nanoparticles: properties, applications, and toxicities. Arab J Chem 12(7):908–931

Khan F, Pham DTN, Kim YM (2020a) Alternative strategies for the application of aminoglycoside antibiotics against the biofilm-forming human pathogenic bacteria. Appl Microbiol Biotechnol 104(5):1955–1976

Khan SA, Shahid S, Lee CS (2020b) Green synthesis of gold and silver nanoparticles using leaf extract of *Clerodendrum inerme*; characterization, antimicrobial, and antioxidant activities. Biomol Ther 10(6):835

Khatoon N, Mishra A, Alam H, Manzoor N, Sardar M (2015) Biosynthesis, characterization, and antifungal activity of the silver nanoparticles against pathogenic Candida species. BioNanoScience 5(2):65–74

Khatoon Z, McTiernan CD, Suuronen EJ, Mah TF, Alarcon EI (2018) Bacterial biofilm formation on implantable devices and approaches to its treatment and prevention. Heliyon 4(12):1–36

Khatoon N, Alam H, Khan A, Raza K, Sardar M (2019) Ampicillin silver nano formulations against multidrug resistant bacteria. Sci Rep 9(1):1–10

Konduri R, Saiabhilash CR, Shivaji S (2021) Biofilm-forming potential of ocular fluid *Staphylococcus aureus* and *Staphylococcus epidermidis* on ex vivo human corneas from attachment to dispersal phase. Microorganisms 9(6):1124

Lara HH, Ixtepan-Turrent L, Jose Yacaman M, Lopez-Ribot J (2020) Inhibition of Candida auris biofilm formation on medical and environmental surfaces by silver nanoparticles. ACS Appl Mater Interfaces 12(19):21183–21191

Lee SM, Song KC, Lee BS (2010) Antibacterial activity of silver nanoparticles prepared by a chemical reduction method. Korean J Chem Eng 27(2):688–692

Lee JH, Kim YG, Cho MH, Lee J (2014) ZnO nanoparticles inhibit Pseudomonas aeruginosa biofilm formation and virulence factor production. Microbiol Res 169(12):888–896

Leid JG, Willson CJ, Shirtliff ME, Hassett DJ, Parsek MR, Jeffers AK (2005) The exopolysaccharide alginate protects *Pseudomonas aeruginosa* biofilm bacteria from IFN-γ-mediated macrophage killing. J Immunol 175(11):7512–7518

Lellouche J, Friedman A, Lellouche JP, Gedanken A, Banin E (2012) Improved antibacterial and antibiofilm activity of magnesium fluoride nanoparticles obtained by water-based ultrasound chemistry. Nanomedicine 8(5):702–711

Leriche V, Sibille P, Carpentier B (2000) Use of an enzyme-linked lectinsorbent assay to monitor the shift in polysaccharide composition in bacterial biofilms. Appl Environ Microbiol 66(5): 1851–1856

Li L, Mendis N, Trigui H, Oliver JD, Faucher SP (2014) The importance of the viable but non-culturable state in human bacterial pathogens. Front Microbiol 5:258

Limoli DH, Jones CJ, Wozniak DJ (2015) Bacterial extracellular polysaccharides in biofilm formation and function. Microb Spectr 3(3)

Lundberg ME, Becker EC, Choe S (2013) MstX and a putative potassium channel facilitate biofilm formation in Bacillus subtilis. PLoS One 8(5):1–10

Mah TF (2012) Biofilm-specific antibiotic resistance. Future Microbiol 7(9):1061–1072

Makvandi P, Gu JT, Zare EN, Ashtari B, Moeini A, Tay FR, Niu LN (2020) Polymeric and inorganic nanoscopical antimicrobial fillers in dentistry. Acta Biomater 101:69–101

Martinez-Gutierrez F, Boegli L, Agostinho A, Sánchez EM, Bach H, Ruiz F, James G (2013) Anti-biofilm activity of silver nanoparticles against different microorganisms. Biofouling 29(6): 651–660

Metwalli KH, Khan SA, Krom BP, Jabra-Rizk MA (2013) Streptococcus mutans, *Candida albicans*, and the human mouth: a sticky situation. PLoS Pathog 9(10):1–5

Minardi D, Cirioni O, Ghiselli R, Silvestri C, Mocchegiani F, Gabrielli E et al (2012) Efficacy of tigecycline and rifampin alone and in combination against *Enterococcus faecalis* biofilm infection in a rat model of ureteral stent. J Surg Res 176(1):1–6

Mir IA, Alam H, Priyadarshini E, Meena R, Rawat K, Rajamani P et al (2018) Antimicrobial and biocompatibility of highly fluorescent ZnSe core and ZnSe@ ZnS core-shell quantum dots. J Nanopart Res 20(7):1–11

Mohanta YK, Biswas K, Jena SK, Hashem A, Abd-Allah EF, Mohanta TK (2020) Anti-biofilm and antibacterial activities of silver nanoparticles synthesized by the reducing activity of phytoconstituents present in the Indian medicinal plants. Front Microbiol 11:1143

Mohanty S, Mishra S, Jena P, Jacob B, Sarkar B, Sonawane A (2012) An investigation on the antibacterial, cytotoxic, and antibiofilm efficacy of starch-stabilized silver nanoparticles. Nanomedicine 8(6):916–924

Mohd Yusof H, Rahman A, Mohamad R, Hasanah Zaidan U, Samsudin AA (2021) Antibacterial potential of biosynthesized zinc oxide nanoparticles against poultry-associated foodborne pathogens: an in vitro study. Animals 11(7):2093

Moser C, Pedersen HT, Lerche CJ, Kolpen M, Line L, Thomsen K et al (2017) Biofilms and host response–helpful or harmful. APMIS 125(4):320–338

Muñoz-Egea MC, García-Pedrazuela M, Mahillo I, Esteban J (2015) Effect of ciprofloxacin in the ultrastructure and development of biofilms formed by rapidly growing mycobacteria. BMC Microbiol 15(1):1–6

Muñoz-Egea MC, García-Pedrazuela M, Mahillo-Fernandez I, Esteban J (2016) Effect of antibiotics and antibiofilm agents in the ultrastructure and development of biofilms developed by nonpigmented rapidly growing mycobacteria. Microb Drug Resist 22(1):1–6

Naha PC, Liu Y, Hwang G, Huang Y, Gubara S, Jonnakuti V et al (2019) Dextran-coated iron oxide nanoparticles as biomimetic catalysts for localized and pH-activated biofilm disruption. ACS Nano 13(5):4960–4971

Naseem T, Durrani T (2021) The role of some important metal oxide nanoparticles for wastewater and antibacterial applications: a review. Environ Chem Ecotoxicol

Nett JE (2016) The host's reply to *Candida* biofilm. Pathogens (Basel, Switzerland) 5(1):33

O'Toole G, Kaplan HB, Kolter R (2000) Biofilm formation as microbial development. Annu Rev Microbiol 54(1):49–79

Pajardi G, Rapisarda V, Somalvico F, Scotti A, Russo GL, Ciancio F, Sgrò A, Nebuloni M, Allevi R, Torre ML (2016) Skin substitutes based on allogenic fibroblasts or keratinocytes for chronic wounds not responding to conventional therapy: a retrospective observational study. Int Wound J 13:44–52

Park SR, Lee HW, Hong JW, Lee HJ, Kim JY, Kim GC, Jeon YC (2014) Enhancement of the killing effect of low-temperature plasma on *Streptococcus mutan*s by combined treatment with gold nanoparticles. J Nanobiotechnol 12(1):1–8

Patra JK, Das G, Fraceto LF, Campos EVR, del Pilar Rodriguez-Torres M, Acosta-Torres LS et al (2018a) Nano based drug delivery systems: recent developments and future prospects. J Nanobiotechnol 16(1):1–33

Patra JK, Das G, Fraceto LF et al (2018b) Nano based drug delivery systems: recent developments and future prospects. J Nanobiotechnol 16:71

Peiqian L, Xiaoming P, Huifang S, Jingxin Z, Ning H, Birun L (2014) Biofilm formation by *Fusarium oxysporum f.* sp. cucumerinum and susceptibility to environmental stress. FEMS Microbiol Lett 350(2):138–145

Peng Z, Ni J, Zheng K, Shen Y, Wang X, He G et al (2013) Dual effects and mechanism of TiO_2 nanotube arrays in reducing bacterial colonization and enhancing C3H10T1/2 cell adhesion. Int J Nanomedicine 8:3093–3105

Peulen TO, Wilkinson KJ (2011) Diffusion of nanoparticles in a biofilm. Environ Sci Technol 45(8):3367–3373

Prasad GL (2009) Biomedical applications of nanoparticles. In: Safety of nanoparticles. Springer, New York, pp 89–109

Priyadarshini E, Rawat K, Bohidar HB (2018) Quantum dots-based Nano-coatings for inhibition of microbial biofilms: a mini review. Nonmagn Magn Quant Dots 87

Qiu W, Zheng X, Wei Y, Zhou X, Zhang K, Wang S et al (2016) D-Alanine metabolism is essential for growth and biofilm formation of *Streptococcus mutans*. Mol Oral Microbiol 31(5):435–444

Qiu M, Singh A, Wang D, Qu J, Swihart M, Zhang H, Prasad PN (2019) Biocompatible and biodegradable inorganic nanostructures for nanomedicine: silicon and black phosphorus. Nano Today 25:135–155

Qu Y, Daley AJ, Istivan TS, Rouch DA, Deighton MA (2010) Densely adherent growth mode, rather than extracellular polymer substance matrix build-up ability, contributes to high resistance of *Staphylococcus epidermidis* biofilms to antibiotics. J Antimicrob Chemother 65:1405–1411

Qvist T, Pressler T, Høiby N, Katzenstein TL (2014) Shifting paradigms of nontuberculous *mycobacteria* in cystic fibrosis. Respir Res 15:1

Ray PC, Yu H, Fu PP (2009) Toxicity and environmental risks of nanomaterials: challenges and future needs. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 27(1):1–35

Rémy B, Mion S, Plener L, Elias M, Chabrière E, Daudé D (2018) Interference in bacterial quorum sensing: a biopharmaceutical perspective. Front Pharmacol 9:203

Romero Diaz R, Picciafuoco S, Paraje MG, Angel Villegas N, Arce Miranda E, Albesa I, Cremonezzi D, Commisso R, Paglini-Oliva P (2011) Relevance of biofilms in pediatric tonsillar disease. Eur J Clin Microbiol Infect Dis 30(12):1503–1509

Sabet M, Mohammadi M, Googhari F (2019) Prominent visible light photocatalytic and water purification activity of PbS/CdS/CdO nanocomposite synthesized via simple co-precipitation method. Nanosci Nanotechnol Asia 9(2):278–284

Sadekuzzaman M, Yang S, Mizan MFR, Ha SD (2015) Current and recent advanced strategies for combating biofilms. Compr Rev Food Sci Food Saf 14(4):491–509

Sahle-Demessie E, Tadesse H (2011) Kinetics and equilibrium adsorption of nano-TiO2 particles on synthetic biofilm. Surf Sci 605(13–14):1177–1184

Selvakumar R, Aravindh S, Ashok AM, Balachandran YL (2014) A facile synthesis of silver nanoparticle with SERS and antimicrobial activity using *Bacillus subtilis* exopolysaccharides. J Exp Nanosci 9(10):1075–1087

Shahana RY, Ganapathy D (2020) Applications of nanotechnology in dentistry. Drug Invention Today 14(5):1–7

Sharma D, Misba L, Khan AU (2019) Antibiotics versus biofilm: an emerging battleground in microbial communities. Antimicrob Resist Infect Control 8(1):1–10

Shkodenko L, Kassirov I, Koshel E (2020) Metal oxide nanoparticles against bacterial biofilms: perspectives and limitations. Microorganisms 8(10):1–21

Singh BR, Singh BN, Singh A, Khan W, Naqvi AH, Singh HB (2015) Mycofabricated biosilver nanoparticles interrupt *Pseudomonas aeruginosa* quorum sensing systems. Sci Rep 5(1):1–14

Singh P, Kim YJ, Zhang D, Yang DC (2016) Biological synthesis of nanoparticles from plants and microorganisms. Trends Biotechnol 34(7):588–599

Singh AK, Prakash P, Singh R, Nandy N, Firdaus Z, Bansal M et al (2017) Curcumin quantum dots mediated degradation of bacterial biofilms. Front Microbiol 8:1517

Srivastava A, Singh BN, Deepak D, Rawat AKS, Singh BR (2015) Colostrum hexasaccharide, a novel *Staphylococcus aureus* quorum-sensing inhibitor. Antimicrob Agents Chemother 59: 2169–2178

Stamo DF, Nagpal P, Chatterjee A (2021) Light-activated quantum dot potentiation of antibiotics to treat drug-resistant bacterial biofilms. Nanoscale Adv 3(10):2782–2786

Stewart PS (2003) Diffusion in biofilms. J Bacteriol 185(5):1485–1491

Su HL, Chou CC, Hung DJ, Lin SH, Pao IC, Lin JH et al (2009) The disruption of bacterial membrane integrity through ROS generation induced by nanohybrids of silver and clay. Biomaterials 30(30):5979–5987

Sun W, Su J, Xu S, Yan D (2012) Trichosporon asahii causing nosocomial urinary tract infections in intensive care unit patients: genotypes, virulence factors and antifungal susceptibility testing. J Med Microbiol 61(12):1750–1757

Sur S, Rathore A, Dave V, Reddy KR, Chouhan RS, Sadhu V (2019) Recent developments in functionalized polymer nanoparticles for efficient drug delivery system. Nano-Struct Nano-Objects 20:100397

Sutherland IW (2001) Biofilm exopolysaccharides: a strong and sticky framework. Microbiology 147(1):3–9

Takei S, Hotomi M, Yamanaka N (2013) Minimal biofilm eradication concentration of antimicrobial agents against nontypeable Haemophilus influenzae isolated from middle ear fluids of intractable acute otitis media. J Infect Chemother 19:504–509

Talarska P, Boruczkowski M, Żurawski J (2021) Current knowledge of silver and gold nanoparticles in laboratory research—application, toxicity, cellular uptake. Nanomaterials 11(9):1–22

Tekade RK, Maheshwari R, Soni N, Tekade M, Chougule MB (2017) Nanotechnology for the development of nanomedicine. Nanotechnology-based approaches for targeting and delivery of drugs and genes. Academic, pp 3–61

Thiruvengadam M, Rajakumar G, Chung IM (2018) Nanotechnology: current uses and future applications in the food industry. 3 Biotech 8(1):74–85

Thoendel M, Kavanaugh JS, Flack CE, Horswill AR (2011) Peptide signaling in the staphylococci. Chem Rev 111(1):117–151

Tiwari V, Mishra N, Gadani K, Solanki PS, Shah NA, Tiwari M (2018) Mechanism of anti-bacterial activity of zinc oxide nanoparticle against carbapenem-resistant *Acinetobacter baumannii*. Front Microbiol 9:1218

Vazquez-Muñoz R, Bogdanchikova N, Huerta-Saquero A (2020) Beyond the nanomaterials approach: influence of culture conditions on the stability and antimicrobial activity of silver nanoparticles. ACS Omega 5(44):28441–28451

Walter JG, Petersen S, Stahl F, Scheper T, Barcikowski S (2010) Laser ablation-based one-step generation and bio-functionalization of gold nanoparticles conjugated with aptamers. J Nanobiotechnol 8(1):1–11

Walters MC III, Roe F, Bugnicourt A, Franklin MJ, Stewart PS (2003) Contributions of antibiotic penetration, oxygen limitation, and low metabolic activity to tolerance of *Pseudomonas aeruginosa* biofilms to ciprofloxacin and tobramycin. Antimicrob Agents Chemother 47(1): 317–323

Wang Y (2021) Liposome as a delivery system for the treatment of biofilm-mediated infections. J Appl Microbiol 131(6):2626–2639

Wang L, Hu C, Shao L (2017) The antimicrobial activity of nanoparticles: present situation and prospects for the future. Int J Nanomedicine 12:1227

Wang DY, Van Der Mei HC, Ren Y, Busscher HJ, Shi L (2020) Lipid-based antimicrobial delivery-systems for the treatment of bacterial infections. Front Chem 7:1–15

Werner E, Roe F, Bugnicourt A, Franklin MJ, Heydorn A, Molin S et al (2004) Stratified growth in *Pseudomonas aeruginosa* biofilms. Appl Environ Microbiol 70:6188–6196

Whiteley M, Bangera MG, Bumgarner RE, Parsek MR, Teitzel GM, Lory S, Greenberg EP (2001) Gene expression in *Pseudomonas aeruginosa* biofilms. Nature 413(6858):860–864

Wiley L, Bridge DR, Wiley LA, Odom JV, Elliott T, Olson JC (2012) Bacterial biofilm diversity in contact lens-related disease: emerging role of *Achromobacter, Stenotrophomonas,* and Delftia biofilm diversity in contact lens-related disease. Invest Ophthalmol Vis Sci 53:3896–3905

Wroe JA, Johnson CT, García AJ (2020) Bacteriophage delivering hydrogels reduce biofilm formation in vitro and infection in vivo. J Biomed Mater Res A 108(1):39–49

Yang K, Shi J, Wang L, Chen Y, Liang C, Yang L, Wang LN (2021) Bacterial anti-adhesion surface design: surface patterning, roughness, and wettability: a review. J Mater Sci Technol 99:82–100

Yaqoob AA, Ahmad H, Parveen T, Ahmad A, Oves M, Ismail IM et al (2020) Recent advances in metal decorated nanomaterials and their various biological applications: a review. Front Chem 8:341

Yeo CK, Vikhe YS, Li P, Guo Z, Greenberg P, Duan H et al (2018) Hydrogel effects rapid biofilm debridement with ex situ contact-kill to eliminate multidrug resistant bacteria in vivo. ACS Appl Mater Interfaces 10(24):20356–20367

Yin IX, Zhang J, Zhao IS, Mei ML, Li Q, Chu CH (2020) The antibacterial mechanism of silver nanoparticles and its application in dentistry. Int J Nanomedicine 15:2555

Yun'an Qing LC, Li R, Liu G, Zhang Y, Tang X, Wang J et al (2018) Potential antibacterial mechanism of silver nanoparticles and the optimization of orthopedic implants by advanced modification technologies. Int J Nanomedicine 13:3311

Zhang XF, Liu ZG, Shen W, Gurunathan S (2016a) Silver nanoparticles: synthesis, characterization, properties, applications, and therapeutic approaches. Int J Mol Sci 17(9):1534

Zlosnik JE, Costa PS, Brant R, Mori PY, Hird TJ, Fraenkel MC, Wilcox PG, Davidson AGF, Speert DP (2011) Mucoid and nonmucoid *Burkholderia cepacian* complex bacteria in cystic fibrosis infections. Am J Respir Crit Care Med 183:67–72

Zhao Y, Tian Y, Cui Y, Liu W, Ma W, Jiang X (2010) Small molecule-capped gold nanoparticles as potent antibacterial agents that target gram-negative bacteria. J Am Chem Soc 132(35): 12349–12356

Zhang X-F, Shen W, Gurunathan S (2016b) Silver nanoparticle-mediated cellular responses in various cell lines: an in vitro model. Int J Mol 17:1603

Use of Nanoparticles in Multidrug Resistant Tuberculosis Diagnosis

17

Aiswarya Chandrasekaran and G. H. R. Eranga Karunaratne

Abstract

Tuberculosis (TB) is one of the main community health concerns causing millions of death every year globally. The irrational use of anti-tuberculosis drugs has led to the development of multidrug resistance (MDR) in *Mycobacterium tuberculosis* (MTB) which has hampered the treatment regimen and patient care in TB and posed a huge challenge in controlling the spread of the disease. Simple, quick, and reliable detection of MDR in MTB is very essential for, appropriate treatment, enhancing patient care and reducing the transmission of TB. However, the conventional methods used so far have been slow and less sensitive while the rapid molecular assays have overcome these limitations. Even though the molecular assays are quick, they are expensive and not appropriate for resource-limited settings. Recently, the trend of using nanoparticles for MDR-TB diagnosis has been emerging along with several advantages like inexpensive, rapid, sensitive, and specific. The advent of nanodiagnosis and the use of nanoparticles have shown potential for transformation and enhanced detection of MDR-TB, thus enhancing treatment regimen and supporting the TB control globally. This chapter discusses the MDR mechanisms in TB with regard to two of the most sensitive first-line anti-tuberculosis drugs (rifampicin and isoniazid) and also the novel nanoparticle based diagnostic assays used to detect MDR-TB.

Keywords

Nanoparticles · Tuberculosis · Multidrug resistance · Diagnosis · Rifampicin · Isoniazid

A. Chandrasekaran · G. H. R. E. Karunaratne (✉)
Faculty of Science, Horizon Campus, Malabe, Sri Lanka
e-mail: ekarunarathne@horizoncampus.edu.lk

S. Hameed, S. Rehman (eds.), *Nanotechnology for Infectious Diseases*,
https://doi.org/10.1007/978-981-16-9190-4_17

17.1 Introduction

Tuberculosis (TB) is a contagious infection caused by aerobic, acid-fast bacillus bacterium *Mycobacterium tuberculosis*. The global tuberculosis report of 2020 by World Health Organization (WHO) reports 1.4 million deaths due to TB in 2019 (WHO 2020). The pathogen transmitted airborne particles are named as infectious droplet nuclei which are usually ranging between 1 and 5 μm in diameter (Muthukrishnan 2021). The *Mycobacterium* genus contains more than 100 species which are grouped into *M. tuberculosis* complex (MTBC) and nontuberculous mycobacteria (Saiman 2004). MTBC species such as *M. tuberculosis, M. bovis, M. microti, M. canetti, M. africanum, M. pinnipedii,* and *M. caprae* cause TB infections to different hosts (Niemann et al. 2000) while the nontuberculous mycobacteria cause pulmonary and extra-pulmonary diseases (Saiman 2004). The TB infection may either be latent or active, where the latent infection is asymptomatic and non-infectious, while the active infection is symptomatic and infectious (Singaram 2016). The development of multidrug resistance and extensive drug resistance (MDR and XDR) in MTB has caused the increased occurrence of ill health among millions of people and remains as the second principal reason for death due to infectious disease globally (Singh et al. 2020). The conventional methods of TB diagnosis such as sputum smear and culture techniques are tedious and time-consuming processes which are also less sensitive.

The emergence of MDR-TB has challenged the tuberculosis control and prevention globally and especially in developing countries. Thus, detecting MDR-TB is crucial in determining the appropriate treatment regimen and monitoring the prognosis of the condition. Many conventional and rapid molecular diagnostic techniques for detection of drug resistance have been developed. However, these techniques have their drawbacks and limited availability. Recently, the use of nanotechnology in diagnosis of MDR-TB has been studied widely. The application of nanoparticles in the diagnosis of MDR-TB has provided promising scope for rapid and accurate detection and thus, enhanced TB control. The unique physio-chemical and optical characteristics of nanoparticles have supported in accurate diagnosis and discrimination of *Mycobacteria* (Muthukrishnan 2021).

The mechanism of MDR-TB towards two of the first-line anti-tuberculosis drugs rifampicin (RIF) and isoniazid (INH) and the use of assays based on nanoparticles are discussed in this chapter. This will help the researchers to develop novel rapid assays using nanoparticles for the diagnosis of MDR-TB.

17.2 Multidrug Resistance (MDR)

Antibiotics play a vital role in fighting against several infectious diseases such as tuberculosis, pneumonia, meningitis, and typhoid fever (Singh et al. 2014). Antibiotics combat against bacteria through several mechanisms such as inhibition of synthesis of nucleic acids, proteins, cell wall, and biofilm formation (Wright 2003). However, the improper use of antibiotics has led to a major health concern to

mankind which is the development of multidrug resistance strains of microorganisms (Munir et al. 2020). Multidrug resistance is generally defined as resistance shown by a microorganism towards an antimicrobial drug administered, despite previous susceptibility to it (Tanwar et al. 2014). Improper usage of antibiotics, long treatment durations for MDR bacteria, and drugs being prescribed as prophylactic treatment for numerous infections are major causes of resistance developed by strains (Munir et al. 2020). As a result, pathogens have acquired resistance towards specific antibiotics and made the treatment process difficult and ineffective. This had led to the requirement of new drug discoveries which includes expensive and complex drug development processes and short patient compliance (Singh et al. 2014). MDR is classified mainly into three categories, namely primary, secondary, and clinical resistance. Primary resistance develops if the organism has never encountered the specific drug in a particular host. Secondary resistance also referred as acquired resistance develops in organism only after encountering with the drug. Clinical resistance is when the pathogen is inhibited by a higher concentration of antimicrobial drug, where the concentration could possibly cause therapeutic failure and reappearance of the pathogen (Tanwar et al. 2014).

17.2.1 Multidrug Resistance in TB

TB is predominantly treated with first-line anti-tuberculosis medications including rifampicin, isoniazid, pyrazinamide, streptomycin, and ethambutol. The misuse of these anti- tuberculosis drugs such as incomplete treatment sequence, receiving wrong dose, wrong treatment duration, and using low quality drugs may cause the bacteria to acquire resistance against these medications (Espinal et al. 2001). As a result, treatment using only first-line anti-TB drugs becomes inefficient. Hence, second-line anti-tuberculosis drugs including fluoroquinolones such as moxifloxacin, ofloxacin, ciprofloxacin, and levofloxacin and injectable anti-tuberculosis drugs such as kanamycin, amikacin, and capreomycin are used (Paul et al. 2020). However, no single drug is 100% effective for MDR-TB (Rai et al. 2015).

MDR-TB is defined as the resistance of the pathogen against at least the major two sensitive anti-tuberculosis drugs: INH and RIF (Singh et al. 2020). MTB has natural resistance towards many antibiotics hence making treatment challenging. Treating MDR-TB necessitates the administration of expensive and harmful second-line medications for approximately 2 years and is accompanied with increased occurrence of morbidity and death (Lu et al. 2021). The extremely hydrophobic cell envelope preventing permeability and the presence of potential resistance determinants encoded in the genome are the reasons for this resistance (Cole et al. 1998). The currently prevailing treatment for TB involves everyday administration of medications for minimum 6 months and this prolonged usage is also a cause for the progress and transmission of drug resistant TB (Muthupandian et al. 2017).

17.2.2 Mechanism of MDR-TB

Antibacterial drug resistance is developed in bacteria through different intrinsic or acquired mechanisms. The intrinsic mechanism is associated with absence of the target, low-affinity targets, reduced cell permeability, antibiotics inactivation, and efflux mechanisms. The acquired mechanisms include mutations of genes aimed by the antibiotics, the exchange of resistance factors found on bacteriophages, plasmids, and movable genetic factors like transposons through conjugation, transformation, and transduction (Singh et al. 2014).

MTB develops natural genetic resistance to anti-tuberculosis drugs without exposure to any antibiotics. However, in the presence of anti-tuberculosis drugs, resistance can arise through any mechanisms including alteration of the drug target protein which inhibits drug binding, decreasing membrane permeability which prevent drug entry, reducing drug metabolism (Palomino et al. 2007), and encoding membrane protein that functions as efflux pump (Zeaur et al. 2012). (Fig. 17.1) Understanding these resistance mechanisms would aid better use of existing drugs and assist the discovery of new therapies (Cole et al. 1998).

MTB has shown different mechanisms of drug resistance including natural drug resistance, cell wall mediated drug resistance, and mutation mediated drug resistance. The natural resistance arises due to random, spontaneous mutations during bacterial cell multiplication with a defined frequency. Unlike most other bacteria, MTB does not show evidence of horizontal gene transfer that leads to acquisition of resistant plasmids or transposons. Multidrug efflux pumps in the cell wall play a significant role in resistance in mycobacteria (Gupta et al. 2006). The encounter with different anti-tuberculous drugs could stimulate expression of particular efflux pumps and it leads to phenotypic resistance mediated by drugs (De Steenwinkel et al. 2010).

Chromosomal mutations are the most common causes associated with MTB drug resistance. Genetic mutations causing RIF resistance in MTB happen at a frequency of 10^{-10} per cell division and result in the presence of 1 in 10^8 cells in drug absent settings. The resistant mutation frequency for INH is roughly 10^{-7} to 10^{-9} per cell division causing prevalence of 1 out of 10^6 cells in drug-free environments (David 1970).

17.2.2.1 Rifampicin Resistance Mechanism

The genetic basis of resistance to RIF in MTB is related to mutations within the 81 bp region in *rpoB* gene which is known as the rifampicin resistance determining region (RRDR) containing codons 507 to 533. (Fig. 17.2) A nucleotide base substitution at the first or second bases in codons 516 (GAC to TAC or GTC) and 526 (CAC to GAC, CGC or TAC) or in the second nucleotide base in codon 531 (TCG to TGG or TTG) is the most common mutation in RIF resistant MTB (Engstrom et al. 2013). RIF binds to RNA polymerase (RNAP) of MTB and inhibits RNA synthesis. The RNAP contains five subunits; 2 copies of α, β, β′ and σ (Stefan et al. 2018) encoded by *rpoA, rpoB, rpoC,* and *rpoZ* genes, respectively. Mutated *rpoB* alters β subunit structure and makes it unrecognizable by RIF. Hence RIF is

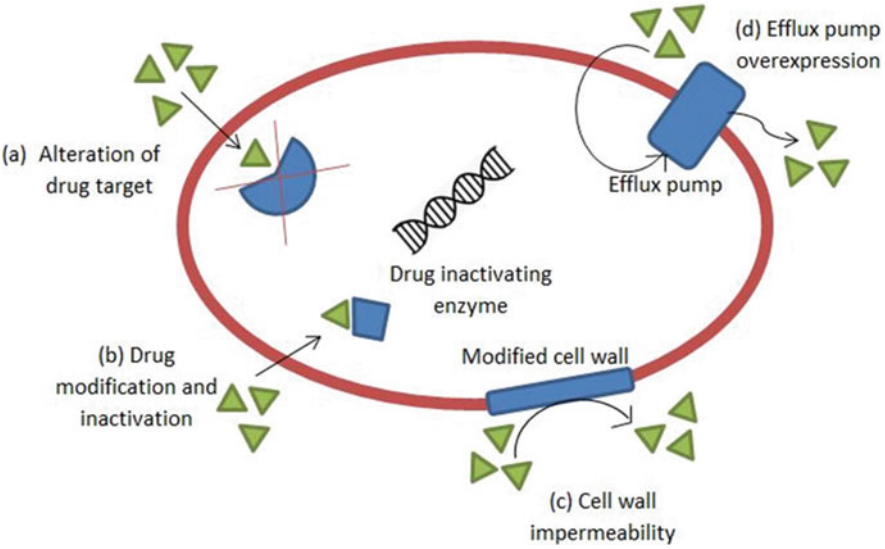

Fig. 17.1 The drug resistance mechanisms shown by *M. tuberculosis* (**a**) Alteration of drug target, (**b**) Drug modification and inactivation, (**c**) Cell wall impermeability, (**d**) Efflux pump overexpression

unable to bind to RNAP, thus causing resistance against RIF. In addition to the *rpoB* gene mutation, MTB also develops RIF resistance through enhanced drugs efflux, reduced drug entry into cell and heterogeneous RIF susceptibility to resist drug pressure (Xu et al. 2021). The PE11 protein is involved in cell wall maintenance in MTB and up-regulation of this protein expression reduces quantity of antibiotic moving into cell (Singh et al. 2016). The CpnT is a membrane channel protein that regulates material intake into cell and the mutant CpnT protein could prevent RIF entering into cells. Overexpression of efflux pump genes such as *Rv0876c, Rv1217c, Rv1218c,* and *Rv1250* has also contributed towards increased RIF resistance in MTB (Xu et al. 2021).

17.2.2.2 Isoniazid Resistance Mechanism

INH is a pro-drug which enters the MTB cell by passive diffusion (Singh et al. 2020). Resistance to INH is linked with several gene mutations such as *katG* gene which codes for enzyme catalase peroxidase, *inhA* gene that codes the enzyme enoyl acyl carrier protein reductase, *ahpC* gene that encodes alkyl hydroperoxide reductase, *kasA* gene which encodes the beta ketoacyl acyl carrier protein synthase (Ahmad and Mustafa 2001), *ndh* gene encoding NADH dehydrogenase (Lee et al. 2001), *iniABC* and *fadE* genes (Unissa et al. 2016). However, most of the recent studies explore the links mainly between INH resistance and mutations in genes *katG*, *inhA,* and *ahpC* of MTB strains (Seifert et al. 2015).

The catalase peroxidase enzyme coded by *katG* gene activates INH. This activation is very much essential to make INH toxic to the MTB because activated INH

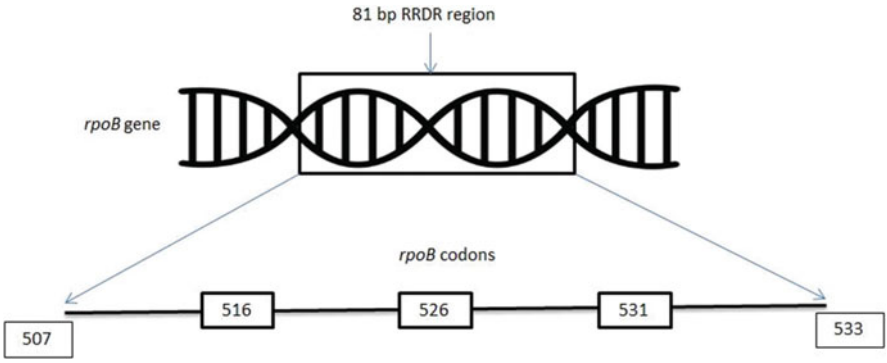

Fig. 17.2 The 81 bp RRDR region of the rpoB gene comprising codons 507 to with 533, mainly depicting the three codons 516, 526 and 531 which carry the mutations associated with RIF resistance

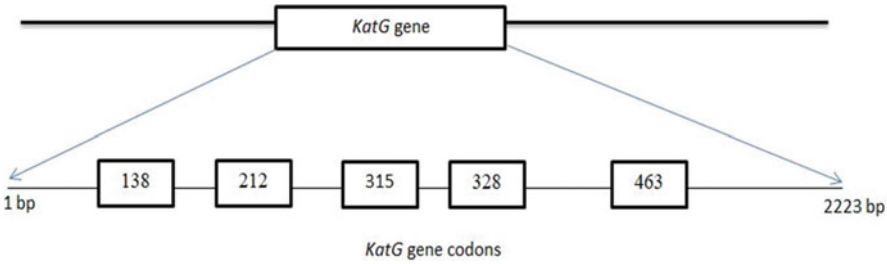

Fig. 17.3 The codons of *KatG* gene where point mutations associated with INH resistance in TB have been reported

inhibits biosynthesis of mycolic acid which is a major mycobacterial cell wall component (Barry et al. 1998). Several modifications in *katG* gene are reported to be related with INH resistance. The very common mutation at codon 315 which confers high-level resistance has been reported to cause amino acid substitution at *katG* Ser315Thr and this substitution favours MTB by interrupting the activation of INH and by retaining a small percentage of catalase peroxidase activity which confers virulence (Rouse et al. 1996). The *katG* gene mutation at codon 315 not only reduced *katG* activity but also increases expression of AhpC protein. This protein favours the bacterial cell by protecting it against oxidative damage and detoxifies organic peroxides. However, this does not offer defence towards INH (Sherman et al. 1996). Further some other *katG* mutations at codon 138, 328, and 463 also lead to high-level INH resistance (Singh et al. 2020). Two novel *katG* gene mutations at codons 212 and 293 have also been reported to cause INH resistance. It has been reported that coexistence of *katG* codon 315 mutations along with codon 463 mutations leads to low level of resistance (Karunaratne et al. 2018) (Fig. 17.3).

Mutations arising at the promoter region of *inhA* operon and *inhA* gene have been extensively related to INH resistance (Seifert et al. 2015). The up-regulation

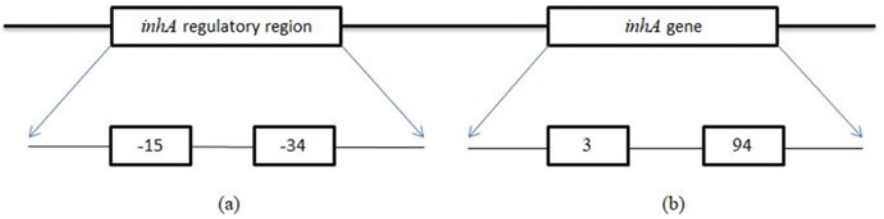

Fig. 17.4 The *inhA* regulatory region and *inhA* gene where point mutations associated with INH resistance in TB have been reported. (**a**) Regions that show mutations in the *inhA* regulatory region, (**b**) Common codons in *inhA* gene which show mutations

mutations at the *inhA* regulatory region led to increase expression of InhA and develop INH resistance through a titration mechanism (Zhang and Yew 2009) and this was the major cause of low-level INH resistance in MTB (Unissa et al. 2016). The point mutation at region −15 of the *inhA* promoter has been described as the most common *inhA* mutation (Seifert et al. 2015). Mutations causing high level of resistance and coexisting with *katG* 315 mutation had been identified at the −34 (C deletion) promoter region of *inhA*. (Fig. 17.4a). The *inhA* gene mutations at codon 3, 94, and 158 have been reported to confer INH resistance (Karunaratne et al. 2018). (Fig. 17.4b). Some studies show that INH resistant bacterial population also exhibited efflux pump-mediated drug tolerance (De Steenwinkel et al. 2010). Furthermore, mutations of *kasA* gene associated with codons 66, 269, 312, and 413 confer low levels of INH resistance (Ramaswamy and Musser 1998). Activated INH binds with NADH co-factor forming adduct of INH-NAD. Point mutations in the *ndh* gene lead to NADH accumulation and NAD exhaustion. The accumulated NADH would hinder the interaction of the INH-NAD adduct with the *inhA* enzyme at its catalytic site (Lee et al. 2001).

17.2.3 Importance of Early Diagnosis of MDR-TB

Development of MDR-TB is greatly related with illness and death and is very challenging since limited therapeutic options are available (Muthupandian et al. 2017). Patients carrying MDR strains of MTB require alternative management schedules with second-line drugs (Wright et al. 2018). However, this sort of patient management is difficult as it increases cost and due to inadequate resources (Karunaratne et al. 2019). The WHO reports that the mortality rate due to antibiotic resistance is high in developing nations than in developed nations (Munir et al. 2020). The frequency of MDR-TB is growing globally due to inadequate treatment adherence, use of low quality medications, and latest straight transmission from MDR infected patients (Zeaur et al. 2012). The rate of occurrence of MDR-TB differs considerably among countries. However, the most predominant countries include India, Russia, and China (Muthupandian et al. 2017). Furthermore, MDR-TB places an economic stress on the pharmaceutical industry by the

requirement of new drug development which is expensive. Therefore, early diagnosis of MDR-TB is crucial in decreasing the mortality rate and spread of the infection by appropriate early stage treatments (Singaram 2016). A quick and trustworthy laboratory diagnosis technique would aid the early diagnosis and control of TB (Singh et al. 2020).

17.3 Nanodiagnosis and Nanoparticles (NPs)

Nanodiagnosis is the application of nanotechnology for early clinical diagnosis of diseases, both in vitro and in vivo, rapidly with increased sensitivity and specificity of the test results with the use of nano devices or nanomaterials (Jain 2005). Nano diagnostics have been playing a promising role in various disease diagnoses such as detection of viral infection like TB, HIV and Hepatitis B (Gupta et al. 2020), carcinogenic cell, antibodies, marker enzymes, particular protein derivatives, and other disease indicator molecules (Yezhelyev et al. 2006). Nanoparticles including gold and carbon nanoparticles, nanoshells, nanotubes, quantum dots, nanopores, and nanocantilever systems play favourable roles in diagnostic activities (Capek 2016). Most of these diagnostic tests are generally based on the interaction between a labelled nanoparticle or a probe specific to the aimed biomolecule, which results in an electrical signal that can be quantified (Gupta et al. 2020). These NPs range between 1 and 100 nm in size and this is related to several other characteristics which make NPs ideal candidates to be employed in the designing of simple, robust, low cost diagnostic assays (Dahiya and Mehta 2021). These characteristics include high surface to volume ratio, surface-enhanced Raman scattering, surface plasmon resonance, ferromagnetic nanoparticles or super-magnetization, enhanced photoluminescence, high heat and electrical conductivity, and strong surface catalytic properties (Singh et al. 2020). These nanoparticle based assays are less expensive and provide easy visual observation, unlike the traditional detection techniques like the fluorescence-based and radioactivity-based assays (Ckumdee et al. 2016).

17.3.1 Types of Nanoparticles

17.3.1.1 Gold Nanoparticles (AuNPs)

The chemical, physical, and optical properties of gold nanoparticles such as inertness and non-toxicity (Pasqua et al. 2009), shape, size, and surface charge make them ideal tools for identification of various pathogens (Sanvicens and Marco 2008). (Fig. 17.5) AuNP was initially used in diagnosis of TB (Baptista et al. 2006). The optical property of AuNPs together with different biomolecules like antibodies or antigens enables it to be used in diagnosis of different pathogens (Choi and Frangioni 2010).

17.3.1.2 Magnetic Nanoparticles (MNPs)

Magnetic nanoparticles are ideal for detection tests as they do not react with chemical reagents or by being exposed to light. They are physically and chemically

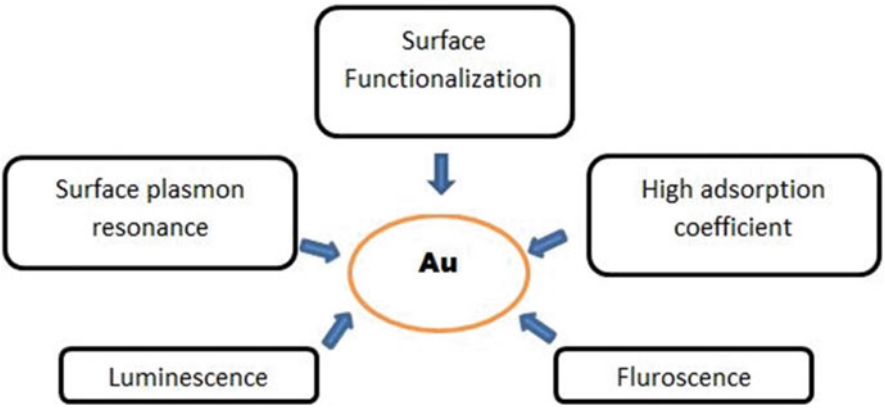

Fig. 17.5 Characteristics of AuNPs which make it suitable for nanodiagnosis

stable and biocompatible. MNPs such as Fe_3O_4 (magnetite) and Fe_2O_3 (maghemite) are biocompatible (Hasan 2014). In nanodiagnosis MNPs tagged with ligands are used to detect the target nucleic acid of pathogens, with the help of diagnostic magnetic resonance device (Kaittanis et al. 2010). It is easy to modify the surface of MNPs using detection components such as immunoglobulins, carbohydrates, and antibiotics, which facilitate MNPs to be successfully utilized in detection of bacterial cells (Singh et al. 2020). Furthermore, the MNPs are cost-effective route for detection of biomolecules (Varadan et al. 2008) and their favourable features allow them to be used in nano-biomedicine.

17.3.1.3 Silicon Nanoparticles (SiNPs)

Silicon nanoparticles are biocompatible and possess modifiable surface chemistry. SiNPs are mainly used in luminescence-based biological imaging, medical imaging, and as labels in diagnostics (Thiessen et al. 2019). Porous SiNPs have been widely used in biosensor in disease diagnosis (Rai et al. 2015).

17.4 Diagnostic Methods for MDR-TB

17.4.1 Conventional Methods

Conventional methods used for diagnosis of drug resistant TB are mostly based on phenotypic methods like drug susceptibility tests (Gupta and Kakkar 2018). These phenotypic methods analyse MTB growth in the presence of a specific antibiotic in conventional cultures which takes a few weeks to yield results thus this process is time consuming (Palomino et al. 2007). The solid culture based systems take around 60 days for the appearance of growth. Later, the mycobacterial growth indicator tube (MGIT) 960 was developed as an automated liquid culture technique which was comparatively faster than the solid culture methods and the MGIT 960 system

required approximately 42 days for detection (Gupta et al. 2020). Meanwhile patients suffering severe medical condition cannot wait that longer for the onset of the treatment. Therefore, modern rapid genotypic methods have been developed to overcome these limitations.

17.4.2 Rapid Molecular Diagnostic Methods

Several modern molecular assays have been developed to detect MDR-TB. An automatic cartridge-based, hemi-nested, real-time PCR assay named GeneXpert MTB/RIF, targeting *rpoB* gene has been used for the simultaneous detection of MTB and rifampicin resistance within 2 h (Dahiya et al. 2020). The drawback of this system is its inability to detect INH mono resistance. In certain circumstances, the mutations conferring RIF resistance located exterior to the RRDR could be simply disregarded and that too is a major drawback of this assay methods. This leaves MDR-TB under diagnosed and could increase the spread of the disease (Andre et al. 2017). In another assay, INH resistant MTB strains and MTC have been simultaneously identified using an allele-specific PCR aiming the *katG* 315 gene and a mutation (*inhA C-15T*) in the regulatory region of the *mabA-inhA* operon (Herrera-León et al. 2005). Furthermore, mutations causing RIF and INH drug resistance have been successfully detected by employing INNO-LiPA Rif.TB assay (Rossau et al. 1997) and GenoType MTBDR plus (Bai et al. 2012) which are two of the line-probe assays for TB. However even though these molecular diagnostic methods are highly sensitive and specifically compared to culture based diagnostics (Luo et al. 2010), there are several limitations such as high cost, need of well-trained personnel, and need of sophisticated technology. Therefore, these techniques are not widely employed in resource-poor settings for routine procedures.

17.4.3 Nanoparticles Based MDR-TB Diagnostic Methods

17.4.3.1 Gold Nanoparticles Based Methods

Gold nanoparticles conjugated with single stranded DNA probes are used to bind to the target nucleic acid of MTB and this is detected with colorimetry and surface enhanced Raman spectroscopy (Baptista et al. 2006). This basic principle has been used in most of the gold nanoparticles (~15 nm) based methods designed so far. A rapid lateral flow strip utilizing AuNP has been used for the recognition of MTB and the most prevalent INH resistance conferring mutation *katG S315T* at the same time. The test employed complementary probes specific for the *IS6110* region specific for MTB and *katG* gene. The complementary probes were conjugated with gold nanoparticles and positive hybridizations were indicated by red colour line formations. This technique has been shown to reliably detect INH resistance within 3 h (Karunaratne et al. 2019). The major benefit of this technique is that it does not require any sophisticated technology and is appropriate for low-resource settings.

However, correct hybridization conditions are crucial to get the accurate result in this strip and will not be used as point of care assay.

Further a loop mediated isothermal amplification (LAMP) along with AuNP probes for detection has been successfully designed for the identification of the gene mutations causing rifampicin resistance in MTB (Veigas et al. 2013). However, the drawback of this technique is that analysis is based on absorbance ratio and spectrophotometric assay. Thus, it would not be suitable for INH resistance diagnosis in TB in field samples. Similarly, a combination of novel LAMP and AuNP probes detection has been used to detect TB and MDR-TB strains as well as the simultaneous detection of the mutations related with INH resistance. In this technique the *katG* gene is subjected to LAMP amplification and then hybridized using specific AuNP probes. In addition, probes specific to the most predominant point mutations related with INH resistance were used as well. AuNP probes on hybridization with LAMP amplicons containing target-specific sequences appeared in red. This method is beneficial as it is rapid, less expensive, sensitive, and specific to MDR-TB detection and it has been designed to be conveniently used for MDR-TB diagnosis in field samples as well (Ckumdee et al. 2016).

A simple nucleic acid lateral flow (NALF) immunoassay was developed with antibodies for the identification of RIF resistance conferred by *rpoB* gene mutation and detection of isoniazid resistance conferred by S315T mutation in *katG* gene. The assay employed anti-biotin monoclonal antibodies conjugated with AuNPs for visual detection. This test design could also be used to detect extensively drug-resistant tuberculosis caused due to nucleotide changes in the *gyrA* and *gyrB* genes (Kamphee et al. 2015). In another study, a lateral flow strip has been designed along with a multienzyme isothermal rapid amplification, for simultaneous detection of MTBC and RIF resistance. In this method AuNPs incorporated with polyclonal anti-antibodies on the lateral flow strip has been used for the visualization (Lu et al. 2021).

In addition to these AuNPs conjugated thiol-modified oligonucleotides have been designed to detect a single base mutation in *rpoB* gene in order to detect rifampicin resistance in MTB (Veigas et al. 2010). Later this same approach was extended to detect the isoniazid resistance caused due to *inhA* C(−)15T mutations using AuNP probes and a multiplex PCR reaction. This system also accompanied a colorimetric assay combined to a conveniently moveable device for screening and analysing data (Pedrosa et al. 2014).

17.4.3.2 Lab-on-Chip Methods

The lab-on-chip method is an emerging technology based on miniaturization of labouratory based assays at nanoscale. AuNPs, MNPs, or carbon nanotubes integrated on a chip had offered very sensitive, portable, and inexpensive tools for quick diagnosis of various contagious ailments (Paul et al. 2020). Microfluidic-multiplexed platforms integrated with electrochemical sensors with functionalized carbon nanotubes, which are capable of detecting DNA from MTB, have been developed recently (Zribi et al. 2016). A novel platform using inexpensive fluidic cartridge and 1.4 nm nanogold-labelled common primers has been formulated for the

diagnosis of MDR-TB. The nanogold labels functioned as an enzymatic site for deposition of silver, which becomes observable to the unaided eye (Wang et al. 2012).

17.4.3.3 Magnetic Nanoparticles Based Methods

RIF resistant MTB was successfully detected using padlock probes and magnetic nanobeads. Magnetic nanobeads have been used in an approach to detect mutation in *rpoB* gene of MTB. Streptavidin bound magnetic nanobeads labelled with biotin have been used here together with an array of 11 padlock probes out of which nine probes were used to identify common mutations in RRDR *rpoB* gene. The padlock probes were specific to mutations in 516, 526, and 531 codons of the *rpoB* gene which is linked with RIF resistance in MTB. A probe for identification of the MTBC was also included in this assay (Engstrom et al. 2013).

17.5 Point of Care (POC) Diagnosis

POC based nanodiagnostic platforms such as magnetic resonance based platform with the use of magnetic nanoparticles (Hoerr and Faber 2014), magnetic barcode based assay system made of magnetic nanoprobes (Liong et al. 2013), and paper strip based platforms utilizing AuNPs and lateral flow assays have been designed for the diagnosis of TB (Yen et al. 2015). The ideal features of a POC assay include ability to function in wide ranges of temperature and humidity, produce results in not more than 20 min and be accessible by health care workers easily without much training (WHO 2014).The advantageous features of POC platforms developed so far are that they allow on field diagnosis of TB since they do not require special conditions such as strict temperature and humidity like in most other techniques. Point-of-care diagnostic can be combined with novel technologies like nanotechnology for early and effective detection of MDR-TB.

17.6 Conclusion

The rapid and accurate detection of MDR-TB is critically important to reduce the infection rate and mortality rate worldwide. Nanoparticles have shown a promising strategy in offering rapid and efficient diagnostic methods in promptly detecting MDR-TB in *mycobacterium* especially towards the first-line anti-tuberculosis drugs RIF and INH at low cost and limited resource availability. However, only very few of these techniques designed so far have the potential to be used as point-of-care assays. Furthermore, these nanoparticle based POC platforms have been designed for the prompt diagnosis of TB and not specifically for MDR-TB diagnosis. The further development of convenient and affordable nanoparticles based POC platform utilizing the interactions between biomolecules such as antibody-antigen reactions for detecting MDR-TB would be a major breakthrough in MDR-TB diagnosis and treatment in future. Most of the assays proposed so far have been successfully

employing gold nanoparticles for the detection and have been designed in conjunction with some molecular techniques. Only few assays have made use of magnetic nanoparticles. Instead of being confined only to gold nanoparticles, future research should also focus more on employing other types of nanoparticles such as carbon nanoparticles and silica nanoparticles in detection of MDR-TB. The use of nanoparticle based assays has offered advantages such as rapid, sensitivity, specificity, and reduced expenses compared to the conventional detection methods. By further developing and adopting these proposed nanoparticles based detection methods, it can be ensured that the patients harbouring MDR-TB strains could be rapidly diagnosed, quarantined, and appropriately treated, thus enhancing patient management, treatment regimen and minimizing non-compliance.

References

Ahmad S, Mustafa AS (2001) Molecular diagnosis of drug-resistant tuberculosis. Kuwait Med J 33(2):120–126

Andre E, Goeminne L, Cabibbe A et al (2017) Consensus numbering system for the rifampicin resistance-associated rpoB gene mutations in pathogenic mycobacteria. Clin Microbiol Infect 23(3):167–172. https://doi.org/10.1016/j.cmi.2016.09.006

Bai Y, Wang Y, Shao C, Hao Y, Jin Y (2012) GenoType MTBDRplus assay for rapid detection of multidrug resistance in Mycobacterium tuberculosis: a meta-analysis. PLoS One 11(3): e0150321. https://doi.org/10.1371/ournal.pone.0150321

Baptista PV, Maria KM, Jolanta PO, Gonc AD, Ricardo F (2006) Gold-nanoparticle-probe-based assay for rapid and direct detection of Mycobacterium tuberculosis DNA in clinical samples. Clin Chem 52(7):1433–1434

Barry CE, Lee RE, Mdluli K, Sampson AE, Schroeder BG, Slayden RA, Yuan Y (1998) Mycolic acids: structure, biosynthesis and physiological functions. Prog Lipid Res 37:143–179

Capek I (2016) Nanosensors based on metal and composite nanoparticles and nanomaterials. In: Parameswaranpillai J, Hameed N, Kurian T, Yu Y (eds) Nanocomposite materials: synthesis, properties and applications. CRC Press, Boca Raton, pp 1–40

Choi HS, Frangioni JV (2010) Nanoparticles for biomedical imaging: fundamentals of clinical translation. Mol Imaging 9:291–310

Ckumdee J, Kaewphinit T, Chansiri K, Santiwatanakul S (2016) Development of au-nanoprobes combined with loop-mediated isothermal amplification for detection of isoniazid resistance in Mycobacterium tuberculosis. J Chem 8. https://doi.org/10.1155/2016/347439

Cole ST, Brosch R, Parkhill J, Garnier T, Churcher C, Harris D, Gordon SV, Eiglmeier K, Gas S, Barry CE, Tekaia F, Badcock K, Basham D, Brown D, Chillingworth T, Connor R, Davies R, Devlin K, Feltwell T, Gentles S, Hamlin N, Holroyd S et al (1998) Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence. Nature 393(6658):537–544. https://doi.org/10.1038/31159

Dahiya B, Mehta PK (2021) Utility of nanoparticle-based assays in the diagnosis of tuberculosis. Nanomedicine 16(15):1263–1268. https://doi.org/10.2217/nnm-2021-0077

Dahiya B, Prasad T, Singh V et al (2020) Diagnosis of tuberculosis by nanoparticle-based immuno-PCR assay based on mycobacterial MPT64 and CFP-10 detection. Nanomedicine (Lond) 15(26):2609–2624

David HL (1970) Probability distribution of drug-resistant mutants in unselected populations of Mycobacterium tuberculosis. Appl Microbiol 20:810–814

De Steenwinkel JE, de Knegt GJ, ten Kate MT et al (2010) Time-kill kinetics of anti-tuberculosis drugs, and emergence of resistance, in relation to metabolic activity of Mycobacterium tuberculosis. J Antimicrob Chemother 65:2582–2589

Engstrom A, Torre TZG, Strømme M, Nilsson M, Herthnek D (2013) Detection of rifampicin resistance in Mycobacterium tuberculosis by padlock probes and magnetic nanobead-based readout. PLoS One 8(4):e62015. https://doi.org/10.1371/journal.pone.0062015

Espinal MA, Laszlo A, Simonsen L, Boulahbal F, Kim SJ, Reniero A, Hoffner S, Rieder HL, Binkin N, Dye C, Williams R, Raviglione MC (2001) Global trends in resistance to antituberculosis drugs. N Engl J Med 344(17):1294–1303

Gupta S, Kakkar V (2018) Recent technological advancements in tuberculosis diagnostics—a review. Biosens Bioelectron 115:14–29. https://doi.org/10.1016/j.bios.2018.05.017

Gupta AK, Chauhan DS, Srivastava K et al (2006) Estimation of efflux mediated multi-drug resistance and its correlation with expression levels of two major efflux pumps in mycobacteria. J Commun Dis 38:246–254

Gupta AK, Singh A, Singh S (2020) Diagnosis of tuberculosis: nanodiagnostics approaches. In: Saxena SK, Khurana SMP (eds) NanoBioMedicine. Springer, Singapore, pp 261–283

Hasan S (2014) A review on nanoparticles: their synthesis and types. Res J Recent Sci 4:1–3. ISSN 2277-2502

Herrera-León L, Molina T, Saíz P, Sáez-Nieto JA, Jiménez MS (2005) New multiplex PCR for rapid detection of isoniazid-resistant Mycobacterium tuberculosis clinical isolates. Antimicrob Agents Chemother 49(1):144–147

Hoerr V, Faber C (2014) Magnetic resonance imaging characterization of microbial infections. J Pharm Biomed Anal 93:136–146. https://doi.org/10.1016/j.jpba.2013.10.034

Jain KK (2005) Nanotechnology in clinical laboratory diagnostics. Chim Acta 358(1–2):37–54

Kaittanis CS, Santra S, Perez JM (2010) Emerging nanotechnology-based strategies for the identification of microbial pathogenesis. Adv Drug Deliv Rev 62(4):408–423

Kamphee H, Chaiprasert A, Prammananan T, Wiriyachaiporn T, Kanchanatavee A, Dharakul T (2015) Rapid molecular detection of multidrug-resistant tuberculosis by PCR-nucleic acid lateral flow immunoassay. PLoS One 10(9):e0137791. https://doi.org/10.1371/journal.pone.0137791

Karunaratne RE, Wijesundera SS, Vidanagama D, Adikaram CP, Perera J (2018) Significance of coexisting mutations on determination of the degree of isoniazid resistance in Mycobacterium tuberculosis strains. Microb Drug Resist 24(6):844–851. https://doi.org/10.1089/mdr.2017.0330

Karunaratne RE, Wijenayaka LA, Wijesundera SS, De Silva KMN, Adikaram CP, Perera J (2019) Use of nanotechnology for infectious disease diagnostics: application in drug resistant tuberculosis. BMC Infect Dis 19:618. https://doi.org/10.1186/s12879-019-4259-x

Lee AS, Teo AS, Wong SY (2001) Novel mutations in ndh in isoniazid-resistant Mycobacterium tuberculosis isolates. Antimicrob Agents Chemother 45:2157–2159

Liong M, Hoang AN, Chung J et al (2013) Magnetic barcode assay for genetic detection of pathogens. Nat Commun 4:1752. https://doi.org/10.1038/ncomms2745

Lu Y, Li MC, Liu HC, Lin SQ, Zhao XQ, Liu ZG (2021) Detecting Mycobacterium tuberculosis complex and rifampicin resistance via a new rapid multienzyme isothermal point mutation assay. Anal Biochem 630:114341. https://doi.org/10.1016/j.ab.2021.114341

Luo T, Zhao M, Li X et al (2010) Selection of mutations to detect multidrug-resistant Mycobacterium tuberculosis strains in Shanghai, China. Antimicrob Agents Chemother 54(3):1075–1081

Munir MU, Ahmed A, Usman M, Salman S (2020) Recent advances in nanotechnology-aided materials in combating microbial resistance and functioning as antibiotics substitutes. Int J Nanomedicine 15:7329–7358

Muthukrishnan L (2021) Multidrug resistant tuberculosis—diagnostic challenges and its conquering by nanotechnology approach—an overview. Chem Biol Interact 337:109397

Muthupandian S, Duche M, Asmelash T, Gebreyesus A, Nanda A, Arokiyaraj S (2017) Nanomedicine as a newly emerging approach against multidrug-resistant tuberculosis (MDR-TB). In: Nayak BK, Nanda, Bhat MA (eds) Integrating biologically-inspired nanotechnology into medical practice. IGI Global, Hershey, p 24

Niemann S, Richter E, Rusch-Gerdes S (2000) Differentiation among members of the Mycobacterium tuberculosis complex by molecular and biochemical features: evidence for two pyrazinamide-susceptible subtypes of *M. bovis*. J Clin Microbiol 38:152–157

Palomino JC, Leao SC, Ritacco V (2007) Tuberculosis, from basic science to patient care, 1st edn. www.Tuberculosistextbook.com

Pasqua AJD, Mishler RE, Shi IIY, Dabrowiak JC, Asefa T (2009) Preparation of antibody-conjugated gold nanoparticles. Mater Lett 63(21):1876–1879

Paul A, Dutta N, Moschou D, Dutta G (2020) Advanced integrative sensing technologies for detection of drug-resistant tuberculosis in point-of-care settings. Sensors Int 1:100036. https://doi.org/10.1016/j.sintl.2020.100036

Pedrosa P, Veigas B, Machado D, Couto I, Viveiros M, Baptista PV (2014) Gold nanoprobes for multi loci assessment of multi-drug resistant tuberculosis. Tuberculosis 94(3):332–337. https://doi.org/10.1016/j.tube.2013.12.009

Rai M, Ingle AP, Bansod S, Kon K (2015) Tackling the problem of tuberculosis by nanotechnology: disease diagnosis and drug delivery. In: Rai M, Kon K (eds) Nanotechnology in diagnosis, treatment and prophylaxis of infectious diseases. Academic, pp 133–149

Ramaswamy S, Musser JM (1998) Molecular genetic basis of antimicrobial agent resistance in Mycobacterium tuberculosis: update. Tuber Lung Dis 79:3–29

Rossau R, Traore H, De Beenhouwer H (1997) Evaluation of the INNO-LiPA Rif. TB assay, a reverse hybridization assay for the simultaneous detection of Mycobacterium tuberculosis complex and its resistance to rifampin. Antimicrob Agents Chemother 41(10):2093–2098

Rouse DA, DeVito JA, Li Z, Byer H, Morris SL (1996) Site-directed mutagenesis of the katG gene of *Mycobacterium tuberculosis*: effects on catalase-peroxidase activities and isoniazid resistance. Mol Microbiol 22:583–592

Saiman L (2004) The mycobacteriology of non-tuberculous mycobacteria. Paediatr Respir Rev 5: S221–S223

Sanvicens N, Marco MP (2008) Multifunctional nanoparticles—properties and prospects for their use in human medicine. Trends Biotechnol 26:425–433

Seifert M, Catanzaro D, Catanzaro A, Rodwell TC (2015) Genetic mutations associated with isoniazid resistance in Mycobacterium tuberculosis: a systematic review. PLoS One 10(3): e0119628. https://doi.org/10.1371/journal.pone.0119628

Sherman DR, Mdluli K, Hickey MJ, Arain TM, Morris SL, Barry CE, Stover CK (1996) Compensatory ahpC gene expression in isoniazid-resistant *Mycobacterium tuberculosis*. Science 272: 1641–1643

Singaram S (2016) Tuberculosis diagnosis—an overview to the conventional diagnostic methodology and need for nano diagnosis. Int J Med Eng Inform 8(1):27. https://doi.org/10.1504/IJMEI.2016.073664

Singh R, Smitha MS, Singh SP (2014) The role of nanotechnology in combating multi-drug resistant bacteria. J Nanosci Nanotechnol 14:4745–4756. https://doi.org/10.1166/jnn.2014.9527

Singh P, Rao RN, Reddy JR et al (2016) PE11, a PE/PPE family protein of Mycobacterium tuberculosis is involved in cell wall remodeling and virulence. Sci Rep 6:21624. https://doi.org/10.1038/srep21624

Singh A, Gupta AK, Singh S (2020) Molecular mechanisms of drug resistance in Mycobacterium tuberculosis: role of nanoparticles against multi-drug-resistant tuberculosis (MDR-TB). In: Saxena SK, Khurana SMP (eds) NanoBioMedicine. Springer Nature, Singapore, pp 285–314

Stefan MA, Ugur FS, Garcia GA (2018) Source of the fitness defect in rifamycin-resistant Mycobacterium tuberculosis RNA polymerase and the mechanism of compensation by mutations in the beta' subunit. Antimicrob Agents Chemother 62(6):e00164–e00118. https://doi.org/10.1128/AAC.00164-18

Tanwar J, Das S, Fatima Z, Hameed S (2014) Multidrug resistance: an emerging crisis. Interdiscip Persp Infect Dis 2014:7. https://doi.org/10.1155/2014/541340

Thiessen AN, Ha M, Hooper RW, Yu H, Oliynyk AO, Veinot JGC, Michaelis VK (2019) Silicon nanoparticles: are they crystalline from the core to the surface. Chem Mater 31:678–688

Unissa AN, Subbian S, Hanna LE, Selvakumar N (2016) Overview on mechanisms of isoniazid action and resistance in Mycobacterium. Infect Genet Evol 45:474–492. https://doi.org/10.1016/j.meegid.2016.09.004

Varadan VK, Chen L, Xie J (2008) Nanomedicine: design and applications of magnetic nanomaterials, nanosensors and nanosystems. Wiley, West Sussex

Veigas B, Machado D, Perdigão J, Portugal I, Couto I, Viveiros M, Baptista PV (2010) Au-nanoprobes for detection of SNPs associated with antibiotic resistance in Mycobacterium tuberculosis. Nanotechnology 21:415101. https://doi.org/10.1088/0957-4484/21/41/415101

Veigas B, Pedrosa P, Couto I, Viveiros M, Baptista PV (2013) Isothermal DNA amplification coupled to Au-nanoprobes for detection of mutations associated to rifampicin resistance in Mycobacterium tuberculosis. J Nanobiotechnol 11:38

Wang H, Chen HW, Hupert ML, Chen PC, Datta P, Pittman TL, Goettert J, Murphy MC, Williams D, Barany F, Soper SA (2012) Fully integrated thermoplastic genosensor for the highly sensitive detection and identification of multi-drug resistant tuberculosis. Angew Chem Int Ed 51(18):4349–4353. https://doi.org/10.1002/anie.201200732

WHO (2014) High priority target product profiles for new tuberculosis diagnostics: report of non consensus meeting. WHO, Geneva

WHO (2020) Global tuberculosis report 2020. World Health Organization, Geneva

Wright GD (2003) Mechanisms of resistance to antibiotics. Curr Opin Chem Biol 7(5):563–569. https://doi.org/10.1016/j.cbpa.2003.08.004

Wright A, Tomlinson GS, Rangaka MX, Fletcher HA (2018) World TB day 2018: the challenge of drug resistant tuberculosis. F1000 Res 7:217. https://doi.org/10.12688/f1000research.14088.1

Xu G, Liu H, Jia X, Wang X, Xu P (2021) Mechanisms and detection methods of Mycobacterium tuberculosis rifampicin resistance: the phenomenon of drug resistance is complex. Tuberculosis 128:102083. https://doi.org/10.1016/j.tube.2021.102083

Yen CW, de Puig H, Tam JO, Gomerz-Marquez J, Bosch I, Schifferli KH, Gehrke L (2015) Multicolored silver nanoparticles for multiplexed disease diagnostics: distinguishing dengue, yellow fever and Ebola viruses. Lab Chip 15(7):1638–1641. https://doi.org/10.1039/c5lc00055f

Yezhelyev MV, Gao X, Xing Y, Hajj AA, Nie S, Regan RMO (2006) Emerging use of nanoparticles in diagnosis and treatment of breast cancer. Lancet 7(8):657–667

Zeaur R, Nakajima C, Raqib R, Zaman K, Endtz HP, Adri GM, Zanden VD, Suzuki Y (2012) Molecular mechanism of rifampicin and isoniazid resistance in Mycobacterium tuberculosis from Bangladesh. Tuberculosis 92:529–534. https://doi.org/10.1016/j.tube.2012.07.005

Zhang Y, Yew WW (2009) Mechanisms of drug resistance in Mycobacterium tuberculosis. Int J Tuberc Lung Dis 13:1320–1330

Zribi B, Roy E, Pallandre A, Chebil S, Koubaa M, Mejri N, Gomez MH, Sola C, Korri-Youssoufi H, Haghiri-Gosnet AM (2016) A microfluidic electrochemical biosensor based on multiwall carbon nanotube/ferrocene for genomic DNA detection of Mycobacterium tuberculosis in clinical isolates. Biomicrofluidics 10(1):014115. https://doi.org/10.1063/1.4940887

Futuristic Potential of Nanoantibiotics Against Multidrug Resistant Tuberculosis

18

Pooja Sanjay Khairnar, Ajit Singh, and Rahul Shukla

Abstract

Tuberculosis (TB) has been a worldwide health issue from the start of sixteenth as well as seventeeth centuries till the date, with new resistance emerging. This sort of epidemic offers a critical challenge to the global control of tuberculosis cases**.** Because of this new form of tuberculosis, the morbidity and mortality rates are significantly higher. In 2019, approximately 8.9–11.0 million individuals became infected by tuberculosis (TB), this figure which really being progressively falling worldwide in the past years. MDR tuberculosis refers to tuberculosis strains that have developed resistance to first-line medication like rifampicin and isoniazid. Extensively drug resistance tuberculosis is a novel tuberculosis variant resistant to the fluoroquinolones and to one or even more injectable second-line antitubercular drugs such as kanamycin, amikacin, and capreomycin. Frequent antibiotic exposure is one of the major mechanisms through which bacteria acquire resistance. Conventional drug delivery has some limitations like poor bioavailability of some drugs, stability issues, time consuming therapy, multidrug resistance, increased drug degradation, less availability of drug at site of infection. Nanoantibiotics are among the new techniques currently investigated for combating the rise of bacteria that are antibiotic resistant. Nanomaterials that exhibit antimicrobial activity on their own or improve the efficacy and safety of antibiotic treatment are referred to as "nanoantibiotics." The use of nanoparticles for antibiotic treatment has various advantages, including regulated as well as generally uniform distribution in the targeted site, increased solubility, combat multidrug resistance, extended and controlled release, reduced adverse effects, and

P. S. Khairnar · A. Singh · R. Shukla (✉)
Department of Pharmaceutics, National Institute of Pharmaceutical Education and Research-Raebareli, Lucknow, UP, India
e-mail: rahul.shukla@niperraebareli.edu.in

S. Hameed, S. Rehman (eds.), *Nanotechnology for Infectious Diseases*,
https://doi.org/10.1007/978-981-16-9190-4_18

increased cellular internalization. Different nanosystems such as SLN, NLC, liposomes, microemulsion, nanoemulsion, dendrimers, nanosuspensions, nano/microparticles are designed to avoid side effects associated with anti-TB medication and have a potential against multidrug resistant TB and extensively drug resistant TB.

Keywords

MDR-TB · XRD-TB · Nanoantibiotics · Liposomes · Nanoparticles · Dendrimers

18.1 Introduction

Despite being primarily a lung infection, TB is a multisystem disease with a broad range of manifestations. Mycobacterium tuberculosis is the bacterium that causes TB. Tuberculosis is among the leading causes of death worldwide, including the major cause due to a particular infectious agent (before HIV/AIDS infection). As per WHO in 2019, approximately 8.9–11.0 million individuals became infected by tuberculosis (TB), this figure which really being progressively falling worldwide in the past years. In 2019, an approximately 1.1–1.3 million people with absence of HIV infection were lost from tuberculosis decrease by 1.7 million in 2000. In 2019, 87% of new cases of TB were reported in the 30 countries with the highest TB burden. Multidrug resistant tuberculosis is indeed a significant threat to global health. However, in year 2019, over one half of million individuals acquired rifampicin resistant tuberculosis (RR-TB) including 78% possessing MDR-TB. People infected with HIV are at a greater risk of mortality from tuberculosis. Anti-TB drugs have been used for ages, and variants resistant to one or more of the drugs have been recorded within every country studies. Drug resistance develops due to wrong anti-TB treatments, use of low-quality medicines, and patients discontinue therapy. In recent decades, there has been a continuous global initiative to eradicate TB. Despite advances in TB control and a drop in new incidence, the people receiving TB preventive therapy have risen in the last several years and have been increased from 1.0 million to 2.2 million and then to 4.1 million in year 2015, respectively.

Mycobacterium tuberculosis is the bacterium that causes tuberculosis (TB) which predominantly affects lungs. The M. tuberculosis complex is comprised of M. tuberculosis, M. pinnipedii, M. bovis, M. mungi, M. canetti, M. africanum, M. caprae, and M. microti. The majority of these species have been identified, but not all cause infections in humans. The major mode of exposure is inhalation of infecting aerosolized droplets. The respiratory system, lymphoreticular system, gastrointestinal (GI) system, central nervous system, skin, liver, reproductive system, and musculoskeletal system are most typically affected organ systems. The bacterium is distinguished from other bacteria by the appearance of different lipids inside their cell wall, such as cord factor, Wax-D, and most important mycolic acid. The cell wall of M. tuberculosis has a high lipid content, which adds some

characteristics to bacterial infection such as survival in stressful conditions such as extreme acidic or basic conditions, resistance to several antibiotics, reduced oxygen condition, challenges in staining with gram stain and some other stains, and intracellular sustainability inside macrophage.

18.2 Pathogenesis of TB

Inhaling the droplets produced by an individual suffering from active form of TB initiates the initial stage of TB. These droplets might persist in the air for a greater span of time. A single droplet aspirated is capable of inducing the disease. The majority of droplets land in upper region of respiratory tract, the site where germs get eliminated, still some of them reach even further down (Nasiruddin et al. 2017). Upon entering the lungs' alveoli, the bacteria are phagocytosed by alveolar macrophages. Toll-like receptor 2 (TLR2), toll-like receptor 4 (TLR4) and mannose receptors, scavenger receptors, CD14, immunoglobulin receptors, receptors for surfactant protein A, and complement receptors are all involved in the uptake mechanism inside the cells (Bhatt and Salgame 2007). Occasionally macrophages struggle to kill bacteria because the microbes synthesize the compounds which inhibit the phagosome-lysosome fusion processes, bypassing exposure to lower pH and also hydrolytic environment in phagolysosomes (Vergne et al. 2004). Mycobacterium amplifies within macrophage during the second stage, subsequently leading to its lysis which is the main cause of cellular damage followed by drawing the blood monocytes and cells associated with inflammatory responses to the region of cell damage (Nasiruddin et al. 2017). Monocytes mature as macrophages which seek to destroy those microbes that are swallowed by the macrophages then proliferate within phagocyte increases the bacterial burden (Szulc-Kielbik et al. 2019). The third stage commences 2–3 weeks following infection. T cells acquire immunity, whereas lymphocytes migrate toward the site of infection. T cells are stimulated when mycobacterial antigens are presented to them, culminating in the production of γ -interferon and other cytokines such as TNF-α, IL-8, IL-12, and certain other proinflammatory cytokines released by macrophages are activated by γ-interferon. M. tuberculosis's rapid development slows at this point, as well as cell-mediated immunity acquired by the host cell. Mycobacterial cell wall has higher lipid composition because it is located outside of the cells which remain completely resistant towards complement assault. Cell-mediated immunity also is accountable for large section of TB pathophysiology. Whenever activated macrophages secrete numerous cytokines, lytic enzymes, and reactive intermediates, tissue impairment can occur. Immune system, particularly macrophages, will encapsulate the microbes within tubercles at this point. The environment existing between the two structures is acidic and anoxic, limiting the progression of mycobacterium. Latency is the most important feature of tuberculosis, it is the equilibrium between both the mycobacterium and the host (Nasiruddin et al. 2017). Many circumstances including malnutrition, immune suppression, steroid usage, and HIV infection can cause tubercles disintegrate and initiate fifth and last phase. Tubercle centers may liquefy for

mysterious reasons, offering an excellent growth medium for the bacterium, which now starts to multiply with faster pace in the extracellular fluid. The tremendous bacterial count and the immune reaction associated with them subsequently induce necrosis in lung tissue surrounding the tubercles to create a cavity (Nasiruddin et al. 2017). The majority of TB infections tend to cease in third stage due to helper T cells (CD4+) and cytotoxic T cells (CD8+) are involved in a cell-mediated immunological response and therefore both play an important function in prevention of tuberculosis.

HelperCD4^{+}cells boost macrophages antimicrobial activity by producing cytokines such as TNF-α and IFN-γ, while CD8+ cells eliminate not only infected macrophages but also Mtb by producing granulysin, perforins, which are mediators with cytotoxic activity (Cooper 2009). Although breadth of our comprehension about the immunological response to Mycobacterium tuberculosis has broadened over years, the kind of the immune response sufficient for efficient immunity which is achievable by vaccination remains a mystery (Horvath et al. 2012).

18.3 Primary, Secondary, and Latent Tuberculosis

Primary tuberculosis occurs whenever a mycobacterium organism interacts with a host for first time (Garg et al. 2015). The Ghon focus of primary tuberculosis is defined as tuberculosis that is localized to the middle section of the lungs. The Ghon focus goes into latency in the majority of affected people this is referred as latent tuberculosis. After immunosuppression in the host, latent TB has been reactivated. Following initial exposure, only a limited number of populations would acquire an active disease. Primary progressive TB is the term used to describe cases such as number of people on long-term steroid usage, individuals with immunosuppression and malnourishment are all at risk for primary progressive TB. Most people with tuberculosis develop the disease after a long latency period (usually a few years after the initial infection). Reactivation of latent TB infection is the very common cause of secondary tuberculosis. Secondary TB lesions appear within the apices of the lungs. After being infected a second time, a lesser proportion of patients acquire secondary TB (re-infection). Secondary TB lesions are analogous in position (there at the lung apices) acquired via reactivation, re-infection, and cavitation which is distinguishes it from main progressive TB that appears to be in the middle zones of lungs without significant tissue cavitation or destruction.

18.4 Treatment for Tuberculosis

18.4.1 First-Line Treatment

These medicines have both high antitubercular efficacy and minimal toxicity.

18.4.2 Second-Line Treatment

These drugs have either limited antitubercular efficacy or greater toxicity, or both. These second-line drugs are mostly utilized for treating patients suffering from multidrug resistance tuberculosis.

Drug resistance has followed several forms:

1. **Primary Resistance:** Primary resistance is defined as the development of drug resistance among tuberculosis patient who have not ever before taken antitubercular treatment.
2. **Secondary/Acquired Drug Resistance:** This is the type of resistance that develops during or after the chemotherapy course as a result of noncompliance with the recommended drug regimen of drug or inaccurate prescription.
3. **Initial/Transitional Drug Resistance:** Drug resistance of this type developed during therapy, and a few colonies of resistant culture are occasionally detected immediately before sputum conversion. Such bacilli cannot grow and have no effect on therapy outcomes.

18.4.3 Multidrug Resistant Tuberculosis [MDR-TB]

Multidrug resistance tuberculosis and XRD-TB (extensively drug resistance tuberculosis) are growing challenges in the control and cure with improper management and misuse of conventional antitubercular medicines are one of the reasons of drug resistance. MDR-TB has developed and spread rapidly due to insufficient or irregular treatment. TB is highly prevalent in HIV/AIDS patients. MDR-TB requires resistance to numerous antitubercular drugs, along with at least one of the two major antitubercular drugs such as isoniazid or rifampicin. Drug-resistant strains currently account for around 10% of all tuberculosis fatalities. India and China are now the countries having the highest number of instances of MDR tuberculosis. The "Revised National Tuberculosis Control Program" (RNTCP) has implemented "Directly Observed Treatment Short-course" (DOTS) treatment. DOTS treatment is a safe and effective way for management of tuberculosis (Singh et al. 2016). The 75% of multidrug resistant cases are caused by exposure to MDR-TB bacteria. The other 25% is acquired which occurs whenever an individual exposed toward resistant TB therapy. The number one cause of acquired MDR-TB is ineffective tuberculosis therapy due to a variety of variables such as antibiotic usage, insufficient dose, and inadequate therapy. Today, it requires nearly 2 years for treating multiple drug resistant tuberculosis, and the medication is very toxic, costly, and complicated, that patients with MDR-TB seem unable to survive. Second-line medicines are used to treat MDR-TB, the second-line medications are fatal or have severe adverse effects.

18.4.4 Extensively Drug Resistant Tuberculosis [XDR-TB]

XRD-TB, i.e., extensively drug resistance tuberculosis is a novel tuberculosis variant resistant to fluoroquinolones as well as one or even more injectable second-line anti-TB drugs which including kanamycin, amikacin, and capreomycin (Singh et al. 2016). XDR-TB is a kind of tuberculosis that is more severe than typical TB. The development of resistance to at least four antitubercular medications, such as isoniazid, rifampicin along with any two of the new antitubercular medicines is necessary for diagnosis. Fluoroquinolones like moxifloxacin, levofloxacin, and second-line injectable aminoglycosides like capreomycin, amikacin, and kanamycin are among the newest drugs associated with XDR-TB.

18.5 Nanoantibiotics

Nanomaterials that exhibit antimicrobial activity on their own or improve the efficacy and safety of antibiotic treatment are referred to as nanoantibiotics (nAbts), and their potential to prevent infections has been investigated and proven, both in vitro and in vivo. The nAbts is one of the potentials uses of nanotechnology that uses physico-chemically conjugated antibiotics with small particles or chemically produced pure form of antibiotic molecules ranging around 100 nm in one dimension (Nano on reflection 2016; Soares et al. 2018). Nanoengineered antibiotic and its advancement impart novel medication approach that widely targets microorganism effectively. The inability to destroy intracellular pathogens due to their low penetration power and bioavailability in the targeted pathogens to achieve a beneficial pharmacological effect is one of the disadvantages of traditional TB drug formulation. Nanomedicine offers the potential to conquer such barriers and increase the therapeutic effectiveness of these drugs. It is being utilized to deliver medicines to areas of the body that were previously difficult to reach. The use of nAbts to treat tuberculosis improves medication effectiveness. By changing the size, charge, shape, functionalization or surface modification, and elemental composition, organ targeting of nanoparticles may be possible. Nanoengineered antibiotic designing depends on the mechanism of action, structure, and its therapy (Mamun et al. 2021). On the other hand, in the absence of core-corona structures, it is possible to link the molecules to nanoparticles surface either chemically or physically. These antibiotics are coupled either with chemically pure nAbts (e.g., Ti, Si, Ag, Au, Fe, etc.) or with surface modified pure nAbts with surface functionalization usually done with different polymers, citrate, PVP, carboxylate, etc. (Mamun et al. 2021). Antibiotics usually neutralize the bacteria by altering the cell membrane's proton-motive force, reducing the bacterium's production and storage capacities, preventing the synthesis of protein, leading to structural breakdown of the cell wall (Kohanski et al. 2010). Scientists are looking for "antibiotic like" substances and structure associated characteristics which kill bacteria in previously unidentified, novel ways. Most of the known antibiotics demand successive dosing with systematic release, but nanoantibiotics have the additional advantage of a targeted, controlled sustained

release which may be achieved with single dosage. The nAbts include "intelligent" multifunctional antibiotics which show interaction with bacterial membrane surfaces in a stimuli-responsive manner, resulting in increased penetration through membranes and deliver drug at target site (Mamun et al. 2021). The incorporation of nano-enabled functionalities to antimicrobials as well as bacteria targeted repurposed drugs altering particular characteristics as desired is broadening the utility of repurposed drugs and antibiotics. Antibiotics with nanoscale engineering play an important role in determining site selective antibacterial activity. Nanomedicine is being utilized to deliver medicines to areas of the body that were previously difficult to reach. The use of nanomedicine to treat tuberculosis improves medication effectiveness (Mamun et al. 2021).

18.6 Solid Lipid Particulate-Based Drug Delivery System

18.6.1 Solid Lipid Microparticles

These are nanoparticulate systems made up of colloidal particles of around 10–1000 nm size, which are mainly composed of biodegradable lipids melted in water or aqueous surfactant. Solid lipid microparticles are characterized by a core matrix of solid-lipid origin which may bring about solubilization of lipid soluble compounds possessing stability due to various surfactants. They are known to achieve effective drug targeting along with improved bioavailability, reduced toxicity, and higher drug loading capacity for hydrophilic and lipophilic drugs.

18.6.2 Solid Lipid Nanoparticles (SLNs)

Around in 1990s, SLNs were presented in the form of an alternate drug delivery strategy to the available conventional colloidal based delivery systems like niosomes, liposomes, polymeric nanoparticulates, and emulsions. Nanoparticles modified with different polymers are known to be more efficacious and exhibit enhanced therapeutic potentials (Shukla et al. 2019). Tumor targeting, minimization of side effects, and prevention of drug resistance can be achieved using nanosystems (Shukla et al. 2019). These nanoparticles contain around 2.5% of lipids such as triglycerides which are widely present in cell membranes. These lipids are primarily produced from fatty acids. Lipids such as fatty acids, partial glycerides, triglycerides along with waxes are common lipids. The usage of physiological lipids for the preparation of SLNs with low risks of acute/chronic toxicities is one of the most significant benefits of SLNs. Other substances that are commonly utilized include lecithin or soya lecithin, as well as non-ionic such as copolymers of propylene oxide or ethylene oxide. Owing to the physiologically abundant lipids employed for their creation, they offer great tolerance, stability, and the capacity to integrate extremely stable hydrophilic or hydrophobic medicines without any obvious harmful consequences. Solid lipid nanoparticles, i.e., SLN are a key development of

nanotechnology along with promising implications in different of fields, including efficient drug delivery, research, cosmeceuticals, and clinical therapeutics. INH-SLN, RIF-SLN, and PZA-SLN for nebulization were developed by Pandey and Khuller. The authors tested the efficacy of these formulations in tuberculosis infection; after just a one-time dose administration to guinea pigs in the form of nebulization, medication remained in the plasma for 5 days and for 7 days in other organs (liver, lung, and spleen), while content of free drug remained in the plasma for 1–2 days. Furthermore, the SLNs enhanced the drug's mean residence duration, pharmacokinetics, and bioavailability after 7 doses of therapy with aerosolized medicines, the SLNs acquired a significant benefit comparable to daily 46 doses of orally taken free medications. There was also no indication of hepatotoxicity (Pandey and Khuller 2005).

18.6.3 Nanostructured Lipid Carrier

Nanostructured lipid carrier not only provides several benefits in terms of drug targeting, but it also improves the systemic availability poorly soluble drugs and protects active components with are sensitive in nature. In the mid-1990s, nanostructured lipid carriers (NLCs) were created as alternate delivery system to the conventionally available carriers to make the particle blends, the solid lipids matrix, which is blended with molten lipids in a ratio of 70:30. Under optimal conditions, they may also load both hydrophilic and lipophilic medicines. NLCs may be made using techniques like high-pressure homogenization for obtaining high content of lipid particle dispersions containing solids ranging from 30 to 80%. This formulation allows for the incorporation of lipophilic and hydrophilic substances and may be delivered via various routes like oral, ophthalmic, pulmonary, and intravenous. Because of their extremely disordered lipid structures, NLCs primarily accommodate drugs. To encapsulate rifampicin, a nanostructured lipid carrier was designed by Song et al. 2015, exhibiting size in range of about 160 nm. The system could entrap rifampicin at a rate more than 90%, with significant uptake reported in NR8383 cells and alveolar macrophages (Song et al. 2015).

18.7 Emulsion Based Drug Delivery Systems

18.7.1 Microemulsion

This nanoparticle is mostly consisting of watery, oil, and emulsifying ingredients having diameter typically varies from 10 to 100 nm. The various compositions and combinations of substances utilized to create this structure impart thermodynamic stability to them, enhancing solubilization, boosting absorption, permeability, and protecting the drugs transported from enzyme hydrolysis. Microemulsions have a large variety of colloidal drug delivery potentials due to these characteristics. Depending upon its composition, microemulsions are of three major types: oil-in-

water (o/w), water-in-oil (o/w), and bicontinuous. With the use of data modelling, ANN, i.e., artificial neural network, a colloidal dosage form for isoniazid and rifampicin oral delivery was developed by Agatonovic-Kustrin et al. 2003, which is helpful for studying ternary and pseudo-ternary colloidal system for the process of microemulsion formation and stability of it (Agatonovic-Kustrin et al. 2003).

18.7.2 Nanoemulsion

This type of emulsion consists of droplets of diameter around 50–1000 nm. Nanoemulsion has two phases, one aqueous and one oil, and employs cosurfactants to assist and build kinetically and thermodynamically stable structure. The NE production is simple and requires little energy, making the operation inexpensive and simple. Given these features, NEs might have uses in a variety of healthcare sectors, including the experimental assessment of several anti-TB medications. Nikonenko et al. (2014) designed a SQ-641-NE phospholipid-based nanoemulsion system that was proven to be effective against Mycobacterium tuberculosis (Singh et al. 2016).

18.8 Vesicular Drug Delivery Systems

18.8.1 Liposomes

Liposomes are vesicles where an aqueous phase is contained within a bilayered lipid membrane, with a diameter ranging from 0.05 to 5.0μm. They may be manufactured and processed by generating structures having varying dimensions, charges, and components. Phospholipids, including synthetic and natural phospholipids, as well as sphingolipids, contribute to the lipidic elements. The biological constitution offers significant biological benefits which including simple decomposition, nonimmunogenicity, good biocompatibility, and nontoxicity, and the potential to incorporate lipophilic and hydrophilic compounds. The ability of liposomes to be ingested and incorporated via phagocytic cells, merged with the lysosomes, and then degradation takes place, liberating its contents within phagocytic cells, is its most vital characteristic. As a result, liposomes are extremely powerful against intracellular infections such as tuberculosis (Eduardo Pérez-Martínez and Zenteno-Cuevas 2020). As liposomes can reach deep into the lungs, they are efficient against intracellular infections. Liposomal phospholipids are taken up and recycled by alveolar type II cells. Liposomes prevent encapsulated drug molecules from decomposing and release them in a controlled manner at specific targets. For controlled and sustained release of antitubercular drugs, peptides, proteins, different liposomes are already developed. Liposomes serve as a carrier for active medicinal compounds such as proteins, peptides, antifungals, immunomodulators, diagnostics, enzymes, vaccines, cancer, ophthalmology, and genetic material. Liposomes are more beneficial due to their wide range of applicability. Liposomes of isoniazid

were prepared by film hydration by using crude soybean lecithin by using method of freeze thaw loading, isoniazid was encapsulated in liposomal nanocarrier. Isoniazid was successfully incorporated within liposomes with 78% efficiency and bioavailability of drug also improved.

18.8.2 Niosomes

Similar to liposomes, niosomes are bilayered membrane vesicles capable of encapsulating lipophilic as well as hydrophilic drugs in a vesicle comprised of lipid membrane. The materials used in manufacturing niosomes are less expensive, more stable, and less hazardous (Eduardo Pérez-Martínez and Zenteno-Cuevas 2020). Niosomes have the ability to transport many medicines, antigens, and hormones. Non-ionic surfactants like span-60 and span-80 can be utilized in niosomes since they can be stabilized by cholesterol and other lipids (Nvs 2011). Niosomes offer greater benefits over liposomes since the ingredients required to formulate them are less expensive and impart stability to the niosomes (Diljyot 2012). They enhance the therapeutic window of the active pharmaceutical ingredient able to achieve drug targeting. Special storage conditions are not required to store them. Given this niosomes can be utilized for the delivering of antitubercular drugs as the carriers (Eduardo Pérez-Martínez and Zenteno-Cuevas 2020). Rani et al. used a lipid hydration process to synthesize rifampicin and gatifloxacin niosomes. They used the BACTEC radiometric method to investigate the bactericidal effects of the niosomal preparation against the sensitive strain (H37Rv) as well as resistant strain (RF8554) of Mycobacterium tuberculosis, which reported inhibitory activity along with decreased growth index which indicates that both gatifloxacin and rifampicin niosomes produced extended release of drug, ideal for minimizing the dosage, reducing the duration of therapy, and increasing patient compliance (Rani et al. 2010).

18.8.3 Lipospheres

Lipospheres are microspheres of lipid with diameters around 0.01–100 mm. The solid core, which is made up of hydrophobic triglycerides having an outer surface embedding phospholipids monolayer, is utilized to transport drugs in a core solid matrix (Islan and Castro 2014). Because of their composition, they are excellent for topical, oral, as well as parenteral drug administration (Eduardo Pérez-Martínez and Zenteno-Cuevas 2020). Lipospheres are synthesized using a variety of processes including hot and cold homogenization, high-pressure homogenization, and solvent evaporation (Elgart et al. 2012). To encapsulate rifampicin, a lipid microsphere was created by Takenaga et al. (2008). Particle size of this lipid microsphere was about 247 nm and it is stable for 4 weeks at various temperature conditions like 4 or 25 °C (Takenaga et al. 2008).

18.9 Miscellaneous

18.9.1 Dendrimers

The dendrimers are polymeric systems with extensive branching having diameters around 2–10 nm. Synthetic nanomaterials are used in the construction of dendrimers. Internal core cavity of dendrimers can also be modified (Shukla et al. 2020). The interaction between dendrimer and drug is either covalent or non-covalent (Shukla et al. 2020). Dendrimers are well-known for their entrapment characteristics (Eduardo Pérez-Martínez and Zenteno-Cuevas 2020). The dendrimer surface has many sites where drug components, ligands, and materials like as polyethylene glycol (PEG) can be attached (Mutalik et al. 2014). Dendrimers are modified with these attachments to allow them to interact with target receptors. Attaching functional groups to the external surface brings about structural modifications, altering target receptor interactions along with enhancement in solubility, miscibility, and reactivity. The internal empty area of these structures can be utilized to enclose or integrate tiny drug moieties so as to exhibit minimal toxicity while the enhancement and regulation of drug release are aided by the functional groups (Eduardo Pérez-Martínez and Zenteno-Cuevas 2020). Molecular dynamics simulations were used to explore the interaction of the anti-TB medication rifampicin with a dendrimer of fourth generation poly(amidoamine) (G4-PAMAM). Overall, these findings indicated that PAMAM dendrimers have potential to be effective delivery system for rifampicin and its analogues, as far as pH-responsive release and stability are concerned (Bellini et al. 2015; Jain et al. 2013).

18.9.2 Nano/Microparticles

These circular structures are produced from several polymers, the most popular of which being poly lactide-co-glycolide (PLGA). Nanoparticles modified with different polymers are known to be more efficacious and exhibit enhanced therapeutic potentials (Shukla et al. 2019). Tumor targeting, minimization of side effects, and prevention of drug resistance can be achieved using nanosystems (Shukla et al. 2019). Nano/microparticles are easy to change based upon the structural moiety of API, organic solvents, and surfactants or the ligands employed in the synthesis and/or attachment. Half-life prolongation and bio-recognition or mucoadhesion enhancement by the bacterial cell wall are achieved by conjugation with biomolecules such as guar gums, lectins, etc. NMPs offer significant benefits over the other structures due to their biological flexibility and availability in a variety of sizes, being an promising system for the delivery of medicament that employs multiple routes of administration (Eduardo Pérez-Martínez and Zenteno-Cuevas 2020).

18.9.3 Microspheres

Microspheres are spherically shaped particles having diameters ranging from 1 to 1000 mm, composed of drug particles distributed in miscible polymers at the macroscopic or molecular level. Microspheres may be manufactured from a variety of biodegradable natural and manmade materials. According to some findings, MCs can be used to deliver antitubercular medicines in both organ tissues and blood plasma (Eduardo Pérez-Martínez and Zenteno-Cuevas 2020).

18.9.4 Carbon Nanotubes

In recent year, these nanoparticles have gained importance as carriers. Carbon nanotubes are a new sector of nano-biotechnology that offers a broad range of potential drug delivery applications. They are many micrometers long and have a diameter of approximately 1–100 nm (Petersen et al. 2011). Carbon nanotubes can be single walled or multi walled (Kaur et al. 2016). Surface of CNTBs are easily functionalized with proteins, peptides, and nucleic acids that effectively target tissue or cell. (Yang and Han 2000). The most significant benefit of carbon nanotubes is their capability to be readily surface functionalized using a number of pharmacological entities as well as functional/chemical groups, depending on required aspects of variety (Banyal et al. 2013). Carbon nanotubes have the ability to act both metallic and non-metallic (Kaur et al. 2014).

18.9.5 Nanosuspension

Nanosuspensions are colloidal dispersions that are typically micron sized (Rana and Murthy 2013). Surfactants are commonly used to stabilize nanosuspension. Nanonization is another effective approach for increasing drug solubility profile. It is defined as “aqueous dispersion of very fine solid particles” and is meant to be administered via various routes such as oral, parenteral, or topical. Dispersed particles size in nanosuspension should be around 200–600 nm (Eduardo Pérez-Martínez and Zenteno-Cuevas 2020). To minimize the drug’s toxicity and poor solubility (0.3 mg/mL), nanocrystalline suspension of clofazimine was developed by Peters et al. (2000). All organs exhibited reduced bacterial count. The findings indicate that the formulation is appropriate for intravenous administration. Since the concentration of drug was discovered to be high in the lungs, liver, and spleen, nanosuspension was proven efficacious against M. avium (Peters et al. 2000). Supercritical carbon-di-oxide aided atomization method (particles with sub-micron sized) was proposed by Reverchon et al. (2002) in order to prepare rifampicin loaded nanosuspension (Reverchon et al. 2002).

18.9.6 Quantum Dots

Semiconductor particles having sizes varying from 1 to 10 nm are known as quantum dots. Depending on their size, they may be made fluorescent in a variety of colors (Sharma et al. 2016). They have remarkable fluorophore properties, and they demonstrate broad emission spectra, due to which quantum dots are used to effectively identify and estimate cells infected with TB simply as well as quickly, within various targeted organs (Banyal et al. 2013).

18.9.7 Nanomicelles

These NAbts are polymeric micelles having a hydrophobic inner core and a hydrophilic exterior layer that range in size from 10 to 200 nm. NMCs offer significant benefits over other forms of NAbts owing to their structural stability, biocompatibility, and high drug loading capacity on exterior surface, along with the interior core possessing solubilized hydrophobic drugs (Eduardo Pérez-Martínez and Zenteno-Cuevas 2020). For rifampicin, Moretton et al. (2010) synthesized polymeric micelles by raising the PEG concentration, large micelles were discovered during the study. The antibiotic rifampicin was proven to get properly entrapped within the system (Moretton et al. 2013).

18.9.8 Polymerosomes

Polymerosomes are nanoparticles enclosed within bilayer membrane made up of hydrophilic polymers which are self-assembled and surround hydrophobic polymers. These comprise an aqueous core within and may transport a wide range of drugs such as hydrophilic, hydrophobic, and amphiphilic drug with excellent colloidal stability (Eduardo Pérez-Martínez and Zenteno-Cuevas 2020).

18.10 Advantages of Nanoantibiotics

Nanoantibiotics set up new standard for delivery of antimicrobial agents like antitubercular drugs to eliminate the limitations of conventional drug delivery systems. Conventional drug delivery has some limitations like poor bioavailability of some drugs, stability issues, time consuming therapy, multidrug resistance, increased drug degradation, less availability of drug at site of infection. There are several advantages of nanoantibiotics.

1. Nanocarriers are designed such as they can be triggered by different stimuli like magnetic, chemical, pH, and heat for the targeted delivery of drug molecules and as biological sensors (Dufresne et al. 2004; Sandhiya et al. 2009).

2. Nanoantibiotics effectively minimize adverse effects of many antitubercular drugs by enhancing their solubility and stability (Moghimi et al. 2005).
3. Many pathogenic bacteria gained resistance to conventional antibiotics thus nanocarrier drug delivery system seems advantageous in such cases (Taubes 2008).
4. The use of nanocarriers to deliver antitubercular drugs helps to improve pharmacokinetics. It also enhances half-life, therapeutic index, extended drug circulation, and regulates drug release (Huh and Kwon 2011).
5. Nanotechnology based antimicrobial drug delivery systems are cost-effective, require less time for therapy, reduce frequency of dosing (Weir et al. 2008).

18.11 Disadvantages of Nanoantibiotics

Even though nanoantibiotics offer considerable advantages and advancements in addressing critical issues in infectious disease treatment, there are significant barriers in transferring this promising technology into therapeutic application. These include carefully examining the nanoantibiotics interactions with tissues, cells, and organs, ultimately result in rectification and identification of appropriate delivery routes to attain the desired treatment efficacy (Sandhiya et al. 2009; Suri et al. 2007). To ensure successful clinical translation, a thorough understanding of the toxicological effects of nanoantibiotics is also necessary (El-Ansary and Al-Daihan 2009). The accumulation of Iv administered NAbts in the lung, lymphatics, colon, spleen, and bone marrow has been demonstrated. Inhaled NAbts can reach the lungs, brain, spleen, heart, and liver through the systemic circulation (Hagens et al. 2007). This becomes especially important for small nanoparticles due to excellent cellular absorption and transcytosis through epithelial and endothelial cells in bloodstream and lymph circulation (Rabea et al. 2003). The toxic effects of nanoantibiotics to public health are unknown at the present, however it is expected to be similar to the nanotoxicity of different nonantibiotic nanomaterials (El-Ansary and Al-Daihan 2009; Poma and Di Giorgio 2008). Many recent researches have shown that therapeutically given antimicrobials NAbts may cause multi-organ nanotoxicity. The interaction of antimicrobial NAbts with cells can cause oxidative stress induced by free radical, which can lead to pulmonary toxicity and hepatotoxicity (De Jong and Borm 2008; Dong et al. 2014). Several metabolic alterations contribute to mitochondrial failure, as well as increased fatty acid beta oxidation, glycolysis, and ketogenesis all of which lead to renal toxicity and hepatotoxicity (Dong et al. 2014). Aside from affecting the circulatory system by changing heart rate (Chalupa et al. 2004), certain NAbts can also greatly influence the reproductive system by increasing seminiferous epithelial detachment (Skovmand et al. 2019) and possibly causing spermatotoxicity (El-Ansary and Al-Daihan 2009; Talebi et al. 2013).

There is no conventional definition for NP dosage in mass, number, surface area, or biological specimens (blood, urine, and inside organ) because NAbts have size-specific characteristics that restricts the application of currently existing in vitro assays in a global manner (Hagens et al. 2007; Kroll et al. 2009). Therefore, it implies that there is a strong demand for novel characterization approaches that are unaffected by NP characteristics and also some biological medium (De Jong and Borm 2008). Toxicogenomic research, when combined with other developing technologies such as proteomics and metabonomic, might elucidate the molecular and genomic mechanisms of NP toxicity (Poma and Di Giorgio 2008).

18.12 Conclusion

Since time immemorial, pulmonary TB has been the second biggest cause of mortality after AIDS, and nowadays it continues to cause huge human agony. The development of multidrug and extensively drug resistance has created a number of challenges that have disrupted global TB control in both developing and developed countries. Adding to the above troubles, drug resistant TB possess greater challenge to effective TB control. Hence it becomes essential to develop newer drugs in order to shorten treatment period, reduce adverse effects, and exhibit longer bioavailability. However, no substantial advances to ATD therapy have been produced aside from few medications such as rifamycins and quinolones. TB vaccine also remains a far cry yet. However, the eradication of transmission of causative organism is a tough task to accomplish owing to diagnostic difficulties, MDR, and poor patient compliance. Number of antitubercular drugs are under research but there are many hurdles in their way. Nanoantibiotics are potential antibacterial agents of a new generation owing to its high surface area to volume ratio and distinctive physicochemical characteristics. Nanotechnology based treatment for tuberculosis has been demonstrated to have a valuable benefit than conventional tuberculosis therapy, with the potential to increase patient compliance and reduce drug treatment. Most significantly, NAbts allow for the combination of various independent and possibly synergistic methods on the similar basis in addition to improving antibacterial efficacy and combat antibiotic resistance. The use of nanoparticles is one of the potential measures being explored right now to enhance TB vaccine. Advances in nanocarrier-based delivery methods provide a commercially viable, feasible, and very promising alternative to prospective TB chemotherapy. The formulation's improved drug bioavailability and therapeutic utility may be achieved even at minimal therapeutic dosages, and the duration of chemotherapy can considerably minimize. Most of these attributes are essential in significantly lowering treatment costs, interactions mostly with anti-HIV medications are reduced, and strengthen management of multidrug resistant tuberculosis and latent tuberculosis. The findings provided above highlighted the fact that nanosystems offer enormous promise for the

Table 18.1 First-line antitubercular drugs (Hari et al. 2010)

Drug	BCS class	MOA	Adverse effects
Rifampicin	BCS-II Class	Rifampicin acts by inhibiting bacterial protein synthesis and transcription by inhibiting DNA-dependent RNA polymerase in a reversible manner	Flu-like symptoms, stomach upset, bleeding. Some other hypersensitivity reactions, rashes, chances of appearance of hepatitis-1
Isoniazid	BCS-III Class	Inhibits synthesis of mycolic acid which is fundamental component of cell wall	Sensations of burning mostly in extremities, hepatitis, tiredness, drug interaction, and fever are all symptoms of hepatitis
Ethambutol	BCS-III Class	Inhibits bacterial arabinogalactan synthesis, which prevents synthesis of bacterial cell wall	Visual imparities may occur
Pyrazinamide	BCS-III Class	The membrane energy is depleted and inhibited	Immunogenic responses, hepatitis, rashes, joint pain
Streptomycin (injectable)	–	Protein synthesis is inhibited by binding to 30S unit of ribosomes	Electrolyte imbalance (e.g., hypokalemia), ototoxicity, nephrotoxicity

treatment of tuberculosis. DOTS are more convenient and reasonable due to their primary advantages such as long shelf life, lowering of dosage frequency, and increase in bioavailability of drug. Another factor that makes nanoparticles more viable is the use of oral and inhalation methods of drug delivery. To summarize, toxicological aspects associated with knowing the destiny of nanocarrier's polymeric components in the body will determine the effectiveness of this technology. As per the above perspective, drug nanocarriers formulated using natural polymeric materials such as chitosan and alginate have promising future, despite that further research is needed. Adequate clinical investigation has to be done in order to assure that nanoparticle-based delivery approaches can enhance patient compliance in TB chemotherapy (Tables 18.1, 18.2, 18.3, 18.4, 18.5, and 18.6, Figs. 18.1 and 18.2).

Table 18.2 Second-line antitubercular drugs

Drug	BCS class	Mechanism of action	Adverse effects
Amikacin Kanamycin Capreomycin (injectable)	BCS-III Class – –	Each of them obstructs the synthesis of proteins	Electrolyte imbalance (e.g., hypokalemia), ototoxicity, nephrotoxicity (Hari et al. 2010)
Para-amino salicylic acid	BCS-IV/II Class	Two processes are inhibited: 1. Incorporation of PABA in FA (folic acid) 2. Metabolism of iron	Gastrointestinal abnormalities, allergic responses, and hypothyroidism (Hari et al. 2010)
Ethionamide	BCS-II Class	Inhibit synthesis of primary component of bacterial cell wall, i.e., mycolic acid	Anorexia, hepatotoxicity, gastric distress, and neurotoxicity (Hari et al. 2010)
Cycloserine	BCS-IV/II Class	D-alanine incorporation in peptidoglycan is inhibited	CNS toxicity, Stevens–Johnson syndrome (Hari et al. 2010)
Fluoroquinolones (levofloxacin, moxifloxacin, ciprofloxacin)	BCS-I Class BCS-I Class BCS-IV Class	Has significant in vitro action against Mycobacterium tuberculosis by inhibiting DNA gyrases and thereby preventing bacterial DNA synthesis	CNS related- insomnia, giddiness, and headache Gastric related-dyspepsia, nausea, vomiting

Table 18.3 New drugs for treatment of tuberculosis (Alladi Mohan and Prabath Kumar 2012)

Drugs under preclinical development stage	Drugs under clinical development stages	
	Phases	Drugs
Capuramucin (SQ-641) Benzothiazone (BTZ-043) Imidazopyridine (Q201) Nitroimidazole (TBA-354)	Phase I	Oxazolidinone AZD5847
	Phase II	Rifamycin: Rifapentine Diarylquinoline: Bedaquiline TMC207 Oxazolidinone: (Sutezolid (PNU-100480) (Linezolid)
	Phase III	Nitro-dihydro-imidazooxazole (Delamanid -OPC67683) Fluoroquinolones (Gatifloxacin, Moxifloxacin) 4-Thioureido-iminomethylpyridinium (Perchlorate) (Perchlozone)

Table 18.4 Drug resistance mechanism of first-line antitubercular drugs

Drug	Gene responsible for resistance	Function of gene	Mechanism of drug resistance
Pyrazinamide	pncA	Pyrazinamidase Nicotinamidase	The majority of Mycobacterium tuberculosis strains exhibiting resistance to pyrazinamide are mutated at pncA, which is responsible for 72–97% of known instances of resistance. Some resistant strains, however, lack the pncA type mutation (Zhang and Yew 2009)
Ethambutol	embB	Arabinosyl transferase	embCAB operon mutations are responsible for development of resistance, specifically in the emb region, rarely in embC, although around 68% resistant strains are direct manifestations of mutations in embB codon 306 (Zhang and Yew 2009)
Isoniazid	katG, inhA	Enoyl ACP reductase, Catalase-peroxidase	Kat GS315T mutation is responsible for resistance to isoniazid in around 94% of cases; mabA or inhA mutations may also account for resistance (Zhang and Yew 2009)
Rifampicin	rpoB	b subunit of RNA polymerase	In 96% of rifampicin resistant cases 81 base pair region of rpoB is found to be mutated, exhibiting both cross resistance and high level resistance (Palomino and Martin 2014)

Table 18.5 Examples of different nanosystems

Nanosystems	Drug	Polymer	Advantages	References
Solid lipid micro and nanoparticles	Rifabutin	Mannose	Mannose coated SLN formulations are comparatively more feasible for alveolar macrophages than uncoated formulations	(Nimje et al. 2009)
	Rifampin	Poloxamers 188, Tween 80, cetyl palmitate	Spherically shaped SLNs with diameter around 100 nm, entrapment efficiency around 82%, zeta potential in negative values, enhanced antibacterial activity against *Mycobacterium fortuitum,* 8 times lower MIC than Rifampin free solution	(Aboutaleb et al. 2012)
	Rifampicin	Tween 80 and Compritol ATO 888	Reduced degradation of isoniazid SLNs and rifampicin SLNs up to 12.35%	(Singh et al. 2013)
	Isoniazid	Tristearin and Phospholipon R 80 H	Spherically shaped SLNs with diameter around 164 nm; polysorbate 80 and sonication were responsible for enhanced entrapment and reduced particle size	(Nair et al. 2011)
	Rifampicin	Sodium taurocholate and stearic acid	Dry SLNs with improved flow properties and release profile	(Maretti et al. 2014)
	Isoniazid	Tween 80, Soya lecithin, stearic acid, compritol ATO 888	Small size (48 nm), high entrapment efficiency (68%), improved bioavailability as compared to free drug solution	(Bhandari and Kaur 2013)
Emulsion systems	Rifampicin, Isoniazid, Pyrazinamide	Oleic acid, ethanol, Tween 80	Rifampicin at pH 7.4 does not follow Fick's law for release	(Mehta et al. 2010)
	Rifampicin, Isoniazid, Pyrazinamide	Brij 96, ruthenium dichloride, nile red	Ruthenium dichloride interacts with isoniazid/pyrazinamide Rifampicin shows interaction with nile red dye	(Kaur and Mehta 2014)
	Rifampicin	Oleic acid, Tween 80, ethanol	Stable microemulsion with controlled/ sustained release	(Mehta et al. 2007)

(continued)

Table 18.5 (continued)

Nanosystems	Drug	Polymer	Advantages	References
	Isoniazid	Oleic acid, ethanol, Tween 80	Stable microemulsion even after incorporating isoniazid, controlled/ sustained release of drug	(Mehta et al. 2008)
	Rifampicin	Sefsol, Tween 80	Stable for around 20 months	(Ahmed et al. 2008)
	Rifampicin	Oleic acid, Tween 80, ethanol	Stable microemulsion, no phase separation	(Mehta et al. 2007)
Liposomal-based preparations	Capreomycin	Di-palmitoyl phosphatidyl- choline (DPPC), distearoyl phosphatidyl choline (DSPC), and hydrogenated phosphatidyl- choline (HPC)	High capreomycin sulfate content, suitable for inhalable formulations	(Ricci et al. 2006)
	Rifabutin	Phosphatidyl choline and phosphatidyl serine	Enhanced antimicrobial action against M. avium	(Gaspar et al. 2000)
	Sparfloxacin	Phosphatidyl glycerol, Phosphatidyl choline, cholesterol	Growth index reduction up to 30% for control and up to 25% for untreated group	(Düzgüneş et al. 1996)
	Isoniazid, Rifampin	di-stearyl phosphatidyl ethanolamine poly (ethylene glycol), egg phosphatidyl choline, monosialogangliosides, cholesterol, O-SAP, dicetyl phosphate	Enhanced accumulation of O-SAP containing liposomes in lungs	(Deol and Khuller 1997)
	Isoniazid, Rifampicin, Ethionamide, Pyrazinamide, Streptomycin	Di-stearoyl phosphatidylcholine and cholesterol	No leakage of drug occurs, constant mean vesicle diameter	(Justo and Moraes 2003)
	Streptomycin	Phosphatidyl choline, Polyethylene glycol, phosphatidyl glycerol, distearoyl phosphatidyl ethanolamine, distearoyl phosphatidyl choline, phosphati-dylinositol, cholesterol	Reduced MAC infection	(Gangadharam et al. 1995)

	Amikacin	Bristol	High amikacin entrapment	(Leitzke et al. 1998)
	Kanamycin	Cholesterol, Di-stearoyl phosphatidyl choline	Entrapment efficiency up to 63%, mean vesicle diameter 132 nm	(Justo and Moraes 2005)
	Isoniazid, Rifampicin	Phosphatidyl choline, cholesterol, dicetyl phosphate	Reduced mycobacterial count in various organs including lungs	(Labana et al. 2002)
	Isoniazid, Rifampicin, Ethionamide, Pyrazinamide	Cholesterol, Di-stearoyl phosphatidyl choline	Improved drug uptake, higher drug entrapment, sustained release pattern	(Bhardwaj et al. 2013)
	Pyrazinamide	Cholesterol, Di-palmitoyl phosphatidyl choline	Improved therapeutic activity	(El-Ridy et al. 2007)
	Isoniazid	Di-palmitoyl phosphatidyl choline (DPPC)	Sustained/controlled release	(Chimote and Banerjee 2010)
	Pyrazinamide	Cholesterol, porous mannitol, Soybean phosphatidylcholine	Liposomes with aerodynamic diameter 4.26–4.39 micrometer, encapsulation efficiency up to 45%	(Rojanarat et al. 2012)
	Rifabutin	Di-palmitoyl phosphatidyl glycerol, Di-palmitoyl phosphatidyl choline	Reduced count of CFUs	(Gaspar et al. 2008)
	DNA-hsp65 vaccine	DNA-hsp65 vaccine	Liposomal system can be utilized to load vaccine	(Rosada et al. 2008)
	Isoniazid, Rifampicin	Dicetyl phosphate, cholesterol, Phosphatidyl choline	Improved loading of rifampicin	(Pandey et al. 2004)
Dendrimer	Rifampicin	Polyethylene glycol, poly propyleneimine	Reduction in release at pH 7.4, reduced cytotoxicity, effective delivery of rifampicin	(Kumar et al. 2006)
	Rifampicin	Polyethylene glycol, poly propyleneimine	Increased drug encapsulation and declined release	(Kumar et al. 2007)
Niosomes	Isoniazid	Diethyl ether, cholesterol, sorbitan monostearate, dicetyl phosphate	Upon increasing cholesterol content drug entrapment increases up to certain extent thereafter it reduces drug dose and frequency, toxicity	(Singh et al. 2011)

(continued)

Table 18.5 (continued)

Nanosystems	Drug	Polymer	Advantages	References
	Rifampicin, Isoniazid	Span 80, polyethylene glycol 2000, Triton X 100	Higher drug entrapment for both the drugs	(Mehta et al. 2011)
	Ethambutol	stearyl amine, dicetyl phosphate, cholesterol	Improved targeting and drug loading (from 12.20 to 25.81%), neutral zeta potential	(El-Ridy et al. 2015)
	Rifampicin	span-85, cholesterol, triton-X-100, span-80, diethyl ether	Effective targeting in lymphatic regions	(Jain et al. 2006)
	Pyrazinamide	Span 85, Cholesterol, dicetyl phosphate, span 60, stearyl amine	Highest loading efficiency using span 60	(El-Ridy et al. 2011)

Table 18.6 Examples of miscellaneous nanosystems

Nanosystems	Drug	Polymer	Advantages	References
PLGA microspheres	Rifampicin	PLGA	Small particles size-3.45 mm	(O'Hara and Hickey 2000)
	Rifampicin	PLGA	Reduced bacterial count, reduced inflammation, and lung damage	(Suarez et al. 2001)
	Rifabutin	PLGA	Reduction in replication of Mycobacterium avium complex (MAC)	(Barrow et al. 2007)
	Rifampicin	PLGA	41% release in 5–6 days, diameter around 72 ± 0.16 mm	(Doan and Olivier 2009)
	Rifampicin	PLGA	Particle size-1.9 mm. effective uptake by NR-8383 cells and localization in phagolysosomes	(Feng et al. 2014) (Onoshita et al. 2010)
Alginate nanoparticles	Isoniazid, Rifampicin, Pyrazinamide	Alginate	Aerodynamic diameter-1.1 mm, entrapment efficiencies up to70–90% for both isoniazid and pyrazinamide, and for Rifampicin around 80–90%; Most of the particles exist in respirable range	(Ahmad et al. 2006)
	Pyrazinamide, Rifampicin, Isoniazid, Ethambutol	Alginate	Higher entrapment of drug, therapeutic drug concentration level was maintained in plasma for more than a week	(Ahmad et al. 2005)
PLGA microparticles	Isoniazid, Rifampicin	PLGA	Effective clearance of bacteria from lungs of experimental murine TB model	(Dutt and Khuller 2001)
	Rifampicin	PLGA	Larger uptake of rifampicin; micron sized particles rapidly excreted compared to nanosized particles	(Ohashi et al. 2009)
Inhalable microparticles	Isoniazid, Rifabutin	–	Intra-cellularly rifabutin and isoniazid level was maintained for 96 h and 24 h, respectively	(Verma et al. 2008)
	Isoniazid, Rifabutin	Poly (lactic acid) (LPLA)	Drug content around 50%, aerodynamic diameter-3.58 mm, Particle size-5 mm, around 70% of drug released within 10 days. Effective targeting of macrophage	(Muttil et al. 2007)

(continued)

Table 18.6 (continued)

Nanosystems	Drug	Polymer	Advantages	References
	Isoniazid, Rifabutin	Poly (D, L-lactic acid)	Large number of particles delivered in lungs with 2 min of exposure	(Sharma et al. 2001)
PLG nanoparticles	Ethionamide	PLGA	Size of nanoparticles-287 nm, drug entrapment efficiency-35%, controlled drug release for 6 days	(Kumar et al. 2011)
	Streptomycin	PLG	Size of nanoparticles-153 nm, drug encapsulation-32.12% and drug loading-14.28%. Streptomycin concentration retained for around 4 days in plasma and almost 7 days in different organs	(Pandey and Khuller 2007)
Oral PLGA nanoparticles	Ethionamide	PLGA	Size-286 ± 26 nm; zeta-potential-13 ± 2.5 mV, loading capacity-38.6 ± 2.3% and drug encapsulation efficiency-35.2 ± 3.1%	(Kumar et al. 2011)
	Isoniazid, Rifampicin, Pyrazinamide, Ethambutol	PLGA	Controlled drug levels maintained in plasma for at least 5–8 days, improved pharmacokinetic parameters	(Pandey and Khuller 2006)
Porous nanoparticle-aggregates particles (PNAPs) of PLGA	Rifampicin	PLGA	Effective deposition in lungs. Initial rapid in vitro release. Measurable rifampicin concentrations in pulmonary tissue for 8 h	(Sung et al. 2009)
Chitosan nanoparticles	Isoniazid	Chitosan	Decreased MIC against Mycobacterium avium. Reduced particle size with increased leucine concentration	(Pourshahab et al. 2011)
Gelatin nanoparticles	Isoniazid	Gelatin	Size ranges from 261 to 380 nm, drug loading-55%, effective targeting to alveolar regions, declined hepatic toxicity	(Saraogi et al. 2011)

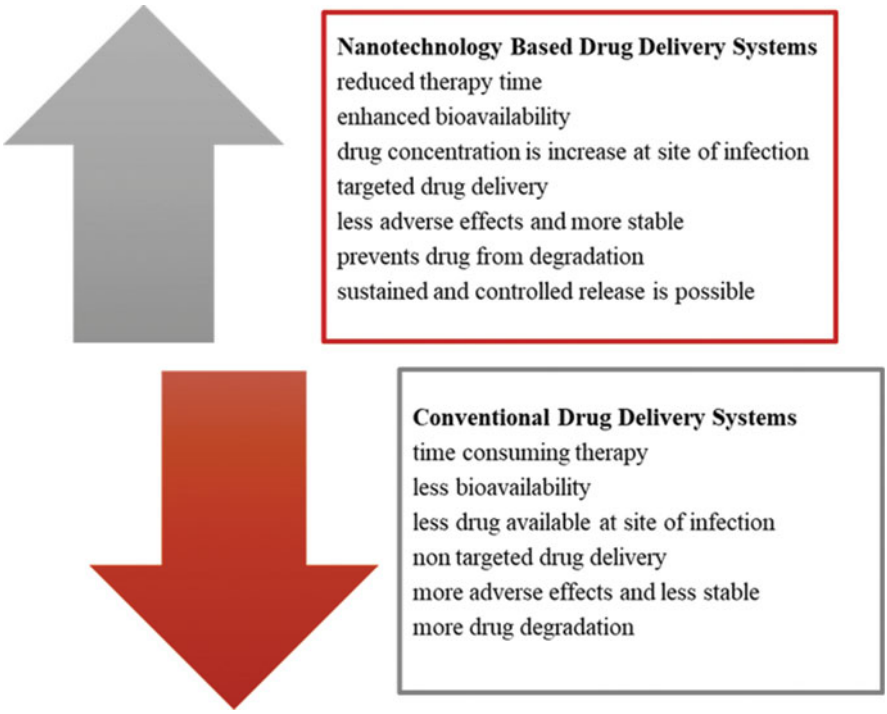

Fig. 18.1 Advantages of Nanotechnology Based DDS Over Conventional DDS

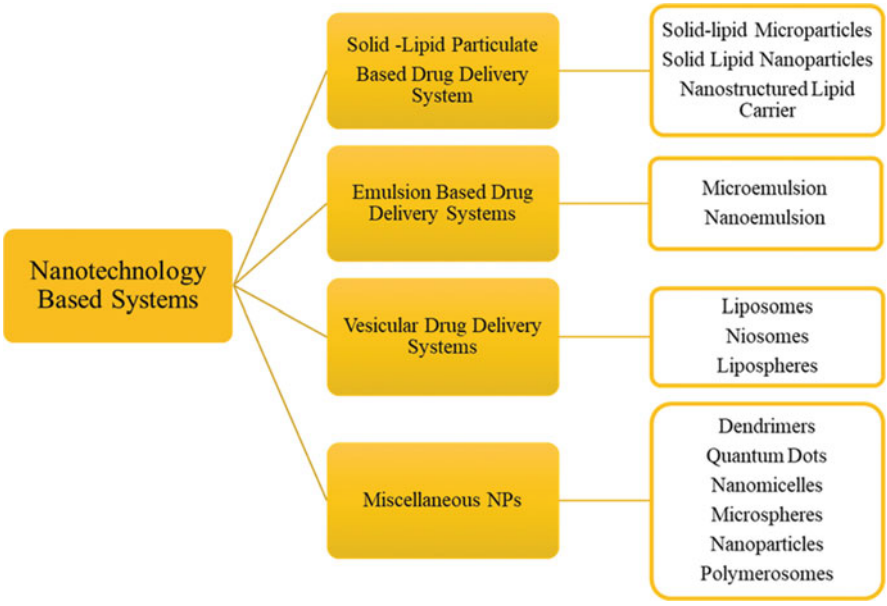

Fig. 18.2 Classification of Nanoantibiotics

Acknowledgements The authors thank the Department Pharmaceuticals (DOP) and the Department of Chemicals and Fertilizers, the Govt of India, for providing research facilities and funding. NIPER-R communication /247.

Author Contributions All authors have given permission for the final version of the manuscript.

Notes The authors claim that there is no competing financial interest.

References

Aboutaleb E, Noori M, Gandomi N, Atyabi F, Fazeli MR, Jamalifar H et al (2012) Improved antimycobacterial activity of rifampin using solid lipid nanoparticles. Int Nano Lett 2(1):33

Agatonovic-Kustrin S, Glass BD, Wisch MH, Alany RG (2003) Prediction of a stable microemulsion formulation for the oral delivery of a combination of antitubercular drugs using ANN methodology. Pharm Res 20(11):1760–1765

Ahmad Z, Sharma S, Khuller GK (2005) Inhalable alginate nanoparticles as antitubercular drug carriers against experimental tuberculosis. Int J Antimicrob Agents 26(4):298–303

Ahmad Z, Pandey R, Sharma S, Khuller GK (2006) Alginate nanoparticles as antituberculosis drug carriers: formulation development, pharmacokinetics and therapeutic potential. Indian J Chest Dis Allied Sci 48(3):171–176

Ahmed M, Ramadan W, Rambhu D, Shakeel F (2008) Potential of nanoemulsions for intravenous delivery of rifampicin. Pharmazie 63(11):806–811

Alladi Mohan D Prabath Kumar JH (2012) Newer anti-Tb drugs and drug delivery systems. Med Update 388–92

Banyal S, Malik P, Tuli HS, Mukherjee TK (2013) Advances in nanotechnology for diagnosis and treatment of tuberculosis. Curr Opin Pulm Med 19(3):289–297

Barrow ELW, Barrow WW, Quenelle DC, Westbrook L, Winchester GA, Staas JK (2007) Efficacy of rifabutin-loaded microspheres for treatment of Mycobacterium avium-infected macrophages and mice. Drug Deliv 14(3):119–127

Bellini RG, Guimarães AP, Pacheco MAC, Dias DM, Furtado VR, de Alencastro RB et al (2015) Association of the anti-tuberculosis drug rifampicin with a PAMAM dendrimer. J Mol Graph Model 60:34–42

Bhandari R, Kaur IP (2013) Pharmacokinetics, tissue distribution and relative bioavailability of isoniazid-solid lipid nanoparticles. Int J Pharm 441(1–2):202–212

Bhardwaj A, Kumar L, Narang RK, Murthy RSR (2013) Development and characterization of ligand-appended liposomes for multiple drug therapy for pulmonary tuberculosis. Artif Cells Nanomed Biotechnol 41(1):52–59

Bhatt K, Salgame P (2007) Host innate immune response to mycobacterium tuberculosis. J Clin Immunol 27:347–362

Chalupa DC, Morrow PE, Oberdörster G, Utell MJ, Frampton MW (2004) Ultrafine particle deposition in subjects with asthma. Environ Health Perspect 112(8):879–882

Chimote G, Banerjee R (2010) In vitro evaluation of inhalable isoniazid-loaded surfactant liposomes as an adjunct therapy in pulmonary tuberculosis. J Biomed Mater Res B Appl Biomater 94(1):1–10

Cooper AM (2009) Cell-mediated immune responses in tuberculosis. Annu Rev Immunol 27:393–422

De Jong WH, Borm PJA (2008) Drug delivery and nanoparticles:applications and hazards. Int J Nanomedicine 3(2):133–149

Deol P, Khuller GK (1997) Lung specific stealth liposomes: stability, biodistribution and toxicity of liposomal antitubercular drugs in mice. Biochim Biophys Acta 1334(2–3):161–172

Diljyot K (2012) Niosomes: a new approach to targeted drug delivery. Int J Pharm Phytopharm Res 53(1):53–59

Doan TVP, Olivier JC (2009) Preparation of rifampicin-loaded PLGA microspheres for lung delivery as aerosol by premix membrane homogenization. Int J Pharm 382(1–2):61–66

Dong S, Gao Z, Shen X, Xue H, Li X (2014) Comparative proteomic analysis shows an elevation of Mdh1 associated with hepatotoxicity induced by copper nanoparticle in rats. J Integr Agric 13(5):1073–1081

Dufresne M-H, Le Garrec D, Sant V, Leroux J-C, Ranger M (2004) Preparation and characterization of water-soluble pH-sensitive nanocarriers for drug delivery. Int J Pharm 277(1–2):81–90

Dutt M, Khuller GK (2001) Chemotherapy of Mycobacterium tuberculosis infections in mice with a combination of isoniazid and rifampicin entrapped in poly (DL-lactide-co-glycolide) microparticles. J Antimicrob Chemother 47(6):829–835

Düzgüneş N, Flasher D, Reddy MV, Luna-Herrera J, Gangadharam PR (1996) Treatment of intracellular Mycobacterium avium complex infection by free and liposome-encapsulated sparfloxacin. Antimicrob Agents Chemother 40(11):2618–2621

Eduardo Pérez-Martínez D, Zenteno-Cuevas R (2020) Nanotechnology as a potential tool against drug- and multidrug-resistant tuberculosis. In: Nanotechnology based approaches for tuberculosis treatment. Elsevier Inc., 37–52 p

El-Ansary A, Al-Daihan S (2009) On the toxicity of therapeutically used nanoparticles: an overview. J Toxicol 2009:754810

Elgart A, Cherniakov I, Aldouby Y, Domb AJ, Hoffman A (2012) Lipospheres and pro-nano lipospheres for delivery of poorly water soluble compounds. Chem Phys Lipids 165(4):438–453

El-Ridy MS, Mostafa DM, Shehab A, Nasr EA, Abd E-AS (2007) Biological evaluation of pyrazinamide liposomes for treatment of Mycobacterium tuberculosis. Int J Pharm 330(1–2): 82–88

El-Ridy MS, Abdelbary A, Nasr EA, Khalil RM, Mostafa DM, El-Batal AI et al (2011) Niosomal encapsulation of the antitubercular drug, pyrazinamide. Drug Dev Ind Pharm 37(9):1110–1118

El-Ridy MS, Yehia SA, Kassem MA-E-M, Mostafa DM, Nasr EA, Asfour MH (2015) Niosomal encapsulation of ethambutol hydrochloride for increasing its efficacy and safety. Drug Deliv 22(1):21–36

Feng R, Zhang Z, Li Z, Huang G (2014) Preparation and in vitro evaluation of etoposide-loaded PLGA microspheres for pulmonary drug delivery. Drug Deliv 21(3):185–192

Gangadharam PR, Ashtekar DR, Flasher DL, Düzgüneş N (1995) Therapy of Mycobacterium avium complex infections in beige mice with streptomycin encapsulated in sterically stabilized liposomes. Antimicrob Agents Chemother 39(3):725–730

Garg T, Rath G, Goyal AK (2015) Biomaterials-based nanofiber scaffold: targeted and controlled carrier for cell and drug delivery. J Drug Target 23(3):202–221

Gaspar MM, Neves S, Portaels F, Pedrosa J, Silva MT, Cruz ME (2000) Therapeutic efficacy of liposomal rifabutin in a Mycobacterium avium model of infection. Antimicrob Agents Chemother 44(9):2424–2430

Gaspar MM, Cruz A, Penha AF, Reymão J, Sousa AC, Eleutério CV et al (2008) Rifabutin encapsulated in liposomes exhibits increased therapeutic activity in a model of disseminated tuberculosis. Int J Antimicrob Agents 31(1):37–45

Hagens WI, Oomen AG, de Jong WH, Cassee FR, Sips AJAM (2007) What do we (need to) know about the kinetic properties of nanoparticles in the body? Regul Toxicol Pharmacol 49(3): 217–229

Hari BNV, Chitra KP, Bhimavarapu R, Karunakaran P, Muthukrishnan N, Rani BS (2010) Novel technologies: a weapon against tuberculosis. Indian J Pharmacol 42(6):338–344

Horvath CN, Shaler CR, Jeyanathan M, Zganiacz A, Xing Z (2012) Mechanisms of delayed anti-tuberculosis protection in the lung of parenteral BCG-vaccinated hosts: a critical role of airway luminal T cells. Mucosal Immunol 5(4):420–431

Huh AJ, Kwon YJ (2011) "Nanoantibiotics": a new paradigm for treating infectious diseases using nanomaterials in the antibiotics resistant era. J Control Release 156(2):128–145

Islan GA, Castro GR (2014) Tailoring of alginate-gelatin microspheres properties for oral ciprofloxacin-controlled release against Pseudomonas aeruginosa. Drug Deliv 21(8):615–626
Jain C, Vyas S, Dixit V (2006) Niosomal system for delivery of rifampicin to lymphatics. Indian J Pharm Sci [Internet] 68. https://link.gale.com/apps/doc/A157068725/HRCA?u=anon~ce8f533d&sid=googleScholar&xid=6c045187
Jain V, Maingi V, Maiti PK, Bharatam PV (2013) Molecular dynamics simulations of PPI dendrimer–drug complexes. Soft Matter 9(28):6482–6496
Justo OR, Moraes AM (2003) Incorporation of antibiotics in liposomes designed for tuberculosis therapy by inhalation. Drug Deliv 10(3):201–207
Justo OR, Moraes AM (2005) Kanamycin incorporation in lipid vesicles prepared by ethanol injection designed for tuberculosis treatment. J Pharm Pharmacol 57(1):23–30
Kaur G, Mehta SK (2014) Probing location of anti-TB drugs loaded in Brij 96 microemulsions using thermoanalytical and photophysical approach. J Pharm Sci 103(3):937–944
Kaur V, Garg T, Rath G, Goyal AK (2014) Therapeutic potential of nanocarrier for overcoming to P-glycoprotein. J Drug Target 22(10):859–870
Kaur M, Garg T, Narang RK (2016) A review of emerging trends in the treatment of tuberculosis. Artif Cells Nanomed Biotechnol. 44(2):478–484
Kohanski MA, Dwyer DJ, Collins JJ (2010) How antibiotics kill bacteria: from targets to networks. Nat Rev Microbiol 8(6):423–435
Kroll A, Pillukat MH, Hahn D, Schnekenburger J (2009) Current in vitro methods in nanoparticle risk assessment: limitations and challenges. Eur J Pharm Biopharm 72(2):370–377
Kumar PV, Asthana A, Dutta T, Jain NK (2006) Intracellular macrophage uptake of rifampicin loaded mannosylated dendrimers. J Drug Target 14(8):546–556
Kumar PV, Agashe H, Dutta T, Jain NK (2007) PEGylated dendritic architecture for development of a prolonged drug delivery system for an antitubercular drug. Curr Drug Deliv 4(1):11–19
Kumar G, Sharma S, Shafiq N, Pandhi P, Khuller GK, Malhotra S (2011) Pharmacokinetics and tissue distribution studies of orally administered nanoparticles encapsulated ethionamide used as potential drug delivery system in management of multi-drug resistant tuberculosis. Drug Deliv 18(1):65–73
Labana S, Pandey R, Sharma S, Khuller GK (2002) Chemotherapeutic activity against murine tuberculosis of once weekly administered drugs (isoniazid and rifampicin) encapsulated in liposomes. Int J Antimicrob Agents 20(4):301–304
Leitzke S, Bucke W, Borner K, Müller R, Hahn H, Ehlers S (1998) Rationale for and efficacy of prolonged-interval treatment using liposome-encapsulated amikacin in experimental *Mycobacterium avium* infection. Antimicrob Agents Chemother 42(2):459–461
Mamun MM, Sorinolu AJ, Munir M, Vejerano EP (2021) Nanoantibiotics: functions and properties at the nanoscale to combat antibiotic resistance. Front Chem 9:348
Maretti E, Rossi T, Bondi M, Croce MA, Hanuskova M, Leo E et al (2014) Inhaled solid lipid microparticles to target alveolar macrophages for tuberculosis. Int J Pharm 462(1–2):74–82
Mehta SK, Kaur G, Bhasin KK (2007) Analysis of Tween based microemulsion in the presence of TB drug rifampicin. Colloids Surf B Biointerfaces 60(1):95–104
Mehta SK, Kaur G, Bhasin KK (2008) Incorporation of Antitubercular drug isoniazid in pharmaceutically accepted microemulsion: effect on microstructure and physical parameters. Pharm Res 25(1):227–236
Mehta SK, Kaur G, Bhasin KK (2010) Entrapment of multiple anti-Tb drugs in microemulsion system: quantitative analysis, stability, and in vitro release studies. J Pharm Sci 99(4): 1896–1911
Mehta SK, Jindal N, Kaur G (2011) Quantitative investigation, stability and in vitro release studies of anti-TB drugs in triton niosomes. Colloids Surf B Biointerfaces 87(1):173–179
Moghimi SM, Hunter AC, Murray JC (2005) Nanomedicine: current status and future prospects. FASEB J 19(3):311–330

Moretton MA, Chiappetta DA, Andrade F, das Neves J, Ferreira D, Sarmento B et al (2013) Hydrolyzed galactomannan-modified nanoparticles and flower-like polymeric micelles for the active targeting of rifampicin to macrophages. J Biomed Nanotechnol 9(6):1076–1087

Mutalik S, Shetty PK, Kumar A, Kalra R, Parekh HS (2014) Enhancement in deposition and permeation of 5-fluorouracil through human epidermis assisted by peptide dendrimers. Drug Deliv 21(1):44–54

Muttil P, Kaur J, Kumar K, Yadav AB, Sharma R, Misra A (2007) Inhalable microparticles containing large payload of anti-tuberculosis drugs. Eur J Pharm Sci 32(2):140–150

Nair R, Vishnu Priya K, Arun Kumar KS, Badivaddin TM, Sevukarajan M (2011) Formulation and evaluation of solid lipid nanoparticles of water soluble drug: isoniazid. J Pharm Sci Res 3(5): 1256–1264

Nano on reflection (2016) Nat Nanotechnol 11(10):828–834

Nasiruddin M, Neyaz MK, Das S (2017) Nanotechnology-based approach in tuberculosis treatment. Tuberc Res Treat 2017(Table 1):1–12

Nimje N, Agarwal A, Saraogi GK, Lariya N, Rai G, Agrawal H et al (2009) Mannosylated nanoparticulate carriers of rifabutin for alveolar targeting. J Drug Target 17(10):777–787

Nvs M (2011) International journal of research in pharmacy and chemistry niosomes: a novel drug delivery system. IJRPC 1(3):498–511

O'Hara P, Hickey AJ (2000) Respirable PLGA microspheres containing rifampicin for the treatment of tuberculosis: manufacture and characterization. Pharm Res 17(8):955–961

Ohashi K, Kabasawa T, Ozeki T, Okada H (2009) One-step preparation of rifampicin/poly(lactic-co-glycolic acid) nanoparticle-containing mannitol microspheres using a four-fluid nozzle spray drier for inhalation therapy of tuberculosis. J Control Release 135(1):19–24

Onoshita T, Shimizu Y, Yamaya N, Miyazaki M, Yokoyama M, Fujiwara N et al (2010) The behavior of PLGA microspheres containing rifampicin in alveolar macrophages. Colloids Surf B Biointerfaces 76(1):151–157

Palomino JC, Martin A (2014) Drug resistance mechanisms in Mycobacterium tuberculosis. Antibiotics 3(3):317–340

Pandey R, Khuller GK (2005) Solid lipid particle-based inhalable sustained drug delivery system against experimental tuberculosis. Tuberculosis (Edinb) 85(4):227–234

Pandey R, Khuller GK (2006) Oral nanoparticle-based antituberculosis drug delivery to the brain in an experimental model. J Antimicrob Chemother 57(6):1146–1152

Pandey R, Khuller GK (2007) Nanoparticle-based oral drug delivery system for an injectable antibiotic—streptomycin. Evaluation in a murine tuberculosis model. Chemotherapy 53(6): 437–441

Pandey R, Sharma S, Khuller GK (2004) Lung specific stealth liposomes as antitubercular drug carriers in Guinea pigs. Indian J Exp Biol 42(6):562–566

Peters K, Leitzke S, Diederichs JE, Borner K, Hahn H, Müller RH et al (2000) Preparation of a clofazimine nanosuspension for intravenous use and evaluation of its therapeutic efficacy in murine Mycobacterium avium infection. J Antimicrob Chemother 45(1):77–83

Petersen EJ, Zhang L, Mattison NT, O'Carroll DM, Whelton AJ, Uddin N et al (2011) Potential release pathways, environmental fate, and ecological risks of carbon nanotubes. Environ Sci Technol 45(23):9837–9856

Poma A, Di Giorgio ML (2008) Toxicogenomics to improve comprehension of the mechanisms underlying responses of in vitro and in vivo systems to nanomaterials: a review. Curr Genomics 9(8):571–585

Pourshahab PS, Gilani K, Moazeni E, Eslahi H, Fazeli MR, Jamalifar H (2011) Preparation and characterization of spray dried inhalable powders containing chitosan nanoparticles for pulmonary delivery of isoniazid. J Microencapsul 28(7):605–613

Rabea EI, Badawy ME-T, Stevens CV, Smagghe G, Steurbaut W (2003) Chitosan as antimicrobial agent: applications and mode of action. Biomacromolecules 4(6):1457–1465

Rana P, Murthy RSR (2013) Formulation and evaluation of mucoadhesive buccal films impregnated with carvedilol nanosuspension: a potential approach for delivery of drugs having high first-pass metabolism. Drug Deliv 20(5):224–235

Rani NP, Suriyaprakash TNK, Senthamarai R (2010) Formulation and evaluation of rifampicin and gatifloxacin niosomes on logarithmic-phase cultures of mycobacterium tuberculosis. Int J Pharma Bio Sci 1(4)

Reverchon E, De Marco I, Della Porta G (2002) Rifampicin microparticles production by supercritical antisolvent precipitation. Int J Pharm 243(1–2):83–91

Ricci M, Giovagnoli S, Blasi P, Schoubben A, Perioli L, Rossi C (2006) Development of liposomal capreomycin sulfate formulations: effects of formulation variables on peptide encapsulation. Int J Pharm 311(1–2):172–181

Rojanarat W, Nakpheng T, Thawithong E, Yanyium N, Srichana T (2012) Inhaled pyrazinamide proliposome for targeting alveolar macrophages. Drug Deliv 19(7):334–345

Rosada RS, de la Torre LG, Frantz FG, Trombone APF, Zárate-Bladés CR, Fonseca DM et al (2008) Protection against tuberculosis by a single intranasal administration of DNA-hsp65 vaccine complexed with cationic liposomes. BMC Immunol 9(1):38

Sandhiya S, Dkhar SA, Surendiran A (2009) Emerging trends of nanomedicine—an overview. Fundam Clin Pharmacol 23(3):263–269

Saraogi GK, Sharma B, Joshi B, Gupta P, Gupta UD, Jain NK et al (2011) Mannosylated gelatin nanoparticles bearing isoniazid for effective management of tuberculosis. J Drug Target 19(3): 219–227

Sharma R, Saxena D, Dwivedi AK, Misra A (2001) Inhalable microparticles containing drug combinations to target alveolar macrophages for treatment of pulmonary tuberculosis. Pharm Res 18(10):1405–1410

Sharma R, Garg T, Goyal AK, Rath G (2016) Development, optimization and evaluation of polymeric electrospun nanofiber: a tool for local delivery of fluconazole for management of vaginal candidiasis. Artif Cells Nanomed Biotechnol 44(2):524–531

Shukla R, Handa M, Lokesh S, Ruwali M, Kohli K, Kesharwani P (2019) Conclusion and future prospective of polymeric nanoparticles for cancer therapy, p 389–408

Shukla R, Sethi A, Handa M, Mohan M, Tripathi PK, Kesharwani P (2020) Chapter 10—dendrimer-based drug delivery systems for tuberculosis treatment. In: Kesharwani P, editor. Nanotechnology based approaches for tuberculosis treatment. Academic, p 163–74

Singh G, Dwivedi H, Saraf SK, Saraf SA (2011) Niosomal delivery of isoniazid—development and characterization. Trop J Pharm Res 10:203–210

Singh H, Bhandari R, Kaur IP (2013) Encapsulation of Rifampicin in a solid lipid nanoparticulate system to limit its degradation and interaction with isoniazid at acidic pH. Int J Pharm 446(1–2): 106–111

Singh J, Garg T, Rath G, Goyal AK (2016) Advances in nanotechnology-based carrier systems for targeted delivery of bioactive drug molecules with special emphasis on immunotherapy in drug resistant tuberculosis—a critical review. Drug Deliv 23(5):1676–1698

Skovmand A, Jensen ACØ, Maurice C, Marchetti F, Lauvås AJ, Koponen IK et al (2019) Effects of maternal inhalation of carbon black nanoparticles on reproductive and fertility parameters in a four-generation study of male mice. Part Fibre Toxicol 16(1):13

Soares S, Sousa J, Pais A, Vitorino C (2018) Nanomedicine: principles, properties, and regulatory issues. Front Chem 6:360

Song X, Lin Q, Guo L, Fu Y, Han J, Ke H et al (2015) Rifampicin loaded mannosylated cationic nanostructured lipid carriers for alveolar macrophage-specific delivery. Pharm Res 32(5): 1741–1751

Suarez S, O'Hara P, Kazantseva M, Newcomer CE, Hopfer R, McMurray DN et al (2001) Airways delivery of rifampicin microparticles for the treatment of tuberculosis. J Antimicrob Chemother 48(3):431–434

Sung JC, Padilla DJ, Garcia-Contreras L, Verberkmoes JL, Durbin D, Peloquin CA et al (2009) Formulation and pharmacokinetics of self-assembled rifampicin nanoparticle systems for pulmonary delivery. Pharm Res 26(8):1847–1855

Suri SS, Fenniri H, Singh B (2007) Nanotechnology-based drug delivery systems. J Occup Med Toxicol 2(1):16

Szulc-Kielbik I, Kielbik M, Przygodzka P, Brzostek A, Dziadek J, Klink M (2019) *Mycobacterium tuberculosis* requires cholesterol oxidase to disrupt TLR2 signalling in human macrophages. Novick D, editor. Mediators Inflamm 2019:2373791

Takenaga M, Ohta Y, Tokura Y, Hamaguchi A, Igarashi R, Disratthakit A et al (2008) Lipid microsphere formulation containing rifampicin targets alveolar macrophages. Drug Deliv 15(3): 169–175

Talebi AR, Khorsandi L, Moridian M (2013) The effect of zinc oxide nanoparticles on mouse spermatogenesis. J Assist Reprod Genet 30(9):1203–1209

Taubes G (2008) The bacteria fight back. Science 321(5887):356–361

Vergne I, Chua J, Singh SB, Deretic V (2004) Cell biology of mycobacterium tuberculosis phagosome. Annu Rev Cell Dev Biol 20:367–394

Verma RK, Kaur J, Kumar K, Yadav AB, Misra A (2008) Intracellular time course, pharmacokinetics, and biodistribution of isoniazid and rifabutin following pulmonary delivery of inhalable microparticles to mice. Antimicrob Agents Chemother 52(9):3195–3201

Weir E, Lawlor A, Whelan A, Regan F (2008) The use of nanoparticles in anti-microbial materials and their characterization. Analyst 133(7):835–845

Yang L, Han J (2000) Electronic structure of deformed carbon nanotubes. Phys Rev Lett 1:2–5

Zhang Y, Yew WW (2009) Mechanisms of drug resistance in Mycobacterium tuberculosis. Int J Tuberc Lung Dis 13(11):1320–1330

Nanoremediation: Role of New Generation Nanomaterials for the Effective Removal of Pharmaceutical Contaminants from Wastewater

19

Aarif Hussain Shah and Mushtaq Ahmad Rather

Abstract

Rapid industrialization and a steadily growing population have put immense pressure on the natural resources, leading to increased release of hazardous and toxic effluents such as pesticides, heavy metals, dyes, pharmaceutical residues, etc. into the environment. These toxic, non-biodegradable, and recalcitrant pollutants can enter food chain and thus pose a serious threat to the human and aquatic life. Pharmaceutical drugs (including their intermediates and transformation products) like antibiotics, analgesics, hormones, endocrine disrupting compounds are considered as new emerging contaminants that have been recently detected in surface water, groundwater, wastewater effluents, drinking water, and even in food sources. Present day conventional wastewater treatment methods are unable to remove these pharmaceutical residues completely. There is a large-scale consumption of antibiotics, antipyretics, etc. due to COVID-19 pandemic that will lead to elevated levels of pharmaceutical residues in the water bodies. Nanomaterials like nanomembranes, nanosorbents, and nanophotocatalysts are potent tools that are used for treatment of water containing emerging contaminants. These new generation nanomaterials are able to remove these contaminants even at low concentrations and under varied operating conditions of pH and temperature.

Keywords

Nanoremediation · Emerging contaminants · Pharmaceutical residues · Nanophotocatalysts · Anti-microbial activity · Advanced oxidation process

A. H. Shah (✉) · M. A. Rather
Department of Chemical Engineering, National Institute of Technology, Srinagar, India
e-mail: aarif_shah@nitsri.ac.in

S. Hameed, S. Rehman (eds.), *Nanotechnology for Infectious Diseases*,
https://doi.org/10.1007/978-981-16-9190-4_19

19.1 Introduction

The presence of abundant and quality water resources is indispensable for the sustainable development of the nations. The constant population growth, limited resources, rapid industrialization, and massive urbanization have led to immense pressure on global water resources (Alcamo et al. 2017). The outcome of all these factors is the generation of wastewater containing diverse pollutants like heavy metals, dyes, pesticides, herbicides, and pharmaceutical residues. Chemical, textile, steel, petroleum, agricultural and pharmaceutical industries, and effluents from hospitals and domestic sewage are the major sources of these pollutants (Hlongwane et al. 2019) Excess water pollution has resulted in shortage of safe drinking water and this crisis is escalating with each passing day.

Pharmaceuticals are very important for maintaining the health of human and livestock population. Thus they form an indispensable tool for the diagnosis and treatment. However, the use of these pharmaceutical compounds has witnessed a huge growth over the last decades, resulting in presence of low levels of these drugs and their metabolites in surface water, groundwater, sewage treatment plant effluents, and even in drinking water. Pharmaceutical compounds, their residues and metabolites are now recognized as "emerging contaminants" (ECs) due to the serious threat they pose to the environment and human health (Fekadu et al. 2019). They are not completely removed from wastewater by conventional wastewater treatment methods. ECs persist in the environment due to their recalcitrant nature and low biodegradability. This has led to a growing concern about their toxicity and potential hazard to the environment, humans, and aquatic life.

The conventional wastewater treatment methods like activated carbon adsorption, reverse osmosis, filtration, sedimentation, chemical oxidation, etc. are not able to completely remove these contaminants. Besides transforming them from one phase to another, these methods also generate toxic intermediates and by-products (Blair 2016). Advanced oxidation processes (AOP) are being developed for effective and economical treatment of wastewater containing PRs. Over the past two decades, ECs are now receiving huge attention from scientific community due to their environmental relevance. This can be observed from the number of publications generated by the countries in the last 20 years.

AOP use ultraviolet (UV) radiation and oxidants like H_2O_2 to generate hydroxyl radicals ($HO^{\cdot}$) and other reactive species like ($O_2^{\cdot -}$, O_3) for treatment of various organic pollutants and pathogens. Heterogeneous photocatalysis is an advanced oxidation process that employs semiconductor catalysts like titanium dioxide (TiO_2), zinc oxide (ZnO), ferric oxide (Fe_2O_3), cadmium sulfide (CdS), gallium phosphide (GaP), and zinc sulfide (ZnS) to degrade various PRs into carbon dioxide (CO_2) and water (H_2O). These nanoparticles have also been reported to show biological activities like anti-fungal, anti-bacterial, and anti-cancerous properties (Rehman et al. 2021a; Al-Jameel et al. 2021; Ravinayagam and Rehman 2020; Alahmari et al. 2021). The use of nanotechnology based AOP for the wastewater treatment has become more important in current scenario as there is a large-scale consumption of medicines globally due to the COVID-19 pandemic. This has

increased the chances of presence of these PRs in water bodies and their subsequent bioaccumulation in aquatic or terrestrial ecosystems.

19.2 Possible Sources of Pharmaceutical Contaminants in the Environment

PRs have been detected in sewage effluents, surface and groundwater, and even in drinking water (Awfa et al. 2018; Lee et al. 2017). Drinking water all over the world has been reported to contain everything from antidepressants to heart medication to birth control pills to caffeine. The main contributing sources of PRs in the environment are human (hospital/household) use, animal use, agriculture/farming use, and industrial manufacturing as shown in Fig. 19.1.

Hospitals are the major sources of PRs released in the environment. Only in few countries in the world PRs are removed by wastewater treatment plants having adopted suitable technologies and in most of countries the residues are discharged in the aquatic environment.

Due to this, there is continuous inflow of PRs in the environment leading to ubiquitous contamination. Pharmaceutical pollution has been found to cause acute and chronic effects in non-target organisms even at low concentrations. The effects

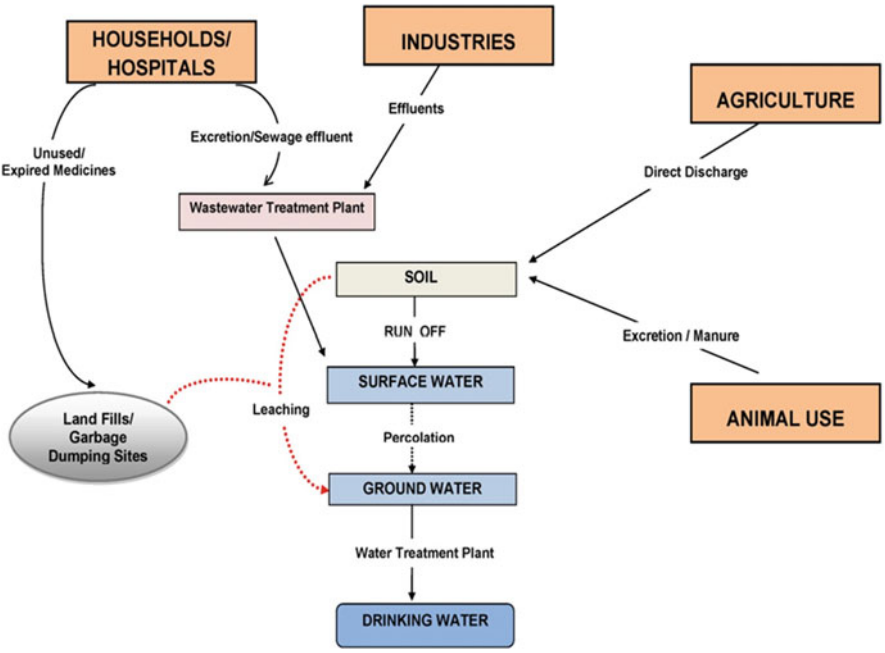

Fig. 19.1 Possible sources of pharmaceutical compounds and residues in the environment (Shah and Rather 2021b)

include endocrine or hormonal system dysfunction, toxic effects on biomolecules, damage to DNA and RNA, oxidative stress, impaired neurotransmission, and membrane lipid oxidation resulting in necrosis and cellular death (Antunes et al. 2013).

India figures among the top five manufacturers of medicines with drug exports growing 30% annually. Every third pill consumed in the world is produced in India and nearly 80% are consumed indigenously (Kallummal and Bugalya 2012). The amount of sewage generated in India far exceeds the treatment capacity; only 31% of the total sewage produced in 2008 (~38,254 million liters per day) was treated (Subedi et al. 2015). In India (year 2017 data), various pharmaceutical residues like ciprofloxacin (11,670 ng/L), erythromycin (9 ng/L), acetaminophen (690 ng/L), ibuprofen (980 ng/L), ampicillin, (12.68 ng/L) etc. have been detected in wastewater treatment plant effluents, rivers, lakes, and groundwater (Balakrishna et al. 2017).

Presently, WHO drinking water quality guidelines do not list pharmaceuticals as pollutants (Al-Odaini et al. 2013). Also the evaluation of the presence, exposure, and the subsequent effect of these residues on the health of general public are hindered by the lack of baseline data. The issue may turn worse due to lack of substantial research on the effect of these PRs on the human health. This may ultimately result in slow and irreversible accumulation of the toxic compounds in the living creatures mainly the humans. Besides, the problem is further alleviated by the inefficiency of conventional wastewater treatment plants in eliminating these residues completely. Since these residues are polar, non-volatile, and non-biodegradable, the conventional treatment processes like sedimentation and biological treatment fail to remove them effectively and efficiently (Bagheri et al. 2016). Consumption of pharmaceutical compounds has increased substantially over the last few decades and the onset of COVID-19 pandemic in the beginning of year 2020 has aggravated this problem to a great extent (Rehman et al. 2021a, b, c, d).

19.3 Adverse Effect of Retained Pharmaceuticals

The presence of pharmaceutical compounds and other emerging contaminants (ECs) in the water poses a serious threat to the environment and biotic health (Ramírez-Malule et al. 2020; Mirzaei et al. 2016). Nearly 2300 toxic and bio-accumulative ingredients have been found in human medicines (Pavithra et al. 2017). Although these drugs are chemically engineered to alter biological activities and functions in humans and animals, they may show similar effect in non-target organisms that have identical metabolic pathways, similar receptors, or biomolecules and get exposed to these drugs (Im Park 2005).

19.3.1 Side Effects in Humans

Drugs are engineered to work at low doses ranging from milligrams per kg to nanograms per kg. After performing the required function, some drugs still cause

Table 19.1 Side effects associated with paracetamol overdose in humans

Side effects	Reference
Fulminant hepatic failure	Sato and Marumo (1992)
Stomach bleeding	Rodríguez and Hernández-Díaz (2001)
Hepatotoxicity leading to acute liver failure (ALF)	Larson et al. (2005)
Gastro-duodenal ulcers and bleeding	Rainsford and Whitehouse (2006)
Hearing loss	Yorgason et al. (2011)
Asthma symptoms in adults and children	Henderson and Shaheen (2013)

potential harmful effects by interacting with non-therapeutic receptors as listed in Table 19.1.

Acetaminophen (Paracetamol) toxicity is dependent upon the pathway of its metabolism. When taken in therapeutic doses, it undergoes detoxification through sulfation, glucuronidation, and by conjugation with glutathione (GSH) as shown in Fig. 19.2a.

Acetaminophen gets conjugated with co-factors forming non-toxic metabolites: acetaminophen glucuronide and acetaminophen sulfate. Around 90% of ingested acetaminophen is detoxified in this way. About 10% of the acetaminophen undergoes oxidation via cytochrome P450 enzymes (CYP 2E1, 1A2, 3A4) forming a highly toxic intermediate N-acetyl-p-benzoquinone (NAPQ1). NAPQ1 undergoes conjugation with glutathione by isoenzymes glutathione S-transferases (GSTs) to form cysteine or mercapturic acid which is excreted.

However, at high doses and/or low intracellular glutathione levels, acetaminophen is converted into NAPQ1 due to the shortage of co-factors for glucuronidation and sulfation. It is electrophilic in nature, highly reactive and produces multiple toxic effects on biomolecules such as proteins, lipids, and nucleic acids. NAPQ1 is not removed through excretion, and reaches toxic concentrations in the liver cells causing damage to DNA, RNA, and proteins. This leads to covalent modification of thiol groups of cellular proteins, decreases in the synthesis of ATP and membrane lipid oxidation, resulting in necrosis and cell death as shown in Fig. 19.2b. Oxidative stress is also induced due to the accumulation of intracellular peroxide resulting in toxic effects by reactive oxygen species (ROS) via Fenton mechanism (Nunes et al. 2014).

19.3.2 Effect on Aquatic Organisms and Food Crops

PRs are reported to show eco-toxic effects in non-target organisms also. Antibiotics like.

enrofloxacin, ciprofloxacin, and tetracycline have been found to be toxic to algae and plants. Tetracycline is also reported to cause inhibition of bacterial protein synthesizing machinery in sewage sludge (Lee et al. 2017). Antibiotic resistance, one of alarming issues facing the world today is also reported to be caused by these PRs. Development of antibiotic resistant genes can lead to aquatic toxicity and can

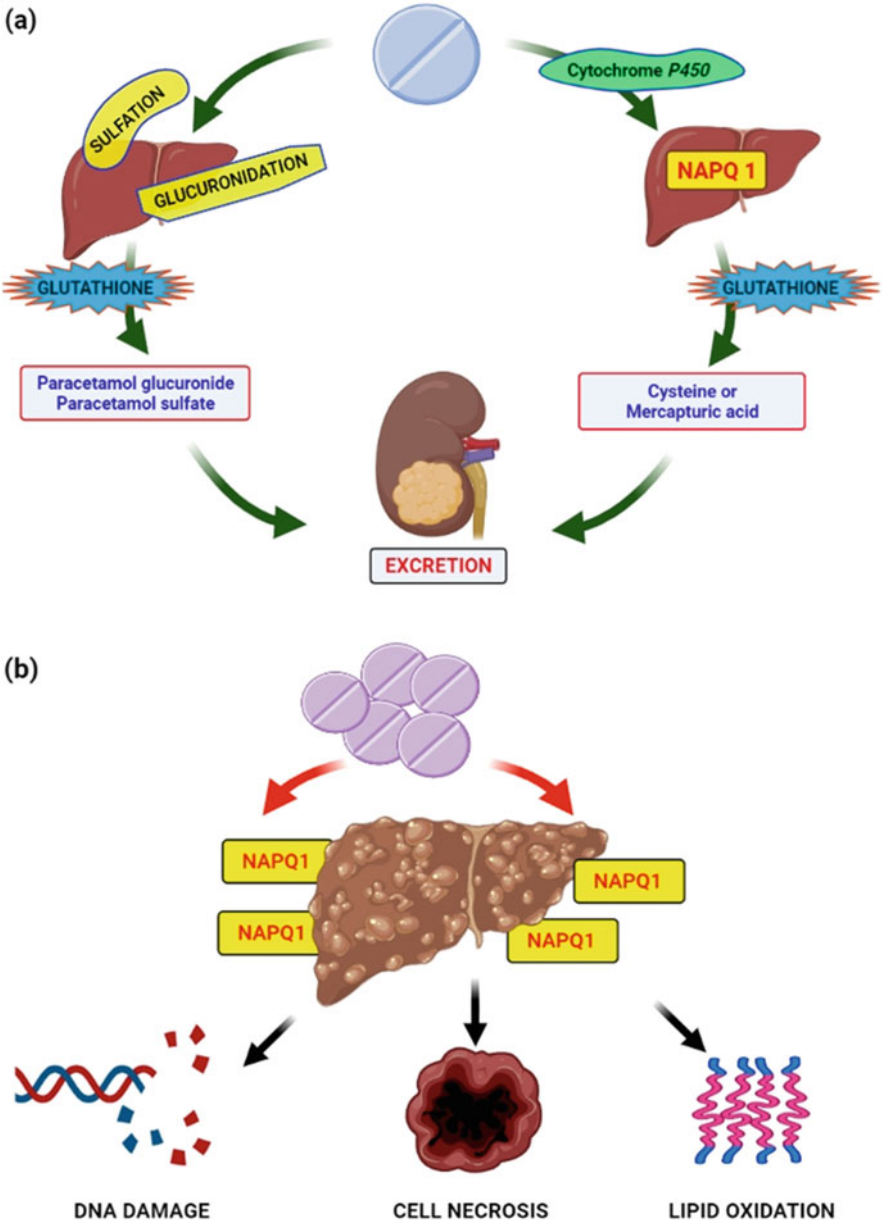

Fig.19.2 (**a**) Detoxification mechanism of paracetamol when taken in therapeutic doses. (**b**) Toxic effects of paracetamol when taken at high doses

Table 19.2 Effect of some pharmaceutical compounds on non-target organisms

Pharmaceutical	Therapeutic class	Reported effect	Concentration
Tetracycline	Antibiotics	Inhibition of growth in *Selenastrum capricornutum* (microalga)	2.2 mg/L
		Growth inhibition in *Daphnia magna*	6617.2 mg/L
		Decreased reproduction and increases mortality subsequent generations in *Daphnia magna*	0.1–5.0 mg/L
Paracetamol	Antipyretics	Affects embryonic development of *Danio rerio*	50 μg/L
		Inhibits elongation growth of roots in *Triticum aestivum* (wheat)	668.8 mg/L
Propranolol	β-blockers	Immobility and mortality in *Ceriodaphnia silvestrii*	2.5 mg/L
Caffeine	Stimulants	Impaired reproduction in *Ceriodaphnia dubia*	44 mg/L
Clofibric acid	Lipid regulators	Increased sperm mortality in *Pimephales promelas*	1 mg/L
Estradiol	Hormones	Induces feminization in *Pimephales promelas*	4 ng/L
Dipyrone	Non-steroidal anti-inflammatory drug (NSAID)	Growth inhibition in *Duplicaria similis*	3.59 mg/L
Fluoxetine	Antidepressants	Immobility in Duplicaria similis	8900 ng/L

cause geno-toxic effects in the micro-organisms. The effects of different pharmaceutical compounds on aquatic organisms are summarized in Table 19.2. Although the effective concentrations at which the toxic effect is reported are much higher than those detected in the aquatic environment, there is still a possibility of synergistic effect among the PRs due to long term exposure.

19.4 Nanoremediation: Pharmaceutical Wastewater Treatment Strategies

Pharmaceutical compounds, their residues and metabolites are not effectively removed by conventional wastewater treatment methods. There are other pharmaceutical mitigation strategies like take-back program, trash disposal, development of green pharmaceuticals, etc. In take-back program, unused or expired medicines can be collected from high pharmaceutical usage sites like hospitals, nursing homes, pharmacies, etc. and thus prevent their improper disposal down the toilet or into the drains (Lee et al. 2017; Blair 2016). Green pharmaceuticals are eco-friendly medicines that are stable inside the patient's body and less harmful to the environment compared to the conventional medicines. They have high activity and degrade well during sewage treatment and photolysis. However, development of such green pharmaceuticals may be cost and seems to be less feasible.

19.4.1 Heterogeneous Photocatalysis

Advanced oxidation processes (AOP) were first proposed for the treatment of drinking water in 1980s. AOP involve the generation of powerful and highly reactive radicals that act as strong oxidizing agents. These radicals include both hydroxyl ($OH^{\cdot}$) and sulfate ($SO_4^{\cdot-}$) radicals and are used to degrade wastewater pollutants to non-toxic substances or less toxic products. Thus these processes are used in removing organic, inorganic, and other recalcitrant pollutants from wastewater (Deng and Zhao 2015).

Heterogeneous photocatalysis is a sustainable and promising method for the degradation of organic contaminants like pharmaceutical residues. It usually involves a semiconductor photocatalyst that absorbs light energy, leading to a series of oxidation and reduction reactions between the semiconductor and liquid or gaseous medium. Photocatalysts have proven to be better tools for degradation of PRs from wastewater, groundwater, surface water, and drinking water. These photocatalysts provide better advantage than the conventional wastewater treatment methods like degradation of recalcitrant PRs, less treatment time, low catalyst dosage, function at natural pH of wastewater, and ability to completely degrade the contaminants into carbon dioxide and water (Hering et al. 2013). Most widely used photocatalysts are TiO_2 and ZnO due to their high reactivity and chemical stability. But these nanomaterials have wide band gap, 3.2 eV in case of TiO_2, which makes them effective in UV radiations only. The photocatalysts are modified to decrease their band gap and make them effective in visible region also (Shah and Rather 2021a; Anjum et al. 2016). Various methods used for catalyst modification are adding metal impurities, dye sensitization, forming nano-composites using semiconductors, which lowers the bad gap energy, creating a narrow band gap which makes the photocatalysts sensitive to visible light also (Lee et al. 2017).

Photocatalysts have not only proven to be effective tools for treatment of wastewater but also have shown to act as anti-microbial agents. TiO_2 and Ag doped TiO_2 have shown efficient anti-microbial activity against pathogens like *E.coli*. (Akhavan 2009; Liu et al. 2008; Rehman et al. 2021a; Shah and Rather 2021c, d) (Figs. 19.3, 19.4, and 19.5).

19.4.2 Mechanism of TiO_2 Based Photocatalysis

Photocatalysts work on the principle of photoexcitation of electrons. The irradiation of photocatalyst with light, having energy higher or equal to the band gap energy of the catalyst, generates holes (h^+) and excited electrons (e^-). The holes (h^+) react with water molecules and form hydroxyl radicals (·OH). The ·OH radicals are highly reactive and powerful oxidizing agents, causing degradation of organic pollutants like PRs into carbon dioxide and water (Anjum et al. 2016; Lee et al. 2017; Vinu and Madras 2011).

TiO_2 semiconductor is widely used photocatalyst owing to its ability to initiate a series of oxidation and reduction reactions on its surface. It has three polymorph

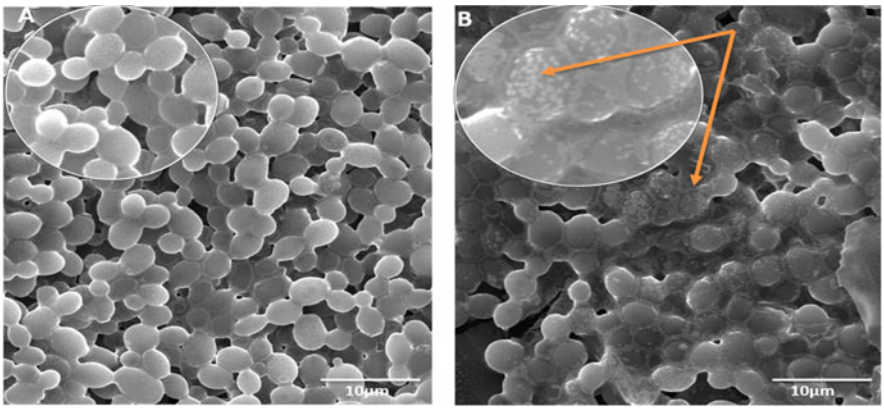

Fig. 19.3 SEM micrographs showing morphogenesis of C. albicans (**a**) control (**b**) MCs treated cells (Rehman et al. 2021b)

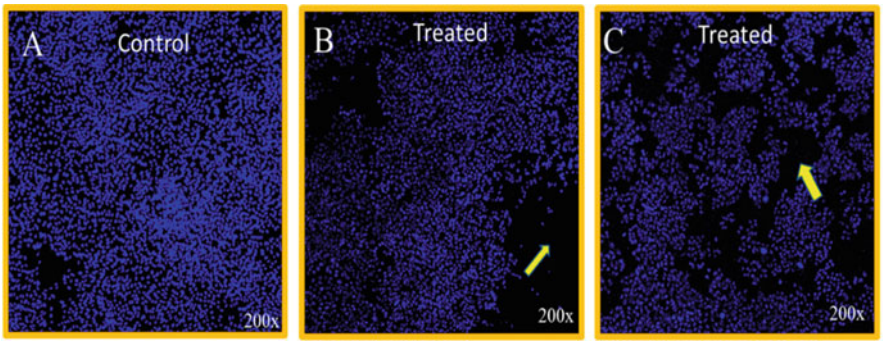

Fig. 19.4 Anticancer activity of manganese based nanoparticles against colon cancer cells (HCT-116). Nuclear disintegration can be observed in B & C (Rehman et al., 2021d)

crystals, anatase, rutile and brookite. Anatase form has been reported to exhibit highest photocatalytic activity among all the three polymorphs and therefore preferred in photocatalysis process. The photoexcited and photogenerated electrons in anatase form of TiO_2 are also reported to show longest lifetime and fastest migration rate, respectively (Awfa et al. 2018).

The presence of a lone electron in the outer orbital of TiO_2 is responsible for its photocatalytic activity. When a light energy ($h\nu$) having intensity is greater than or equal to the bandgap energy (the energy difference between the valence band and the conduction band) of TiO_2 (3.2 eV for anatase), the lone electron gets excited from the valence band (VB) to the empty conduction band (CB) within femto (10^{-15}) seconds (Chong et al. 2010; Foo and Hameed 2010). The wavelength of such energy usually corresponds to $\lambda < 400$ nm and leads to generation of an electron–hole pair (e^- and h^+), leaving behind an unfilled empty valence band. The electrons and holes participate in a series of oxidation-reduction reactions on the photo-activated surface

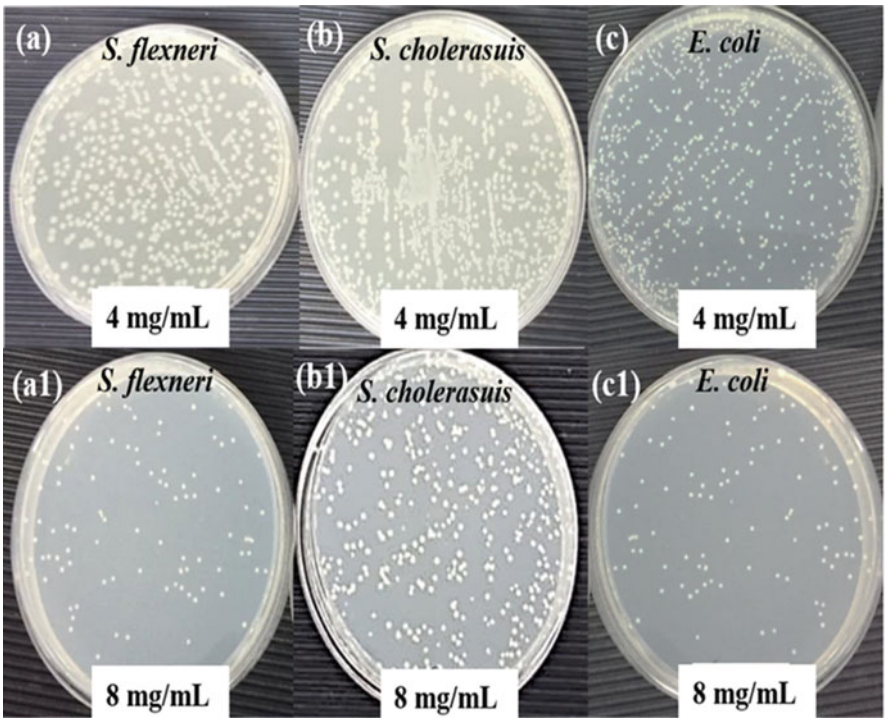

Fig. 19.5 Antibacterial activity of Ag doped WO_3 nanoparticles (Baig et al. 2020)

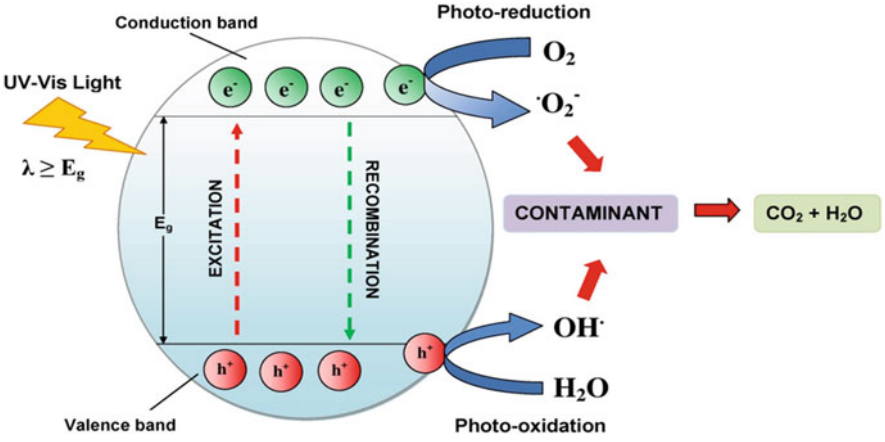

Fig. 19.6 Mechanism of electron–hole pair formation in TiO_2 (Shah and Rather 2021e)

of the semiconductor catalyst resulting in the formation of highly reactive species such as hydroxyl radical (·OH) and superoxide radical anion ($\cdot O_2^-$) as shown in

Table 19.3 Key redox reactions that occur at the surface of TiO_2 photocatalyst

Reactions		Eq.no.
Photoexcitation	$TiO_2 + h\nu \rightarrow e^- + h^+$	1
Charge-carrier trapping of e^-	$e^-_{CB} \rightarrow e^-_{TR}$	2
Charge-carrier trapping of h^+	$h^+_{VB} \rightarrow h^+_{TR}$	3
Electron–hole recombination	$e^-_{TR} + h^+_{VB}(h^+_{TR}) \rightarrow e^-_{CB}$ + heat	4
Oxidation of hydroxyls	$(OH^-)_{ads} + h^+ \rightarrow {}^{\cdot}OH_{ads}$	5
Photoexcited e^- scavenging	$(O_2)_{ads} + e^- \rightarrow O_2^{\cdot-}$	6
Protonation of superoxides	$O_2^{\cdot-} + OH^{\cdot} \rightarrow HOO^{\cdot}$	7
Co-scavenging of e^-	$HOO^{\cdot} + e^- \rightarrow HO_2^-$	8
Formation of H_2O_2	$HOO^- + H^+ \rightarrow H_2O_2$	9
Photodegradation by $OH^{\cdot}$	$R - H + OH^{\cdot} \rightarrow R'^{\cdot} + H_2O$	10
Direct photoholes	$R + h^+ \rightarrow R^{+\cdot} \rightarrow$ Intermediate(s)/Final Degradation Products	11
Overall reaction	Organic contaminants $\overset{TiO_2/h\nu}{\rightarrow}$ Intermediate(s) $\rightarrow CO_2 + H_2O +$ Inorganic ions	12

Fig. 19.6. The key reactions and the mechanism involved in the photocatalysis are summarized in Table 19.3 (Awfa et al. 2018; Chong et al. 2010; Dong et al. 2015).

As shown in Eqs. (1)–(3), the surface trapped VB electrons (e^-_{TR}) and CB holes (h^+_{TR}) are generated after the photonic excitation of the catalyst. It has been reported that their recombination occurs much more slowly than in the bulk (Dong et al. 2015; Chong et al. 2010). The presence of electron scavenger species is vital for slowing down the recombination and successful photooxidation of organic compounds. Eq. (4) shows the recombination of photoexcited electron with the valence band hole in the absence of electron scavengers. The recombination occurs in nanoseconds and is accompanied by dissipation of heat.

Mostly photocatalytic degradation of emerging contaminants and other organic compounds is carried out in the presence of photocatalyst, oxygen, and water. Surface adsorbed hydroxyl radicals ($\cdot OH_{ads}$) are formed when the positive holes react with the surface OH^- groups on the TiO_2 surface as shown in Eq. (5). The presence of oxygen results in the formation of superoxide radicals ($O_2^{\cdot -}$) and prevents electron–hole pair recombination (Eq. 6). Further protonation of $O_2^{\cdot -}$ forms hydroperoxyl radical ($HO_2^{\cdot}$) that can react further to form H_2O_2 (Eqs. 7–9). The degradation of emerging contaminants/organic pollutants (R) is carried out by the photogenerated holes and hydroxyl radicals (Eqs. 10 and 11). The $HO_2^{\cdot}$ radical formed in the photocatalysis process is also reported to act as a scavenger and along with other radical species can prolong the recombination time of h^+_{TR}. Also the presence of water and dissolved oxygen is important for the photocatalysis process. The photodegradation of liquid phase organic contaminants is hindered in the absence of water molecules as highly reactive hydroxyl radicals (OH·) are not generated (Chong et al. 2010).

Many studies have been conducted to understand the degradation mechanism of various emerging contaminants over TiO_2 surface. During heterogeneous photocatalysis, liquid phase organic contaminants are broken down to their corresponding intermediates. As shown in Eq. (12), these intermediates are further mineralized to carbon dioxide, water, and inorganic ions (from heteroatoms) (Awfa et al. 2018; Chong et al. 2010; Dong et al. 2015). Figure 19.6 shows the mechanism of formation of electron–hole pair in TiO_2 nano-photocatalyst

TiO_2 has been doped with metals like Pt, Au, Ag, and Pd, showing excellent photocatalytic activities compared to unmodified TiO_2. ZnO, ZnS, ZnSe, CuO, CdS are other nano-catalysts that have been doped with dopants like Co, Ni, Al, Cu, Eu, Mn, Fe, Cr, Mg, Ga to form nano-composites having higher photocatalytic degradation and biological activities (Chandrakar et al. 2015; Jamal et al. 2012; Letti et al. 2017; Akhtar et al. 2020; Azam et al. 2020; Aldakheel et al. 2020; Rehman et al. 2019a, b, c, 2020a, b).

Nanoparticles like spinel cobalt ferrite ($CoFe_2O_4$) doped with neodymium and cerium have been synthesized that show photocatalytic activity as well as antimicrobial properties. The activity of neodymium and cerium doped cobalt ferrite nanoparticles against *Candida albicans* was found to increase with the increasing concentration of dopants. (Rehman et al. 2021c, d; Rehman et al. 2019; Elsharif et al. 2019; Saba et al. 2021; Qureshi et al. 2021; Nahvi et al. 2021; Almessiere et al.

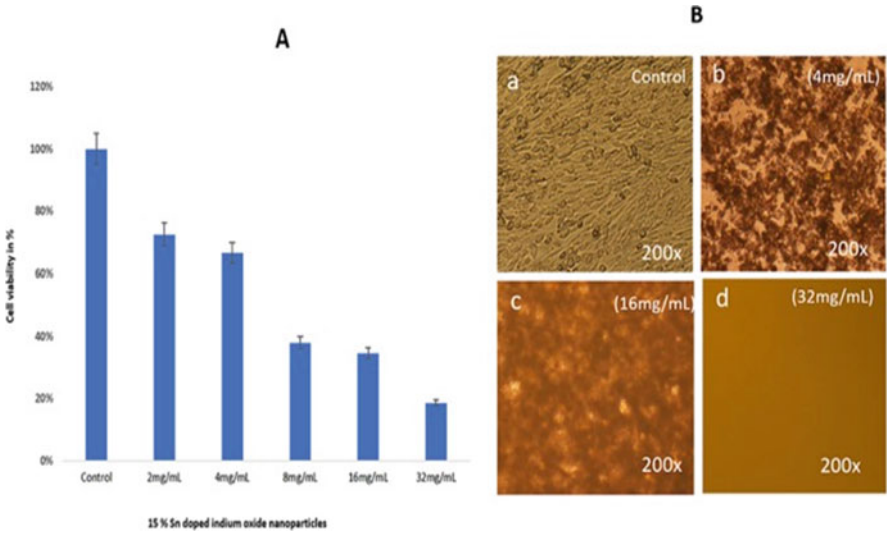

Fig. 19.7 Effect of Sn-In_2O_3 nanoparticles on cancerous cells (**a**) MTT assay (**b**) effect on cell morphology (Rehman et al. 2020b)

2020a, b). Tin doped indium oxide nanoparticles have also been reported to show anti-cancerous activity on human colon cancer cells (HCT-116). The nanoparticles caused significant changes in cell morphology like disruption of cell membrane and nuclear condensation (Rehman et al. 2020a, b), as shown in Fig. 19.7.

Photocatalytic degradation of various pharmaceuticals like analgesics, antipyretics, antibiotics, anesthetics, β blockers, and lipid regulators has been studied extensively. This is due to their high consumption world over and subsequent detection in aquatic environment. TiO_2 photocatalytic degradation has proven to be a successful process for the efficient removal of PRs from wastewater. Mostly those medicines have been selected for the degradation studies that are consumed in large quantities worldwide and thus have high chances of showing presence in the aquatic environment. These medicines include paracetamol (acetaminophen), diclofenac, amoxicillin, naproxen, aspirin, ibuprofen, metoprolol, cetirizine, etc.

TiO_2 photocatalytic degradation studies involve the optimization of various parameters like initial concentration of the medicine, catalyst dosage, pH, nature of the catalyst (doped/undoped), light source, and effect of organic matter. In addition to these parameters, effect of factors like stirring speed, temperature of the reaction mixture, type of gas (and rate) used for sparging of the solution, and design of the photoreactor vessel on the photocatalytic degradation have also been studied (Bianchi et al. 2017; Jallouli et al. 2018)

19.5 Conclusion, Future Prospects, and Recommendations

Heterogeneous photocatalysis is an effective advanced oxidation treatment method for the removal of non-biodegradable and recalcitrant contaminants from wastewater. The conventional wastewater treatment methods are not able to remove these emerging contaminants completely. Thus the presence of these persistent pharmaceutical residues in aquatic environment may lead to an alarming situation due to their unintentional consumption and assimilation by humans and other organisms. Consumption of pharmaceutical compounds has increased substantially over the last few decades and the onset of COVID-19 pandemic in the beginning of year 2020 has aggravated this problem to a great extent. The colossal consumption of various medicines is being witnessed due to this pandemic and according to WHO, more than 230 million cases have been reported worldwide till date. This will ultimately give rise to elevated levels of these PRs in water bodies. There is need to carry out a comprehensive study regarding the presence of these PRs in water bodies and revisit the current water treatment technologies. Heterogeneous TiO_2 photocatalysis is an efficient treatment method for the removal of these ECs as demonstrated by numerous studies. Also toxicity studies should be carried out to determine the safety of intermediates and by-products generated during photocatalytic degradation.

References

Akhavan O (2009) Lasting antibacterial activities of Ag–TiO2/Ag/a-TiO2 nanocomposite thin film photocatalysts under solar light irradiation. J Colloid Interface Sci 336(1):117–124. https://doi.org/10.1016/j.jcis.2009.03.018

Akhtar S, Rehman S, Asiri SM, Khan FA, Baig U, Hakeem AS, Gondal MA (2020) Evaluation of bioactivities of zinc oxide, cadmium sulfide and cadmium sulfide loaded zinc oxide nanostructured materials prepared by nanosecond pulsed laser. Mater Sci Eng C 116:111156

Alahmari F, Rehman S, Almessiere M, Khan FA, Slimani Y, Baykal A (2021) Synthesis of Ni0. 5Co0. 5-xCdxFe1. 78Nd0. 02O4 ($x \leq 0.25$) nanofibers by using electrospinning technique induce anti-cancer and anti-bacterial activities. J Biomol Struct Dyn 39(9):3186–3193

Alcamo J, Henrichs T, Rösch T (2017) World water in 2025: global modeling and scenario analysis for the world commission on water for the 21st century

Aldakheel RK, Rehman S, Almessiere MA, Khan FA, Gondal MA, Mostafa A, Baykal A (2020) Bactericidal and in vitro cytotoxicity of moringa oleifera seed extract and its elemental analysis using laser-induced breakdown spectroscopy. Pharmaceuticals 13(8):193

Al-Jameel SS, Rehman S, Almessiere MA, Khan FA, Slimani Y, Al-Saleh NS, Manikandan A, Al-Suhaimi EA, Baykal A (2021) Anti-microbial and anti-cancer activities of Mn0. 5Zn0. 5DyxFe2-xO4 ($x \leq 0.1$) nanoparticles. Artif Cells Nanomed Biotechnol 49(1):493–499

Almessiere MA, Slimani Y, Rehman S, Khan FA, Güngüneş ÇD, Güner S, Shirsath SE, Baykal A (2020a) Magnetic properties, anticancer and antibacterial effectiveness of sonochemically produced Ce3+/Dy3+ co-activated Mn-Zn nanospinel ferrites. Arab J Chem 13(10):7403–7417

Almessiere MA, Slimani Y, Rehman S, Khan FA, Polat EG, Sadaqat A, Shirsath SE, Baykal A (2020b) Synthesis of Dy-Y co-substituted manganese-zinc spinel nanoferrites induced antibacterial and anti-cancer activities: comparison between sonochemical and sol-gel auto-combustion methods. Mater Sci Eng C 116:111186

Al-Odaini NA, Zakaria MP, Yaziz MI, Surif S, Abdulghani M (2013) The occurrence of human pharmaceuticals in wastewater effluents and surface water of Langat River and its tributaries,

Malaysia. Int J Environ Anal Chem 93(3):245–264. https://doi.org/10.1080/03067319.2011.592949

Anjum M, Miandad R, Waqas M, Gehany F, Barakat MA (2016) Remediation of wastewater using various nano-materials. Arab J Chem 12:4897–4919. https://doi.org/10.1016/j.arabjc.2016.10.004

Antunes SC, Freitas R, Figueira E, Gonçalves F, Nunes B (2013) Biochemical effects of acetaminophen in aquatic species: edible clams Venerupis decussata and Venerupis philippinarum. Environ Sci Pollut Res 20(9):6658–6666. https://doi.org/10.1007/s11356-013-1784-9

Awfa D, Ateia M, Fujii M, Johnson MS, Yoshimura C (2018) Photodegradation of pharmaceuticals and personal care products in water treatment using carbonaceous-TiO_2 composites: a critical review of recent literature. Water Res 142:26–45. https://doi.org/10.1016/j.watres.2018.05.036

Azam M, Kumar V, Siddiqui K, Jan AT, Sabir JS, Rather IA, Rehman S, Haq QM (2020) Pharmaceutical disposal facilitates the mobilization of resistance determinants among microbiota of polluted environment. Saudi Pharm J 28(12):1626–1634

Bagheri H, Afkhami A, Noroozi A (2016) Removal of pharmaceutical compounds from hospital wastewaters using nanomaterials: a review. Anal Bioanal Chem Res 3(1):1–18. https://doi.org/10.22036/abcr.2016.12655

Baig U, Gondal MA, Rehman S, Akhtar S (2020) Facile synthesis, characterization of nano-tungsten trioxide decorated with silver nanoparticles and their antibacterial activity against water-borne gram-negative pathogens. Appl Nanosci 10(3):851–860

Balakrishna K, Rath A, Praveenkumarreddy Y, Guruge KS, Subedi B (2017) A review of the occurrence of pharmaceuticals and personal care products in Indian water bodies. Ecotoxicol Environ Saf 137:113–120. https://doi.org/10.1016/j.ecoenv.2016.11.014

Bianchi CL, Sacchi B, Pirola C, Demartin F, Cerrato G, Morandi S, Capucci V (2017) Aspirin and paracetamol removal using a commercial micro-sized TiO 2 catalyst in deionized and tap water. Environ Sci Pollut Res 24(14):12646–12654. https://doi.org/10.1007/s11356-016-7781-z

Blair BD (2016) Potential upstream strategies for the mitigation of pharmaceuticals in the aquatic environment: a brief review. Curr Environ Health Rep 3(2):153–160. https://doi.org/10.1007/s40572-016-0088-x

Chandrakar RK, Baghel RN, Chandra VK, Chandra B (2015) Synthesis, characterization and photoluminescence studies of Mn doped ZnS nanoparticles. Superlattice Microst 86:256–269

Chong MN, Jin B, Chow CW, Saint C (2010) Recent developments in photocatalytic water treatment technology: a review. Water Res 44(10):2997–3027. https://doi.org/10.1016/j.watres.2010.02.039

Deng Y, Zhao R (2015) Advanced oxidation processes (AOPs) in wastewater treatment. Current pollution reports. Springer. https://doi.org/10.1007/s40726-015-0015-z

Dong H, Zeng G, Tang L, Fan C, Zhang C, He X, He Y (2015) An overview on limitations of TiO_2-based particles for photocatalytic degradation of organic pollutants and the corresponding countermeasures. Water Res 79:128–146. https://doi.org/10.1016/j.watres.2015.04.038

Elsharif AM, Youssef TE, Al-Jameel SS, Mohamed HH, Ansari MA, Rehman S, Akhtar S (2019) Synthesis of an activatable tetra-substituted nickel phthalocyanines-4 (3H)-quinazolinone conjugate and its antibacterial activity. Adv Pharmacol Sci 2019:5964687

Fekadu S, Alemayehu E, Dewil R, Van der Bruggen B (2019) Pharmaceuticals in freshwater aquatic environments: a comparison of the African and European challenge. Sci Total Environ 654:324–337. https://doi.org/10.1016/j.scitotenv.2018.11.072

Foo KY, Hameed BH (2010) Decontamination of textile wastewater via TiO2/activated carbon composite materials. Chong MN, Jin B, Chow CW, Saint C (2010) Recent developments in photocatalytic water treatment technology: a review. Water research 44(10), 2997–3027. https://doi.org/10.1016/j.watres.2010.02.039, https://doi.org/10.1016/j.cis.2010.06.002

Henderson AJ, Shaheen SO (2013) Acetaminophen and asthma. Paediatr Respir Rev 14(1):9–16. https://doi.org/10.1016/j.prrv.2012.04.004

Hering JG, Waite TD, Luthy RG, Drewes JE, Sedlak DL (2013) A changing framework for urban water systems

Hlongwane GN, Sekoai PT, Meyyappan M, Moothi K (2019) Simultaneous removal of pollutants from water using nanoparticles: a shift from single pollutant control to multiple pollutant control. Sci Total Environ 656:808–833. https://doi.org/10.1016/j.scitotenv.2018.11.257

Im Park J 2005 Pharmaceuticals in the environment and management approaches in Korea. 기본연구보고서 2005:1–155

Jallouli N, Pastrana-Martínez LM, Ribeiro AR, Moreira NF, Faria JL, Hentati O et al (2018) Heterogeneous photocatalytic degradation of ibuprofen in ultrapure water, municipal and pharmaceutical industry wastewaters using a TiO2/UV-LED system. Chem Eng J 334:976–984. https://doi.org/10.1016/j.cej.2017.10.045

Jamal A, Rahman MM, Khan SB, Faisal M, Akhtar K, Rub MA, Asiri AM, Al-Youbi AO (2012) Cobalt doped antimony oxide nano-particles based chemical sensor and photo-catalyst for environmental pollutants. Appl Surf Sci 261:52–58. https://doi.org/10.1016/j.apsusc.2012.07.066

Kallummal M, Bugalya K (2012) Trends in India's trade in pharmaceutical sector: some insights. Center for WTO Studies Working Paper, 200(2)

Larson AM, Polson J, Fontana RJ, Davern TJ, Lalani E, Hynan LS, Reisch JS et al (2005) Acetaminophen-induced acute liver failure: results of a United States multicenter, prospective study. Hepatology 42(6):1364–1372. https://doi.org/10.1002/hep.20948

Lee CM, Palaniandy P, Dahlan I (2017) Pharmaceutical residues in aquatic environment and water remediation by TiO_2 heterogeneous photocatalysis: a review. Environ Earth Sci 76(17):611. https://doi.org/10.1007/s12665-017-6924-y

Letti CJ, Costa KA, Gross MA, Paterno LG, Pereira-da-Silva MA, Morais PC, Soler MA (2017) Synthesis, morphology and electrochemical applications of iron oxide based nanocomposites. Adv Nano Res 5(3):215–230. https://doi.org/10.12989/anr.2017.5.3.215

Liu Y, Wang X, Yang F, Yang X (2008) Excellent antimicrobial properties of mesoporous anatase TiO_2 and Ag/TiO_2 composite films. Microporous Mesoporous Mater 114(1–3):431–439. https://doi.org/10.1016/j.micromeso.2008.01.032

Mirzaei A, Chen Z, Haghighat F, Yerushalmi L (2016) Removal of pharmaceuticals and endocrine disrupting compounds from water by zinc oxide-based photocatalytic degradation: a review. Sustain Cities Soc 27:407–418. https://doi.org/10.1016/j.scs.2016.08.004

Nahvi I, Belkahla S, Asiri SM, Rehman S (2021) Overview and prospectus of algal biogenesis of nanoparticles. In: Microbial nanotechnology: green synthesis and applications. Springer, Singapore, pp 121–134

Nunes B, Antunes SC, Santos J, Martins L, Castro BB (2014) Toxic potential of paracetamol to freshwater organisms: a headache to environmental regulators? Ecotoxicol Environ Saf 107:178–185

Pavithra KG, Kumar PS, Rajan PS, Saravanan A, Naushad M (2017) Sources and impacts of pharmaceutical components in wastewater and its treatment process: a review. Korean J Chem Eng 34(11):2787–2805. https://doi.org/10.1007/s11814-017-0255-2

Qureshi A, Blaisi NI, Abbas AA, Khan NA, Rehman S (2021) Prospectus and development of microbes mediated synthesis of nanoparticles. In: Microbial nanotechnology: green synthesis and applications. Springer, Singapore, pp 1–15

Rainsford KD, Whitehouse MW (2006) Paracetamol [Acetaminophen]-induced gastrotoxicity: revealed by induced hyperacidity in combination with acute or chronic inflammation. Inflammopharmacology 14(3–4):150–154. https://doi.org/10.1007/s10787-006-1389-8

Ramírez-Malule H, Quiñones-Murillo DH, Manotas-Duque D (2020) Emerging contaminants as global environmental hazards. A bibliometric analysis. Emerg Contam 6:179–193

Ravinayagam V, Rehman S (2020) Zeolitic imidazolate framework-8 (ZIF-8) doped TiZSM-5 and mesoporous carbon for antibacterial characterization. Saudi J Biol Sci 27(7):1726–1736

Rehman S, Almessiere MA, Tashkandi N, Baykal A, Slimani Y, Jermy R, Ravinayagam V, Yaman C (2019) Fabrication of spinel cobalt ferrite (CoFe2O4) nanoparticles with unique earth element cerium and neodymium for anticandidal activities. ChemistrySelect 4(48):14329–14334

Rehman S, Al Salem Z, Al Jindan R, Hameed S (2019a) Microbial natural products: exploiting microbes against drug-resistant bugs. In: Pathogenicity and drug resistance of human pathogens. Springer, Singapore, pp 393–404

Rehman S, Gunday ST, Alsalem ZH, Bozkurt A (2019b) Synthesis and characterization of novel azole functionalized poly (glycidyl methacrylate) s for antibacterial and anticandidal activity. Curr Org Synth 16(7):1002–1009

Rehman S, Ansari MA, Alzohairy MA, Alomary MN, Jermy BR, Shahzad R, Tashkandi N, Alsalem ZH (2019c) Antibacterial and antifungal activity of novel synthesized neodymium-substituted cobalt ferrite nanoparticles for biomedical application. Processes. 7(10):714

Rehman S, Almessiere MA, Khan FA, Korkmaz AD, Tashkandi N, Slimani Y, Baykal A (2020a) Synthesis and biological characterization of Mn0. 5Zn0. 5EuxDyxFe1. 8-2xO4 nanoparticles by sonochemical approach. Mater Sci Eng C 109:110534

Rehman S, Asiri SM, Khan FA, Jermy BR, Ravinayagam V, Alsalem Z, Al Jindan R, Qurashi A (2020b) Anticandidal and in vitro anti-proliferative activity of sonochemically synthesized indium tin oxide nanoparticles. Sci Rep 10(1):1–9

Rehman S, Ravinayagam V, Nahvi I, Aldossary H, Al-Shammari M, Amiri MS, Kishore U, Al-Suhaimi EA (2021a) Immunity, sex hormones, and environmental factors as determinants of COVID-19 disparity in women. Front Immunol 12:680845

Rehman S, Almessiere MA, Al-Jameel SS, Ali U, Slimani Y, Taskhandi N et al (2021b) Designing of Co0. 5Ni0. 5GaxFe2-xO4 ($0.0 \leq x \leq 1.0$) microspheres via hydrothermal approach and their selective inhibition on the growth of cancerous and fungal cells. Pharmaceutics 13(7):962

Rehman S, Ansari MA, Al-Dossary HA, Fatima Z, Hameed S, Ahmad W, Ali A (2021c) Current perspectives on mycosynthesis of nanoparticles and their biomedical application. In: Modeling and control of drug delivery systems. Academic, pp 301–311

Rehman S, Almessiere MA, Al-Suhaimi EA, Hussain M, Yousuf Bari M, Mehmood Ali S, Al-Jameel SS, Slimani Y, Khan FA, Baykal A (2021d) Ultrasonic synthesis and biomedical application of Mn0. 5Zn0. 5ErxYxFe2− 2xO4 nanoparticles. Biomolecules 11(5):703

Rodríguez LAG, Hernández-Díaz S (2001) The risk of upper gastrointestinal complications associated with nonsteroidal anti-inflammatory drugs, glucocorticoids, acetaminophen, and combinations of these agents. Arthritis Res Ther 3(2):1–4

Saba I, Wani K, Syed A, Rehman S (2021) Role of microbial nanotechnology in bioremediation of heavy metals. In: Microbial nanotechnology: green synthesis and applications. Springer, Singapore, pp 307–319

Sato C, Marumo F (1992) Paracetamol (Acetaminophen) bioactivation by liver microsomes—its role in hepatotoxicity. Springer, Dordrecht, pp 188–97. https://doi.org/10.1007/978-94-011-2982-4_23

Shah AH, Rather MA (2021a) Effect of thermal treatment on the phase composition and surface properties of WO3-TiO2 nanocomposites synthesized via hydro-thermal method. ChemistrySelect 6(5):987–994. https://doi.org/10.1002/slct.202004160

Shah AH, Rather MA (2021b) Pharmaceutical residues: new emerging contaminants and their mitigation by nano-photocatalysis. Adv Nano Res 10(4):397–414. https://doi.org/10.12989/ANR.2021.10.4.397

Shah AH, Rather MA (2021c) Anti-microbial nanocarriers: role of nanomaterials in food preservation, quality improvement and control. In: Microbial nanotechnology: green synthesis and applications. Springer, Singapore, pp 343–359. https://doi.org/10.1007/978-981-16-1923-6_17

Shah AH, Rather MA (2021d) Intracellular and extracellular microbial enzymes and their role in nanoparticle synthesis. In: Microbial nanotechnology: green synthesis and applications. Springer, Singapore, pp 41–59. https://doi.org/10.1007/978-981-16-1923-6_3

Shah AH, Rather MA (2021e) Effect of calcination temperature on the crystallite size, particle size and zeta potential of TiO2 nanoparticles synthesized via polyol-mediated method. Mater Today 44:482–488. https://doi.org/10.1016/j.matpr.2020.10.199

Subedi B, Balakrishna K, Sinha RK, Yamashita N, Balasubramanian VG, Kannan K (2015) Mass loading and removal of pharmaceuticals and personal care products, including psychoactive and illicit drugs and artificial sweeteners, in five sewage treatment plants in India. J Environ Chem Eng 3(4):2882–2891. https://doi.org/10.1016/j.jece.2015.09.031

Vinu R, Madras G (2011) Photocatalytic degradation of water pollutants using nano-TiO_2. In: Energy efficiency and renewable energy through nanotechnology. Springer, London, pp 625–677. https://doi.org/10.1007/978-0-85729-638-2_19

Yorgason JG, Luxford W, Kalinec F (2011) In vitro and in vivo models of drug ototoxicity: studying the mechanisms of a clinical problem. In: Expert opinion on drug metabolism and toxicology. Taylor & Francis, Milton Park. https://doi.org/10.1517/17425255.2011.614231

Part V

Nano Diagnostic Tools

Bacteriophage-Based Biosensors: Detection of Bacteria and Beyond

20

Jan Paczesny, Mateusz Wdowiak, and Enkhlin Ochirbat

Abstract

Pathogenic infections cause tremendous health threats and socioeconomic burdens worldwide. Conventional methods for bacteria detection are laborious, time-consuming, expensive, and require particular devices and highly qualified specialists. Sensitive, selective, inexpensive, quick, and user-friendly biosensors are in urgent demand to prevent and detect bacterial infections in many fields, e.g., healthcare, food industry, or terrorism prevention. Among biorecognition elements utilized in biosensors, bacteriophages are highly promising due to their numerous advantages, such as host specificity, cheap and simple production, resistance to external factors, and ease of immobilization. Here we reviewed currently used methods for bacteria detection, pointing their advantages and disadvantages. We paid particular attention to bacteriophage-based methods, including phage-based sensors and phage display method.

Keywords

Bacteria · Bacteriophage · Biosensor · Detection · Applications

J. Paczesny (✉) · M. Wdowiak
Institute of Physical Chemistry Polish Academy of Sciences, Warsaw, Poland
e-mail: jpaczesny@ichf.edu.pl

E. Ochirbat
Institute of Physical Chemistry Polish Academy of Sciences, Warsaw, Poland

Warsaw University of Technology, Warsaw, Poland

S. Hameed, S. Rehman (eds.), *Nanotechnology for Infectious Diseases*,
https://doi.org/10.1007/978-981-16-9190-4_20

20.1 Introduction

Although quality of living is constantly improving through technological progress, bacterial infections remain a major problem in the modern world. Approximately 13% of the deaths are related to bacterial diseases (Punina et al. 2015). Furthermore, bacteria are also involved in specific types of cancers (Elsland and Neefjes 2018) and various metabolic disorders, including obesity, which affects 39% of adults (Castañer and Schröder 2018).

Bacteria are a significant threat for children or elders and in the developing countries, where respiratory diseases such as tuberculosis cause millions of deaths (Harding 2020). A major source of bacterial infections is food and water poisoning, causing 1.8 million casualties worldwide in 2005 (ECDC 2009). In a publication from 2009, it was shown that only in the USA number of infections and illnesses caused by foodborne pathogens reached 76 million. 325,000 cases were reported to hospitals, and 5200 died (Buzby and Roberts 2009). There are also socioeconomic costs related to outbreaks of an epidemic caused by food hazards. The report from 2011 showed 9.4 million episodes of foodborne illnesses in the USA (Scallan et al. 2011). 3816 people got sick, and 54 died due to *Escherichia coli* O104:H4 outbreak in Europe in 2011 (King et al. 2012). The total financial burden was estimated to reach 3 billion Euros. In 2015, the Center for Disease Control and Prevention (CDC) reported 15,202 foodborne infected patients, 950 hospitalizations, and 15 deaths (Wan et al. 2019). World Bank study conducted in 2018 in low- and middle-income countries estimated the cost of food-born illnesses at 110 billion USD and treatments cost at 15 billion USD annually (Jaffee et al. 2019).

Another major cause of bacterial infections is hospitals. According to a World Health Organization report from 2011, 4.1 million patients are affected by healthcare-related illnesses each year in Europe (World Health Organization 2011). Furthermore, only in the USA, nosocomial infections cause 100,000 deaths each year (Cimiotti et al. 2012). Due to the impact of the COVID-19 pandemic, the number of hospital-acquired infections (HAI) increased by 34–47% in 2020 compared to the number of cases that occurred in 2019 (Weiner-Lastinger et al. 2022). According to the report published by Quince Market Insights in July 2021, the HAI market reached over 12 billion USD in 2020 (Global hospital-acquired infections 2021).

The appearance of multidrug-resistant bacteria strains makes this problem even more urgent. The knowledge about antibiotic resistance mechanisms is still unsatisfying, and our main weapon against bacteria lost its potential (Paczesny et al. 2020). Due to the lack of enough funding in antibiotic development and the uncontrollable use of antibiotics, the danger of antibiotic resistance is increasing radically. The CDC's latest estimation of death and infection in the USA conducted in 2018 suggested that more than 2.8 million antibiotic-resistant infections occur each year, and more than 35,000 people die because of it (US Department of Health and Human Services, CDC 2019). In 2015, antibiotic-resistant Gram-negative pathogens caused losses estimated at 287 million EUR (Touat et al. 2019). The cost of critical measures for antimicrobial resistance containment is estimated to be

9 billion USD globally (Jit et al. 2020). Research conducted by the World Bank Group estimated that the global economic cost of antibiotic resistance will range between 1.0 and 3.4 trillion USD in 2030, which is 1.1–3.8% of global GDP (Miller-Petrie et al. 2017).

Cost for biodefense and prevention from threats of biological warfare and bioterrorism also cause enormous expenses. For instance, statistics from 1997 indicated that the cost of prevention from brucellosis was estimated to be around $477.7 million per 100,000 persons exposed and anthrax was $26.2 billion per 100,000 people exposed (Kaufmann et al. 1997). These costs have multiplied over the last two decades (Christian 2013).

Therefore finding an efficient way to overcome problems caused by pathogens is paramount. There is also a need for a rapid and specific method to detect and recognize bacteria. Most methods that are currently in use rely on culturing, biochemical tests, or molecular protocols (e.g., PCR, *polymerase chain reaction*, amplification). Although these approaches are useful, there is still no method allowing to combine short time of the analysis and very low detection limit (e.g., 1 CFU (colony forming unit) per mL), even at the expense of the cost of analysis.

20.2 Culturing-Based Methods

The bacteria identification based on cultivation aims to get pure culture from repeated collection and seeding of an isolated colony. General-purpose agar-based media is commonly used to cultivate various pathogens, but some bacteria require more specific culture media for more accurate identification. For instance, "differential" culture media relies upon the metabolic difference of the pathogens by using a biochemical or pH indicator to detect them. "Selective" culture media has antimicrobials that inhibit the commensal flora from increasing the growth of certain bacteria of interest (Váradi et al. 2017).

Chromogenic media is frequently used as a microorganism identification method since it is cheap and straightforward. The chromogenic media method requires culturing bacteria samples using appropriate broth or agar media enriched with colorless or fluorescence chromogenic enzyme substrates. The substrates are colorized by specific bacterial enzymes (Váradi et al. 2017). The chromogenic media method is commonly employed in clinical laboratories since it requires a small workload and increases the chances of identification due to colored colonies, especially when multiple species are present in the sample.

Commercially available biochemical tests are frequently used after isolation to identify genus and species levels. Commercial kits such as Analytical Profile Index (API) kits can be applied to carry out the inoculation and reading of biochemical panels manually, so do automated tests such as the BD Phoenix or the Vitek 2. These systems can identify bacteria in 2–3 h and execute automated antimicrobial susceptibility testing (Jorgensen and Ferraro 2009).

Even though these methods cost less and provide quantitative and qualitative information about the bacteria, they require a lot of work and time for media

preparation, dilution, plating, incubation, counting, isolation, and characterization. The main disadvantage of the chromogenic media method is that this method is usually time-consuming and requires up to a few days to obtain the results (Franco-Duarte et al. 2019). Also, in some cases, it requires additional examination using other analytical, often instrumental, methods. At times, biochemical properties inaccurately indicate the genomics of a given species (Janda and Abbott 2002), and results can be false positives considering similar species (Justé et al. 2008).

20.3 Molecular Methods

Molecular methods present multiple tools and techniques for bacteria characterization, detection, and identification (Ferone et al. 2020). They brought remarkable insights by detecting previously unidentified bacteria, classifying uncultivable bacteria, and allowing the metagenomics study of diverse bacterial communities on a large scale. Most molecular techniques for bacteria detection and identification are based on DNA analysis, extending from rather simple DNA amplification-based methods, such as polymerase chain reaction (PCR), real-time PCR, random amplification of polymorphic DNA PCR (RAPD-PCR) to more intricate approaches that rely upon restriction fragment analysis, targeted gene, and whole-genome sequencing (Galluzzi et al. 2007). Molecular methods can be classified as amplification methods (PCR, quantitative real-time PCR (qPCR), and reverse transcription PCR (RT-PCR)), DNA microarrays, hybridization-based detection methods (FISH), and whole-genome sequencing (WGS). These methods are culture-independent and enable bacteria identification at the genus level. It is crucial to understand the basic operating principles of each method, as well as their uses and limitations (Ferone et al. 2020).

Gene amplification and target gene sequencing is an effective method for bacteria identification. Over the past years, PCR amplification and gene sequencing have been utilized for detecting and identifying bacteria from colonies. Gene sequencing is a more objective method of bacteria identification, which does not regard fastidious growth or cell viability. This method provides reliable results and enables an increase in the diversity of bacterial taxa (Moshirabadi et al. 2019). Amplification methods provide relatively quick results, but there is a risk of cross-contamination associated with their sensitivity.

The 16S ribosomal RNA (16S rRNA) gene, the 26S rRNA gene, or particular genes encoding bacterial toxins are sequenced to detect bacteria. The 16S rRNA, a 1500 base pair gene common to all bacteria, is the most frequently utilized gene target for bacterial identification due to its high specificity to each species (Petti 2007). Real-time PCR is qualitative, more sensitive, and accurate compared to conventional PCR techniques. qPCR with fluorescence intensity enables the analysis of DNA amplification in real-time and does not require any post-PCR detection, which explains its broad usage in clinical and research fields. For instance, real-time-based 16S rRNA PCR was applied to identify and quantify microorganisms in chronic wound tissue and saliva sample (Melendez et al. 2010). Quantitative

real-time PCR (qPCR) and reverse transcription real-time PCR (RT-qPCR), and other amplification methods were used to identify foodborne pathogens, such as *Listeria monocytogenes, E. coli* O157:H7, *S. aureus, Campylobacter jejuni, Salmonella* spp., and *Shigella* spp. (Law et al. 2014). Random amplification of polymorphic DNA (RAPD), on the other hand, uses short primers with random sequences that result in the amplification of arbitrary, repetitive regions of template DNA. Since the short primers for RAPD-PCR are intended to bind randomly to the template, this method does not oblige any prior information of the target genome sequence. RAPD-PCR can be utilized not only to detect bacterial genetic variability but also to discover and detect unidentified microorganisms (Franco-Duarte et al. 2019).

Microarray is an ordered assemblage of samples (DNA, RNA, protein, tissue) that can be probed with target molecules to generate gene expression or diagnostic information. Microarray analysis can simultaneously detect and characterize numerous bacteria. Several microarray methodologies are available for application, such as printed and in situ—synthesized microarrays, electronic and suspension bead microarrays, and high-density bead arrays. Generally, the ssDNA sequence is synthesized and immobilized as discrete features or spots on the microarray surface. The "unknown" target sequence of interest is fluorescently labeled and then hybridized to the probe microarray. Hybridization between the immobilized probe and the labeled target enhances the fluorescence intensity. The fluorescence scanner measures the intensity, and the collected data is analyzed further (Miller and Tang 2009).

Fluorescence in situ hybridization (FISH) is considered a less time-consuming and reliable cytogenetic technique for bacteria detection and identification at the genus or species level. The principle of the FISH method relies upon the binding of short (18–25 base pair), fluorescence-labeled target-specific DNA or nucleic-acid mimicking peptide-nucleic-acid (PNA) probes to the ribosomal RNA with subsequent analysis under the fluorescence microscope. The FISH analysis offers information on spatial resolution, morphology, identification, and fast differentiation of bacteria from a mixed-species solution. The method offers rapid and reliable detection at the genus and species level, minimal technical equipment necessity, and cost-effectiveness. The main drawbacks are a need for specifically targeted investigation, trained and experienced personnel, and lower sensitivity than PCR (Frickmann et al. 2017).

Whole-genome sequencing (WGS) is becoming a highly applicable technique that provides rapid detection and identification of bacteria, viruses, and fungi due to advancements in sequencing technologies (Tagini and Greub 2017). WGS technologies permit valuable data about difficult-to-grow pathogens and drug resistance, bacteria's evolution and spread, possible virulence factors, candidate drug complexes, and a deep understanding of infection mechanisms. WGS technologies can compete with standard methods in speed, specificity, expense, and monitoring/investigating outbreaks of infectious diseases. Currently, WGS is commonly used in addition to real-time diagnostics in medical laboratories. Apart from detecting, identifying, and characterizing bacteria, WGS is applied to design diagnostic tools,

assess multidrug resistance, examine and track the emergence of pathogens in hospital environments (Punina et al. 2015).

20.4 Probes for Bacteria Detection

Probes techniques such as Southern blot, Northern blot, and Western blot are relatively old yet not overused methods for detection. Southern blot was developed based on Southern sequencing, which was the first used DNA sequencing technique. This sequencing method relies on isolating the DNA from the "target" sample, amplification reaction using specific primers with controlled termination of amplification by dehydrogenated nucleoside triphosphates, agarose gel electrophoresis, and gel visualization by the usage of ethidium bromide (Yuen et al. 1993). Then, protocols were modified to detect specific DNA sequences in DNA samples. At first, the DNA sample is cut by restriction nucleases. DNA fragments are separated by size through agarose gel electrophoresis, then transferred to nitrocellulose membrane and crosslinked the membrane via exposure to ultraviolet radiation. The critical step in Southern blotting is exposing the crosslinked membrane to a hybridization probe—a single-stranded DNA fragment complementary to the sequence of interest, usually tagged with a fluorescent dye or radioactive marker. After hybridization, membranes are blocked, washed, and then visualized (Marcadet et al. 1989). The main advantage of Southern blot is that it detects unculturable, usually environmental, bacteria (Cecchini et al. 2012).

Northern blot is commonly used to analyze the gene expression by detecting RNA in the sample. In principle, it is similar to Southern blotting, but electrophoresis gels have to contain formaldehyde to limit RNA secondary structure. Probes are complementary to the RNA sequence of interest. Still, they can be DNA, RNA, or oligonucleotides, usually labeled with radioactive isotopes, but chemiluminescence probes are becoming more and more common in use (Streit et al. 2009). Northern blot is not directly used for bacteria detection. Still, it allows detection of some particular bacterial small RNAs (sRNAs) in total RNA extract (Beckmann et al. 2010), which makes a fine way for examining gene expression. Its drawback is the impermanence of the analytical material, for it is tough to avoid RNase contamination.

Western blot, also known as the protein immunoblot, allows for the detection of specific proteins. In this method, proteins are separated by size via electrophoresis, usually in polyacrylamide gel, then transferred on the membrane and blocked. A protein of interest is targeted by incubation with a primary antibody. Then a secondary antibody targets the primary one. The secondary antibody is visualized through colorimetric, chemiluminescence, immunofluorescence, or radioactivity assays, indirectly detecting a target protein (Mahmood and Yang 2012). Because bacteria produce species-specific proteins, such as toxins, it is possible to detect and recognize them with Western blotting protocol (Bone et al. 2017). The main advantage of this technique is its simplicity and unambiguity of the results. Unfortunately, the analysis may require about a week to complete, making it an extremely

lengthy procedure. Also, analyzed proteins tend to form complexes. This phenomenon may cause the antibody-binding site to become unavailable, or even worse—the protein complex may be visualized and mistakenly recognized as an additional target protein (Mahmood and Yang 2012).

20.5 Microscopic Methods

The optical microscope is a fundamental detection device for bacteria identification. Obtained images allow determining the shape, following motion, and categorizing species by their morphological contrast (Dige et al. 2007). However, only using microscopy for bacteria detection is not enough. In natural samples, smaller cells can be missed due to the density of larger cells. Distinguishing cells from other objects or living cells from dead cells can also be challenging (Franco-Duarte et al. 2019). Another major disadvantage of microscopy is that none displays the microorganisms' phylogenetic diversity (Franco-Duarte et al. 2019). Also, microscopic techniques do not allow for the display of phylogenetic diversity.

In most cases, microscopic methods are used with fluorescent dyes due to more specific visualization and uncomplicated performance. Dyes such as 4′,6-diamidino-2-phenylindole (DAPI; maximum absorption is at 400 nm), acridine orange (absorption maximum 500 nm), SYBR® Green I (maximum absorption 497 nm) bind to the DNA of the bacteria and fluorescence after the UV exposure, making bacteria detectable (Franco-Duarte et al. 2019). Flow cytometry can also be applied for detecting individual cells. This method enables the possibility to count and evaluate individual cells' size, shape, and features. Cells are suspended in a fluid flow and passed through a detector, collecting fluorescence or scattered light. Clausen et al. used a label-free technique of electrical impedance flow cytometry to distinguish Gram-negative from Gram-positive bacteria successfully and accurately determined the concentration of the bacteria solution (Clausen et al. 2018).

20.6 Spectroscopic Methods

Spectroscopy is the study of matter and its interactions with electromagnetic radiation. Spectroscopic techniques are used in nearly all technical areas of science and technology for quantitative and qualitative analyses (Ferone et al. 2020). This multivariate, reproducible methodology is used to solve numerous analytical problems due to its non-destructive, simple, and precise approach, enabling broad amounts of information acquired in a single measurement (Mariey et al. 2001). Spectroscopic techniques vary based on the examined species (molecular or atomic spectroscopy), the type of radiation–matter interaction to be monitored (absorption, emission, or scattering), as well as the used range of the electromagnetic spectrum. Spectroscopic methods require a combination of spectral pre-processing and different chemometric techniques to analyze and differentiate bacteria quantitively.

One of the latest developments in applying new spectroscopic techniques is the Fourier transform infrared spectroscopy (FTIR), an adjustable, rapid, non-invasive, and effortlessly operated method (Mariey et al. 2001). This method provides a label-free approach to the comprehensive interpretation of the chemical compounds and the physical state of the whole sample. It is possible to acquire precise, thorough information about nucleic acids, carbohydrates, lipids, and proteins only in one measurement with a small sample volume (Kosa et al. 2017). Also, FTIR enables an efficient biochemical characterization of the entire biological systems. The major advantage of the FTIR method is the capacity to examine numerous compounds at once. FTIR application to examine microorganisms leads to quite a complicated spectrum with the principal compounds' overlapping absorption bands. Hence, a detailed statistical analysis is indispensable to extract only the essential data from spectra (Franco-Duarte et al. 2019). Also, this method does not require cell lysis to evaluate the biomolecules and is considered eco-friendly since toxic compounds are not used. Besides the achievement on the field of screenings, FTIR can be applied to monitor various processes in real-time (Kosa et al. 2017).

However, for the analysis of microbial diversity, regular infrared radiation is much more applicable (Santos et al. 2010). The near-infrared (NIR) spectral region was used in the food microbiology industry to detect and identify of *Lactococcus lactis*, *Listeria innocua*, *Pseudomonas fluorescens*, *Pseudomonas putida*, and *Pseudomonas mendocina* on chicken breast muscle (Alexandrakis et al. 2012). The major drawback of using NIR spectroscopy in food or microbiological analyses is the samples' sensitivity to temperature shifts or the occurrence of photodegradation triggered by the light sources. Furthermore, the infrared (IR) signal is frequently dominated by the signal from the water, which is almost always present in the culture media and food products (Ferone et al. 2020).

Raman spectroscopy is another popular spectroscopic method recognized for its non-invasive and rapid recognition and characterization of various analytes, including bacteria. Its principle is the inelastic scattering of monochromatic light. Inelastic scattering implies the shifts of photon frequency in monochromatic light upon contacting the sample. The sample absorbs light photons and then reemits. The reemitted photons' frequency is altered compared with the original monochromatic frequency (the Raman effect). This shift provides information about molecules' vibrational, rotational, and other low-frequency transitions to create a structural fingerprint. This structural fingerprint can be used to distinguish microorganisms, with the accuracy that allows differentiating species, or even the strains, in a short period (Ferone et al. 2020). Additionally, the Raman signal is not affected by water, but fluorescence signals can give high background because of amino acids and nucleic acids (Ferone et al. 2020). Even though Raman spectroscopy has high specificity, it has inadequate sensitivity.

Surface-enhanced Raman spectroscopy (SERS) enables greater sensitivity in detecting low concentration analytes by intensifying electromagnetic fields created from the excitation of localized surface plasmons. Comparing to the standard Raman, a signal can be boosted from 10^3 to 10^6 times using SERS (Kneipp and Kneipp 2006). Bacteria detection using SERS can be carried directly by analyzing

the intrinsic vibrational fingerprint of bacteria (Witkowska et al. 2017; Witkowska et al. 2018); or via indirect detection by using a nanotag as a quantitative reporter (Huang et al. 2021). SERS signal relies upon the active substrate's material since each substrate has unique enhancement effects on the samples. The shape and size of the nanoparticles, the active substrate's material, distance, and the number of probes adsorbed on the active substrate affect the signal (Ferone et al. 2020). Wei et al. successfully detected and identified *E. coli*, *S. aureus*, and *Salmonella* spp. using SERS coupled with silver colloidal nanoparticles. The distinctive differences of each pathogen were observed in the SERS spectral data, and a short time was required for the assay (Wei et al. 2018).

20.7 Chromatographic Methods

Mass spectrometry (MS) based techniques are an important microbial-typing tool because of the rapidity, low expense, ease of use, and effectiveness of all kinds of bacteria, archaea, and fungi. Mass spectrometry can be associated with multiple ionization and separation methods, such as gas chromatography (GC) and liquid chromatography (LC) (Fox 2006), matrix-assisted laser desorption ionization time-of-flight mode (MALDI-TOF) (Jang and Kim 2018), electromigration techniques (Buszewski et al. 2017), or electrospray ionization (ESI) (Zhang et al. 2011).

The LC combined with MS (LC-MS) transformed the analytical determination of metabolome, thus, enabled bacteria identification (Warren 2018). The use of relatively low temperatures and the fact that the sample doesn't need to be volatile, the preparation process of the sample is much simpler, and the costs decreased. Samples are introduced into the solvent then separated within the column with the stationary phase (Holčapek et al. 2012). LC depends on the gravity force to move the mobile phase across the column, but for HPLC, pressures reach 50–350 bars. Moreover, it can be utilized at higher temperatures (high-temperature liquid chromatography) or in monolithic columns (Teutenberg 2009).

MALDI-TOF MS, a new generation tool, is widely used for the identification of microorganisms in the most advanced clinical laboratories. In this procedure, the microbial cells' ionization is ionized by the laser pulses. Then the electric field accelerates created ions in a vacuum system (Doern and Butler-Wu 2016). An acquired spectra profile is unique for a particular microorganism and can be utilized as its molecular fingerprint. The identification is proceeded by comparing the molecular fingerprint with the database (Jang and Kim 2018). Nowadays, MALDI-TOF is commonly used as a complementary procedure to the culture methods. When combined, these two methods provide a quick and specific (to the species-level) detection (Váradi et al. 2017).

20.8 Electrokinetic Separation Methods

Capillary electrophoresis (CE)–MS merges the separation process of electrophoresis with MS detection. Comparing to GC and LC, it provides more efficient separation, faster analysis, allows for small volumes of sample required, inexpensive reagents, and separation of cations, anions, and uncharged molecules in one run. This method is applied to examine the metabolome of various bacteria, in which results were intriguing in the detection and quantification of numerous metabolite classes (Soga et al. 2002). CE lacks sensitivity due to the small sample volumes. At the same time, combined with MS, it has a limited number of accessible commercial libraries, and last but not least, decreased retention time reproducibility.

CE combined with capillary isoelectric focusing (CIEF) was used to isolate and identify bacteria species having different sizes and shapes (Armstrong et al. 1999). This experiment showed that intact biological cells could be successfully isolated via methods typically limited to macromolecules. Another combination is CE fused with fluorescence that can be utilized to monitor the separation process, operational conditions, and microbial dynamics regarding cell aggregation (Armstrong et al. 2002). The primary benefit of these methods is the capacity to control parameters (size, shapes, and charges) for isolation and detection.

Electrical field-flow fractionation (EIFFF) is an alternative method. It depends on the separation of sample components in a channel because the various electrical fields result in a distinct layer of each component. Two main walls of the channel are utilized by the EIFFF device to generate a difference in the potential, which allows for the separation of charges (Desai and Armstrong 2003).

20.9 Biosensors

Biosensors appear as the most promising devices for the detection of microorganisms. Biosensor-based methods are perceived to have great potential for further development (Arora et al. 2011; Velusamy et al. 2010). According to IUPAC, "*a biosensor is a device which uses specific biochemical reactions mediated by isolated enzymes, immunosystems, tissues, organelles or whole cells to detect chemical compound usually by electrical, thermal or optical signals.*" Antibodies, enzymes, and nucleic acids are commonly utilized as bio-receptors (Chen et al. 2017a).

Biosensors are divided into physical and chemical biosensors depending on the transducer used to detect the target analyte. Physical biosensors sense shifts in mass, resonance frequency, refractive index, fluorescence and are further categorized as optical and mechanical biosensors. Optical biosensors measure the analyte by its interaction with photons, such as fluorescence or phosphorescence emissions. Optical biosensors are divided into labeled and label-free. Mechanical biosensors detect the analytes by examining the shift in mass during the recognition stage. These sensors have several beneficial characteristics, such as no sample preparation step and label-free detection comparing to other sensors. The most frequently employed

mechanical biosensors are quartz crystal microbalance or cantilever technology (Brindha et al. 2018).

Chemical biosensors detect the shifts in the chemical reactions during the interactions between the analytes and biorecognition elements. Chemical biosensors are further classified into electrochemical and biochemical sensors. Electrochemical biosensors analyze the differences in electrical properties, such as current, potential, or impedance at the electrode surface during the binding step. Based on the detection technique, electrochemical biosensors are categorized into labeled and label-free. Labels, such as enzymes, metal particles, or nanoparticles, are employed to target the analytes in labeled biosensors. In label-free biosensors, the attachment of biomolecules to the surface of the electrode cause shifts in electrical parameters. Electrochemical biosensors are categorized into amperometric, potentiometric, voltammetric, conductometric, and impedimetric (Gothandam 2018).

Analytes in biosensors range from ions and molecules, through nucleic acids and proteins, up to the whole viruses and bacteria. Biosensors can detect bacteria by targeting bacterial components, such as DNA, RNA, intracellular proteins, exotoxins. This method requires sample processing and additional reagents, which raises costs and time. An alternative method to detect bacteria is to target whole bacteria cells. This direct method does not require additional reagents, which is more suitable for quick and inexpensive point of care testing. For the whole bacteria detection, impedimetric and optical methods are frequently applied (Ahmed et al. 2014).

Even though biosensors are rapid and specific, they are not consistently applied in bacteria detection due to cost, limit of detection, complex matrix, and difficulty in detecting more than one bacteria simultaneously (Velusamy et al. 2010). Primarily, much depends on the chosen type of bioreceptor element that can be more or less sensitive to contaminants (Neethirajan et al. 2018).

20.10 Bacteriophage-Based Methods

20.10.1 Bacteriophages

Bacteriophages are viruses that attach to particular bacterial receptor proteins to infect the host cells. Most known bacteriophages belong to *Caudovirales*, whose representatives are characterized by dsDNA genome and icosahedral, tailed capsid with the fibers attached to the tail (Ackermann 2007). The size of the virion is usually about 50–200 nm. However, some filamentous phages (e.g., M13) may reach even 400 nm length (Sharma et al. 2017). Recently, even bigger bacteriophages were discovered from marine water.

Based on their life cycles and means of propagation, phages are classified into two categories. Lysogenic (temperate or reductive) phages fuse their genetic materials into the bacterial genome and are inherited by daughter cells during binary fusion. Lytic (virulent or productive) phages undergo four steps process during infection: (1) binding to the receptors (protein or sugar moieties) of the bacterium

due to host specificity properties; (2) injection of genomic materials into the cytoplasm of the bacteria; (3) viral replication via bacterial transcription, translation, and replication; (4) newly assembled phages leave the cell through bacterial lysis with the help of choline and endolysins proteins, causing the death of the host cell. This process is the basis of phage therapy for targeting pathogens (Kortright et al. 2019).

Bacteriophages are gaining recognition as a promising recognition element in the area of rapid detection of bacteria. Bacteriophages demonstrate advantageous qualities such as excellent specificity, robustness, toughness, and cheap preparation, making them popular biorecognition elements in biosensors and other assays for bacteria detection (Paczesny et al. 2020; Richter et al. 2018). The most crucial advantages of phage-based methods for bacteria detection are as follows:

- Phages are ubiquitous and highly specific to bacteria (Koskella and Meaden 2013) but cause no major threat to humans (Tian et al. 2021).
- Because of being "molecular parasites" (Breaker et al. 1994), phages need to infect a viable host to multiplicate by using its transcriptional machinery. This fact allows us to distinguish between living and dead bacteria, which is usually a significant issue for bacteria detection protocols. However, it may be phages absorbed on the surface of the dead cell (Krueger 1931).
- Phages can self-amplify, which makes their "production" simpler and cheaper than, e.g., antibodies,
- Phages targeting particular bacterial species may be isolated from various environments, such as hospital sewage water (Farooq et al. 2020), environmental water or sewage samples (Yan et al. 2017; Bhardwaj et al. 2016, 2017), or the soil (Cross et al. 2015). The isolation process is quick and cheap, can be provided in every biological laboratory without even identifying the isolates.
- Phages display more shelf life due to their resistant nature to external factors, which decreases the environmental limitations and allows regeneration of the sensor surface (Zourob 2010).
- Finally, phages are biological entities that evolve. This allows them to compete in the arms race with bacteria (de Jonge et al. 2019) and overcome developing resistance mechanisms. An intriguing example is the discovery of anti-CRISPR (Trasanidou et al. 2019).

Phage-based biosensors rely on two different approaches. The first one is the generation of the analytical signal upon the capturing of bacteria. These are usually surface-sensing elements or phage-based probes. Their main advantage is the speed of the analysis, yet a single event is difficult to detect. While being one of the major problems, this fact is responsible for relatively high detection limits (LOD). The sensitivity of these types of biosensors can be improved by using phage-based bioconjugates, layered sensors, and methods utilizing parts of phages without additional pre-incubation steps. This might be done by developments in biorecognition elements themselves (e.g., by ordering of phages within sensing layers) or by utilizing ultrasensitive transducers (e.g., optoelectronic-based).

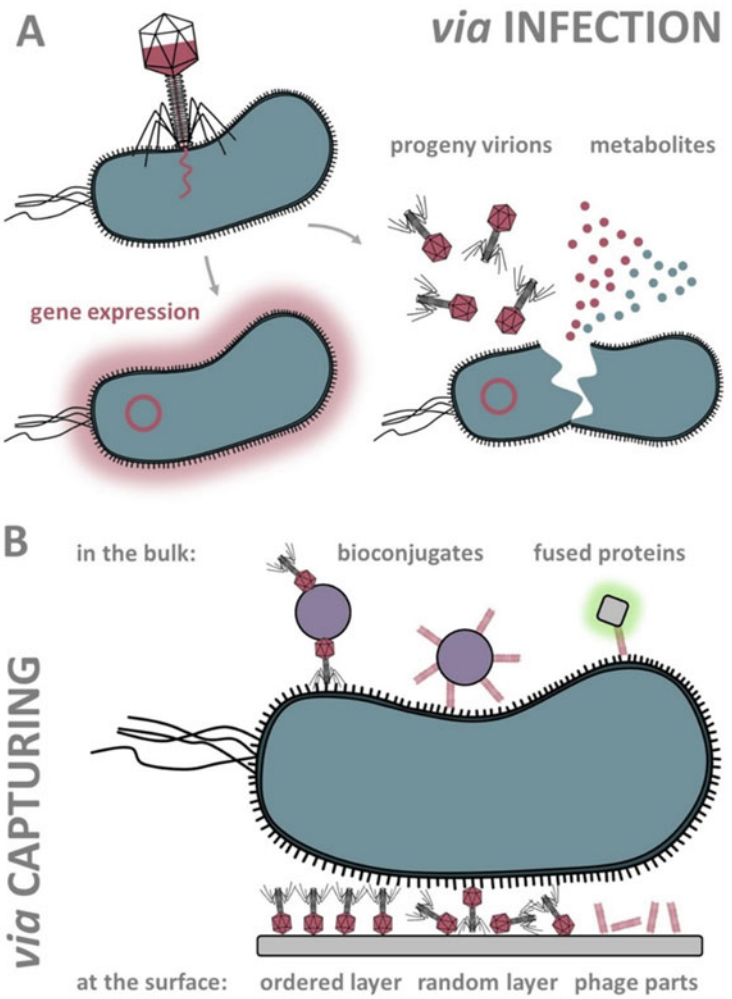

Fig. 20.1 Designs of most commonly used phage-based biosensors. (**a**) Bacteria detection by depositing phages on sensor surface or phage-based probes is rapid, but signal amplitude is usually low. On the contrary, (**b**) infecting bacteria and using its molecular mechanism increases sensitivity by producing progeny virions, expression of reporter genes, or releasing bacterial metabolites due to lysis. However, the process is time-consuming. The figure was inspired by (Paczesny et al. 2020) based on Creative Common CC BY 4.0 license

Alternative designs are based on the infection of the target bacteria, which generates the measurable signal by affecting the cell's metabolism (the release of progeny virions, products of reporter genes, or metabolites). These methods already showed some ultra-sensitivity, but they are lengthy. Schemes showing the phage-based approaches for bacteria detection are shown in Fig. 20.1.

20.10.2 Methods Targeting Bacterial Metabolites

Certain metabolites can be used as target analytes in phage-mediated bacterial detection. These metabolites are discharged from the cytoplasm due to the cell membrane burst caused by the completion of the lytic cycle. Phage application allows more specificity and species-level recognition. For instance, T7 phage-based biosensor platform depended on the detection of β-galactosidase released during the lysis of bacterial cells. The released enzyme cleaved the substrate resorufin β-D-galactopyranoside resulting in a fluorescent product. This approach resulted in LOD of 10 CFU/mL *E. coli* BL21 within 8 h (Tilton et al. 2019). He et al. proposed a phage-affinity approach to detect *P. aeruginosa* based on bioluminescence detection of released intracellular adenosine triphosphate (ATP). The concentration of *P. aeruginosa* was determined via firefly luciferase-ATP bioluminescence reaction. The proposed method resulted in LOD of 2×10^2 CFU/mL. The separation and detection process required 2 h (He et al. 2017).

A further step for developing such an approach was introducing genetically modified phages to obtain both sensing and signal-generating elements. Reporter phages are genetically modified phages used as gene importers to inject a specific gene into the bacteria's genome. The most suitable genes are fluorescent coding proteins or other easy-to-detect products expressed inside the host cells during the infection. Genes, such as bacterial *lux* (Loessner et al. 1996) or firefly *luc* (Lankes et al. 2007) bioluminescence genes, *inaW* gene-ice nucleation (Goodridge and Griffiths 2002), *lacZ* gene-β-galactosidase (Bremer et al. 1984), and I*gfp* gene (Poul and Marks 1999) were used as a reporter for various pathogens detection.

A review published by Pizarro-Bauerle and Ando (Pizarro-Bauerle and Ando 2020) presented the current state of the art of engineered bacteriophages' practical applications. The report summarized the most recent applications of genetically modified phages in the clinical settings, food industry, agriculture, and material science. Here, we present the most significant reports consisting of biosensing using genetically modified phages.

A report from 2000 by Irwin et al. described the usage of *Salmonella*-targeting bacteriophage encoding ice nucleation protein (INP) to infect the bacteria. After supercooling with a phase-sensitive dye, the quantitative analysis of bacteria solutions was conducted. This allowed for the detection of *Salmonella* spp. with a minimum detectable level of about 2 CFU/mL within 3 h (Irwin et al. 2000).

Wisuthiphaet et al. developed an *E. coli* detection method by using a genetically modified T7-ALP phage that causes overexpression of alkaline phosphatase after the infection. The detection of *E. coli* BL21 bacteria was provided via fluorescence imaging of ELF-97 alkaline phosphatase substrate used to stain the bacteria retained on the filter. The LOD was around 10^2 bacteria per gram of model beverage and the time of analysis was about 6 h (Wisuthiphaet et al. 2019). Another recent publication by Wisuthiphaet et al. showed a rapid colorimetric pathogen detection method in a food matrix. T7 phage engineered with *phoA* gene was used to detect *E. coli* in food matrices. The technique consisted of phage-induced expression of an exogenous enzyme, alkaline phosphatase, incubation in nitro blue tetrazolium/5-bromo-4-

chloro-3-indolyl phosphate (NBT/BCIP) color dye, and filtration through a 0.2-micron polycarbonate membrane. Reported results showed LOD around 10 CFU/mL and 10^2 CFU/mL (coconut water and spinach leaves, respectively) within 5 h of enrichment and up to 2 h of incubation with phages (Wisuthiphaet et al. 2021).

A paper by Nugen et al. reported detection of *E. coli* using T7 containing NanoLuc luciferase expression cassette. This method requires the addition of luciferin NanoGlo substrate to detect the chemiluminescent signal (Pulkkinen et al. 2019). Modified phages were prepared by synthetic biology approach— PCR fragments and in vitro DNA assembly were used. This protocol provided relatively fast and straightforward preparation of modified phages. The LOD was about 5×10^2 CFU/mL after 2 h of incubation. Later on, the same research group proposed an approach to develop a new protocol for analysis of drinking water against generic *E. coli* (according to the US standards, there can be no "coliforms" in 100 mL of drinking water). They developed a phage-based membrane filtration approach by using luciferase and alkaline phosphatase for detection and quantification. 100 mL of drinking water sample was filtered on the cellulose filter. After 8 h of incubation, *E. coli* specific T7 phages carrying a reporter gene were added. In both cases, reporter genes were fused with genes encoding cellulose-specific carbohydrate-binding modules (CBM). After 1.5 h of incubation, enzymatic substrates were added to visualize the colonies. The overall time of this procedure was about 10 h, making it significantly faster than the plating method (24 h) and providing the most satisfying limit of detection of 1 CFU/100 mL (Hinkley et al. 2018a).

To make the analysis time shorter, the same group proposed another approach for detecting *E. coli* by using a genetically modified T7 coliphage carrying NanoLuc reporter gene. The authors managed to fuse NanoLuc with CBM and detected immobilized fusion protein resulted from a single CFU of *E. coli*. This approach enables rapid and low-cost detection of *E.coli* in 100 mL of the water sample. The final LOD was about 10 CFU/mL (Hinkley et al. 2018b). According to Wisuthiphaet, such detection limits can be acquired only in simple matrices. In complex matrices, the background signals may cover the signals from bacteria detection (Wisuthiphaet et al. 2019). Eventually, Hinkley et al. developed a rapid, selective, and sensitive phage-based detection by combining previously developed membrane filtration method along with phage infection to generate luminescence in the presence of *E. coli*. The proposed approach resulted in LOD around 20 CFU in 100 mL of water sample within 5 h (Hinkley et al. 2020).

Certain procedures can be done without any external substrates. Vinay and coworkers proposed the detection of *E. coli* and *S. enterica* ser. Typhimurium using HK620 and P22 phages with introduced *gfp* gene. Vinay et al. used genetically modified HK620 and P22 phages carrying *gfp* gene to detect *E. coli* and *S. enterica* Typhimurium by measuring fluorescence using flow cytometry. This method enabled rapid and sensitive detection as low as 10 cells/mL without enrichment procedure within 1 h (Vinay et al. 2015). Following research focused on the genetic modification of phages HK620 and HK97 to express the entire lux operon—*lux*AB genes coding luciferase and *lux*CDE coding fatty acids reductases. Although the

reported LOD was not satisfying (10^4 bacteria/mL), incorporating the *lux*CDABE cassette into the COMBITOX instrument was successful. When upgraded, this instrument may become a useful tool for accommodating several bio-detector systems to detect bacteria, toxins, and heavy metals (Franche et al. 2017). A report published by Kim et al. presented quite a different approach for utilization of *lux*CDABE operon. Their phage-of-choice was phiV10 phage, targeting *E. coli* O157:H7. The amount of time required for the detection was 40 minutes, and the LOD of the biosensor was as low as 1 CFU/mL in the case of pure culture. For food samples, the LODs were 10 CFU/cm^2 (romaine lettuce), 13 CFU/mL (apple juice), and 17 CFU/g (ground beef) (Kim et al. 2017). In 2016 Wu et al. fused the tetracysteine (TC)-tag with small outer capsid protein of the wild-type PP01 bacteriophage and used them to infect *E. coli* O157:H7 host cells. Then the progeny PP01-TC phages were fluorescently labeled, and a flow cytometry procedure was used to measure the fluorescence. The LOD in the complex fluid (apple juice) provided the LOD of 1 CFU/mL within 1 h (Wu et al. 2016). Recently, the same research group developed a rapid, sensitive, and multiplex detection method targeting *E. coli* O157:H7, *S. typhimurium,* and *P. aeruginosa* using dual-modified M13KE phage. The M13KE phage-displayed the targeting peptide on the minor coat protein pIII and the streptavidin-binding peptide on the major coat protein pVIII. The LOD of this method was 10^2 cells/mL in 40 mL of sample volume via flow cytometry (Wu et al. 2021).

Wang et al. proposed the electrochemical detection of *E. coli* using T7 phage expressing the *lacZ* gene encoding β-galactosidase. The substrate was 4-aminophenyl-β-galactopyranoside (4-APG). 4-APG forms an electroactive product when cut by β-galactosidases. This product was detected by differential pulse voltammetry. The detection limit was in the range of 10^2 CFU/mL within 7 h (Wang et al. 2017).

FASTPlaqueTB assay for detecting *Mycobacterium tuberculosis* in sputum uses the lytic virulence of the phages as a "sensor." Phage particles containing a luciferase gene are commonly used as reporters due to the highly sensitive detection of the bioluminescent signal luciferase generates. In addition, green fluorescent protein (GFP) and several other reporter genes are considered suitable. GFPs retain major advantages, such as high stability, low toxicity, and the fact that fluorescence is triggered by excitation light, eliminating the additional substrate as required for luciferases (Harada et al. 2018).

20.10.3 Detection of Progeny Virions

Bacteriophages offer a "built-in" amplification system—after the infection of the host cell and multiplication using its translational machinery, progeny virions destroy the host cell and are released. These progeny virions can be used for the detection of bacteria. Such amplification improves the detection limit, for much more objects can be detected.

To provide a faster time of analysis and better sensitivity, phage amplification methods for detecting progeny virions are usually combined with the PCR technique. Luo and others developed an assay that allows detection of 10 CFU *Acinetobacter baumannii* in 100 μL serum within 4 h without DNA extraction and purification process by using phage p53 at the concentration of 10^3 PFU/mL (Luo et al. 2018). Later, the same group used qPCR combined p53 phage recognition of *A. baumannii* LB8 isolated from sputum samples. They designed the primer pairs to recognize the phage or the bacteria. It allowed for a detection limit of around 1 CFU/mL within 6 h (Luo et al. 2020). A novel bacteria detection method for *Salmonella* Enteritidis based on phage vB_SenS_PVP-SE2 and qPCR was developed by Garrido-Maestu. The proposed method required 10 h to obtain LOD of 8 CFU in 25 g of chicken meat (Garrido-Maestu et al. 2019). Sergueev reported the detection of zoonotic bacteria *Brucella abortus* in mixed cultures and blood samples with the LOD of around 1 CFU/mL within 72 h (Sergueev et al. 2017). One of the most spectacular examples was published by Anany et al. (Anany et al. 2018), who developed a rapid and inexpensive method for constructing phage-based bioactive paper by utilizing inkjet printing to detect foodborne pathogens. The reported LOD of this dipstick assay was between 10 and 50 CFU/mL within 8 h.

Mido et al. proposed combining phage amplification with immunoassay protocol. Progeny MS2 phages coupled with specific antibodies were immobilized on the surface of magnetic beads. Upon the addition of the detector antibody, binding to MS2, the fluorescence was measured. A fluorescence-based method allowed for detection after 3 h of incubation with the LOD of around 10^2 cells/mL (Mido et al. 2018).

Phage titration is the most archaic and the simplest approach to detect and count progeny virions. Specific amounts of phages solution with different concentrations are dropped onto the agar plate with bacteria. Bacteria get lysed where virions are presented, which is visible as the holes in the bacterial layer. These holes are called plaques, and they mark the number of phages in the stock solution. Said et al. used this method to observe changes in *Salmonella typhi* pathogen during nutritional deficiency circumstances. The phage infectivity rate was much more suitable than the traditional plate culturing technique. This method enabled the detection of active pathogens, which are normally undetectable by conventional approaches (Ben et al. 2019). In 2006 Ulitzur and Ulitzur reported the usage of mutant phages (mutants that cannot form plaques at concentrations lower than their reversion rate and temperature-sensitive mutants) as a method for bacteria detection and determination of their antibiotic susceptibility. The method is based on plaque formation as the endpoint of the phage lytic cycle. The LOD ranged between 1 and 10 viable bacteria cells within 3–5 h (Ulitzur and Ulitzur 2006). Jassim and Griffiths reported an interesting *P. aeruginosa* detection method using Pseudomonas Phage NCIBM 10116 for standard plague counting method combined with live/dead fluorescent measurement. This resulted in the highly specific analysis in a reasonable period (1 cell/mL within 4 h) that allows viable cells monitoring (Jassim and Griffiths 2007).

20.10.4 Utilization of Whole Virions for Bacteria Detection for Biosensors

There are disadvantages to approaches depending on the completion of the phage lytic cycle. First, such methods require choosing only the virulent phages. Moreover, progeny phages are generated in rare cases while prophages are integrated into the host's gene. Finally, bacterial phage-infections-preventing mechanisms, such as CRISPR-Cas, influence the process (Faure et al. 2019). Also, methods relying on genetically modified phages have some drawbacks that need to be considered. Reporter phage-based methods require deep understanding and expertise. It is also necessary to continuously adjust and enhance approaches depending on the target bacteria. The infection rate of genetically modified phages tends to be weaker than wild types (Pires et al. 2016). Finally, the release of genetically modified phages into the environment could cause unpredictable effects on the biosphere (Bárdy et al. 2016).

All this resulted in developing phage-based methods for bacteria detection, which generates an analytical signal upon capturing target cells. This can be done both in bulk via bioconjugates (cf. following section) and at the surface (Fig. 20.1).

A transducer is an essential part of a sensor that detects and converts signals from bio-receptors into measurable signals. The contact between the target analyte (here bacteria) and the surface is necessary for many analytical techniques, e.g., SERS, microbalance-based, magnetoelastic-based, or electrochemical methods. Phages immobilized at the surface must maintain their infectivity, binding affinity, and selectivity. The most frequently applied immobilization techniques include adsorption, entrapment, cross-linking, covalent coupling, and affinity. Physical adsorption is the fastest and simplest way, but there is the risk of desorption and low surface coverage. Hence, for phage immobilization purposes covalent bonding method is considered more favorable.

Richter et al. and Zhou et al. developed a phage deposition method on an electroconductive sensing layer. Experiments were carried out based on the surface charge and dipole moment of phage particles. The application of an alternating electric field on the sensing layer enabled head down–tails up orientation of phages considering the negative charges of the capsid. T4 phage was successfully oriented on a gold surface by utilizing an electric field coupled with chemical modification of the sensing layer. Chemical attachment of bacteriophage onto the biosensor surface considerably increases the overall detection's stability and performance (Harada et al. 2018; Richter et al. 2016, 2017; Zhou et al. 2017).

Recently the number of reports on electrochemical approaches for bacteria detection increases rapidly (Farooq et al. 2020; Wang et al. 2017; Zhou et al. 2017; Neufeld et al. 2003; Neufeld et al. 2005; Li et al. 2018; Sedki et al. 2020; Xu et al. 2020). Electrochemical methods offer satisfying sensitivity, low-cost analysis, a vast field of possibilities for miniaturization. Here we present a couple of the latest reports on bacteriophage-based electrochemical methods for bacteria detections (Ferapontova 2020; Xu et al. 2019; Janczuk-Richter et al. 2019).

Sedki et al. described utilization of M13 phage immobilized on the electrodes combined with electrochemical impedance spectroscopy to target coliforms with the LOD of around 14 CFU/mL within 30 min (Sedki et al. 2020). This research presents a single phage balance highly specific with a wide range of hosts—multiple strains of *E. coli* can be detected, while there is no response to non-*E. coli* bacteria. Niyomdecha et al. used M13 phages displaying *Salmonella*-specific peptide immobilized on the electrode and applied it into a capacitive flow injection system. Their sensor provided measurements with sensitivity ranging from 2×10^2 to 1×10^7 CFU/mL within 40 min. The sensor was reusable up to 40 times, thanks to the alkaline eluting solution (Niyomdecha et al. 2018). Recently the review of available M13 phage-based biosensors was published (Moon et al. 2019).

Yue et al. developed a rapid and sensitive electrochemiluminescent (ECL) bioassay using highly specific virulent phage PaP1 as biorecognition element for detecting *P. aeruginosa* without employing labels. Glass carbon electrode was covered with phage-fused carboxyl graphene and luminol used as a source of chemiluminescence. This approach resulted in LOD of 56 CFU/mL and the amount of time required for detection was 30 minutes (Yue et al. 2017).

Xu et al. detected viable bacteria by chemically immobilizing T4 bacteriophages on the surface of the extended gate connected to a metal oxide semiconductor field-effect transistor (MOSFET) device. The obtained LOD was around 14 CFU/mL within 35 min (Xu et al. 2020).

An alternative approach is magnetoelastic biosensors. Mass sensitivity is a crucial measurement criterion for magnetoelastic biosensors. The amplitude of vibrations changes when the analyte is deposited on the sensing surface. The first sensor prepared according to this protocol, targeting methicillin-resistant *S. aureus* (MRSA) strain, reached the limit of detection of 3×10^3 CFU/mL within 30 min (Hiremath et al. 2015). In 2017 the same research group confirmed their sensor was detecting MRSA strain even in the presence of other competing bacteria (Hiremath et al. 2017). Chen et al. (2017b) and Mack et al. (2017) described the detection of *S. enterica* and *S. typhimurium* at the surface of chicken and lettuce, respectively.

Recently, Halkare et al. developed a new label-free method for detecting *E. coli* B40, using T4 phages as biorecognition elements on a plasmonic fiber-optic platform. The novelty of this method relies upon capturing the analyte before subjecting the sensing layer to bacteriophages. Application of this method resulted in detection concentration of 10^3–10^7 CFU/mL in environmental matrices within less than 4.5 h with high specificity to only *E. coli* B40 (Halkare et al. 2021).

Srivastava's group proposed to use the sensing layers based on bacteriophages in SERS-based sensors. T4 phages were immobilized on a silicon platform along with the thin silver film. The authors reported the detection of *E. coli* in the concentration of 1.5×10^2 CFU/mL (Srivastava et al. 2015). In 2018 Rippa et al. immobilized bacteriophages on the surface of a substrate made of plasmonic nanocavities, obtaining the plasmonic quasicrystals (Rippa et al. 2018). The same group presented the meta structures for SERS-based detection, conjugated with *Tbilisi* bacteriophages targeting *Brucella* spp. With the sensitivity on the single-cell level, within 1 h, the authors were able to detect bacteria in the concentration of over 10^4

CFU/mL (Rippa et al. 2017). Lai et al. obtained an approximate LOD in detecting *Bacillus* spp. with gamma phages (Lai et al. 2017).

20.10.5 Phage-Based Bioconjugates

The micromolar concentration of a chemical compound makes over 10^{10} molecules or ions to be detected in every single mL. The situation looks quite different for bacteria when the need is to detect single cells in relatively large volumes (with the goal of 1 CFU/mL). Because of the small number of "objects," the number of signal-generating events is low. The other problem is the relatively low probability of the attachment of bacteria to the sensing surface covered with immobilized phages and long search time.

Moving from the detection at the surface towards the bulk solves these issues. Bioconjugates offer shorter search time, more capturing events, and a broader range of analytical techniques for signal acquisition. The application of P9b phage conjugated with the gold nanoparticles in the SERS protocol for the detection of *P. aeruginosa* was reported in 2020 (Franco et al. 2020). Gold nanoparticles solution can also be used to prepare a colorimetric sensor because its color changes the aggregation. Another important feature of gold is that it covalently combines with the thiols (-SH) (Li et al. 2006). In the research by Peng and Chen, M13 phages were chemically modified to exposed SH groups. Phages were also genetically modified to display the receptors against different species of bacteria (i.e., *P. aeruginosa*, *Vibrio cholerae*, *Xanthomonas campestris*). The pellet of centrifuged bacteria with bounded M13 phages was resuspended in a buffer containing gold nanoparticles (AuNPs). AuNPs got attached to thiol groups presented on the viral capsids. The presence of phages in the pellet resulted in the change of color of gold nanoparticles solution, indirectly confirming the presence of target bacteria. The procedure took about 30 min and provided the limit of detection around 10^2 cells/mL (Peng and Chen 2019). SiO_2@AuNP nanoparticles were also used to get bacteriophages immobilized on their surface. Darkfield microscopy was chosen for the analysis of the conjugates. While attached to *S. aureus* SA27 cells, the conjugates got aggregated, which caused a strong light scattering. The authors reported a detection limit of *S. aureus* of around 8×10^4 CFU/mL in up to 20 min (Imai et al. 2019).

Janczuk et al. proposed the utilization of bifunctional T4 phage-based bioconjugates to first magnetically separate target bacteria and then enumerate them using flow cytometry. The reported LOD of *E.coli* was around 10^4 CFU/mL. The separation protocol and flow cytometry analysis took about 15 minutes (Janczuk et al. 2017). Yan et al. combined bacteriophages deposited on the magnetic particles with immunoassay, targeting *S. aureus* in complex fluid (apple juice). This approach resulted in a LOD of around 9×10^3 CFU/mL within 90 min without any pre-enrichment (Yan et al. 2017).

Metal-organic frameworks (MOF) crystallites were used to generate the analytical signal in phage-based bioconjugates. IRMOF-3 ($Zn_4O(NH_2\text{-BDC})_3$) (BDC = benzene-1,4-dicarboxylic acid) and NH_2-MIL-53(Fe) (MIL = *Matériaux*

de l'Institut Lavoisier) are examples of fluorescent MOFs. When conjugated to phages, they were used as probes for *Staphylococcus arlettae* and *S. aureus* detection (Bhardwaj et al. 2016, 2017). Upon capturing bacteria, MOF crystallites were partially concealed, and less excitation energy reached them. This resulted in a decrease in the fluorescence signal, which was reversely related to the number of bacteria in the sample. The obtained LODs were 10^2 CFU/mL and 31 CFU/mL, respectively.

Farooq et al. developed a highly efficient sensing surface integrating bacterial cellulose and carboxylated multiwalled carbon nanotubes (c-MWCNTs) with immobilized phages targeting *S. aureus*. They achieved the LOD of 3–5 CFU/mL within 30 min, which is the best balance between LOD and time for now-on, and were able to distinguish living and dead bacterial cells (Farooq et al. 2020).

Particles were also used as carriers for active compounds to be detected. In the interesting example, $Cu_3(PO_4)_2$ nanoflowers were loaded with glucose oxidase, horseradish peroxidase, thionine, then gold nanoparticles were incorporated, and finally, T4 phages were attached to these gold nanoparticles. The detection process occurred at the surface of the electrode utilizing differential pulse voltammetry. First, bacteria were non-specifically immobilized at the surface. Loaded nanoflowers are bound only to bacteria selected by the used phages. In the vicinity of the electrode, the cascade of the electrochemical reactions involving electroactive compounds carried by nanoflowers occurred, resulting in signal generation. The LOD of this method was in the range of 1 CFU/mL and the time of analysis within 140 min (Li et al. 2018).

20.10.6 Parts of Virions as Sensing Elements

There are particular issues with whole-phage biosensors as sensing elements. First, the size of the virions marks the miniaturization limits. For instance, magnetophoretic separation requires the sub-micrometer size of virions conjugated with magnetic particles to maintain efficiency. The techniques such as surface plasmon resonance also need the binding to take place in the proper spacing from the transducer. Analytical signals can be hindered due to the size of phages that create a distance that is too long to be examined. Finally, lytic bacteriophages eventually destroy target bacterial cells, making prolonged analysis almost impossible. The first bacteria to be bounded may be lysed even before the end of the procedure.

These problems can be solved by preparing biosensors using only the bacteria-capturing parts of the virions. The recombinant tail fiber protein (P069) was used to detect *P. aeruginosa* in two approaches. The first one relied on the conjugation of P069 protein with the magnetic beads, then on adding them to the target bacteria in the sample. After the incubation and the magnetic separation, cells were washed and then lysed. Bacteria were detected indirectly, based on the amount of released ATP, recognized by using the bioluminescence protocol. In the second approach, the solid substrate was covered with the P069 fused with the fluorescent marker. The resulting

parameters of the analyses were the following: the LOD of 6.7×10^2 CFU/mL within 60 min for the bioluminescent methods and LOD of 1.7×10^2 CFU/mL within 80 min (He et al. 2018).

Another solution made the cell-binding domain (CBD) of P108 bacteriophage fused with GFP for recognition of MRSA strains (Wang et al. 2020). Target cells were separated by magnetic beads conjugated with CBD and incubated. The analysis was provided with flow cytometry protocol. The procedure provided the LOD of around 40 CFU/mL and a time of analysis of about 1 h. The efficiency of CBD-GFP protein was compared with GFP-CTP1L-bacteriophage endolysin targeting *Clostridium tyrobutyricum (*Gómez-Torres et al. 2018*)*. The GFP-CTP1L protein allowed for the recognition of 17 of 20 examined *Clostridium* strains. The suggested method also detects the clostridial spores in the sample.

Recently, Cunha et al. reported the development of a method as sensitive as magnetoresistive sensors, as portable as a lab-on-chip platform, and with the specificity comparable to the phage receptor-binding proteins. This method used protein *gp18*, a phage RBP, to detect *E. faecalis* I809 and protein *gp109* for detecting *S. aureus* Sa12. In the case of both examined bacterial strains, the detection limit was about 10 CFU/mL within less than 2 h (Cunha et al. 2021).

Braun et al. created an approach for detecting pathogens by developing a single-tube centrifugation assay that simplifies the analysis of suspect colonies. Two types of enzyme-linked phage RBP assay (ELPRA) were used to identify vegetative cells of *B. anthracis*. Counting from the moment of colony collection, this assay can be completed within less than 30 min. The assay for now-on is rather qualitative than quantitative—allows to distinguish if *B. anthracis* spores were present in the sample (Braun et al. 2021).

20.10.7 Phage Display Method

Another aspect of phage-based recognition is a method developed by George P. Smith in the 80s, known as *phage display* (Smith 1985). This method allows using phages as universal recognition elements (not only for bacteria detection) instead of antibodies. The usage of antibodies is relatively expensive because of their preparation, and very often, the specificity of these sensors is not satisfying enough. G. P. Smith was the first one to obtain phages displaying specific peptides on the surface (Burton 1995).

Filamentous phages (M13, f1, or fd) are usually used in phage display (Ebrahimizadeh and Rajabibazl 2014), with several examples of using icosahedral phages (e.g., T4 or T7). Several types of filamentous phage-based phage display can be distinguished. Their classification is based on the surface protein used, i.e., pIII or pVIII (Nemudraya et al. 2016). These proteins were chosen because of their location in the virion and presence in a couple of many copies. pIII protein is located in the distal part of the virion in the number of copies of 3–5, while pVIII is present in about 2700 copies and is a major protein building viral capsid. Also, both of these proteins have an N-terminal signal sequence, so a foreign peptide sequence can be

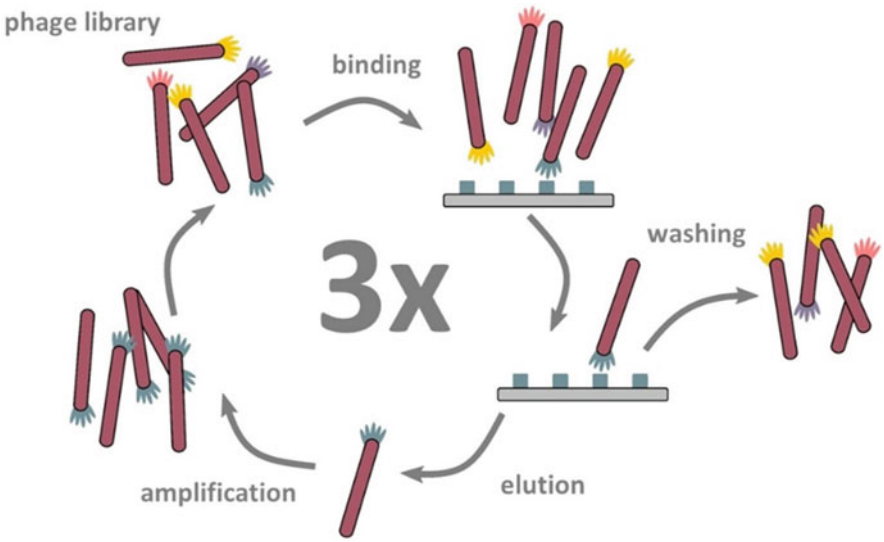

Fig. 20.2 The scheme presents the biopanning of phage display library. Adapted from the ref. (Paczesny and Bielec 2020) on the Creative Common CC BY 4.0 license

placed between the signal peptide and actual pIII/pVIII protein, forming the transcriptional fusion (Nemudraya et al. 2016).

The purpose of the phage display is to obtain a library of bacteriophages expressing various peptides. A library is defined as a heterogeneous mixture of phage clones carrying different genetic inserts (Burton 1995). First, a surface needs to be covered with objects to be detected. Then, bacteriophages with inserted sequences of random oligopeptides or proteins are incubated with their target. Once the incubation is over, unbound/unspecific virions are washed off. At the same time, bound phages are eluted and amplified (Nemudraya et al. 2016). Figure 20.2 presents this procedure schematically.

This approach made a pathway to the alternative for using antibodies, so-called phage antibodies—phages with a domain of chosen oligopeptide or protein displayed. Because obtaining phage displaying molecule is a routine procedure, within a short time, it is possible to distribute a library of virions expressing numerous types of antibodies, making the research quicker and cheaper (Petrenko and Vodyanoy 2003).

Phage display is also a solution for one of the greatest limitations of phage therapy—bacteria getting resistant to phage infections (Labrie et al. 2010). When treated with the same bacteriophage, bacteria strain would eventually become resistant to infections by this particular phage to improve fitness. A phage requires a virulence factor, which is a surface receptor, lipopolysaccharide, pili, or secretion system for a successful infection (Nobrega et al. 2018). Phage display provides the selection of virions able to infect phage-resistant bacteria. Moreover, the correlation between phage and antibiotic sensitivity was observed. This phenomenon involves

two strategies for fighting multidrug resistant bacteria strains—directly killing them by bacteriophages or using bacteriophages to make bacteria antibiotic-sensitive again (Kortright et al. 2019).

Once improved, phage display, formerly designed for molecular biology, became an attractive alternative approach for traditional blood morphology tests and in vivo blood analyses. One of the first came the antibodies targeting the antigens determining the blood types, which are anti-AB0 and anti-Rh antibodies (Marks et al. 1993). Antibodies against the cluster of differentiation (CD, AITP, GPIa, GPIII antigens, or 11-dehydro-thromboxane B2 cloning factor) were obtained. Another application is the diagnostic of immune diseases. In this field, the most important are antiTNFα or anti-CD52 antibodies, but also antibodies targeting some neurological disorders or tumors. Tumor targeting bacteriophage-based particles were already developed for various types of cancer diseased, e.g., B-cell lymphoma, colon, breast, or thyroid cancers (Bazan et al. 2012).

Also, modified bacteriophages were used as the nanocarriers for tumor-associated antigens (TAAs) or TAA-mimic molecules. The importance of this application is that, in general, tumors produce immunosuppressing factors that inhibit the immunological response. TAAs and TAA-mimic molecules are presented to immune system agents, first by exposure to the MHC (major histocompatibility complex), then through CD4+ and CD8+ T lymphocytes to B lymphocytes to induct cytotoxic response via production of TAA-specific antibodies (Goracci et al. 2020). TAA-displaying phages are sometimes considered anti-cancer vaccines (Fig. 20.3). It is also possible to modulate the activity of immune cells, CD11c/CD18 (integrin $\alpha_X\beta_2$) from the surface of APC (antigen-presenting cells) were fused with 12xhistidine tag and crosslinked to liposomes, creating the artificial tumor cells. By treating patients that way resulted in regression of primary cancer (Goracci et al. 2020).

20.10.8 Phage-Based Detection of Other Analytes

The detection capabilities of bacteriophages are not limited to just bacteria. They can be used to detect small molecules, such as ions (Yang et al. 2018) or organic compounds (Yoo et al. 2020). The report from Kim et al. (Kim et al. 2020) suggested the applications of bacteriophages to detect medical chemicals. The M13 phage variants were used: a wild-type virus and two displaying specific peptides changing the hydrophobic/hydrophilic balance of virions. A color pattern made an additional value for the determination of the response. This colorimetric method allowed the detection of endocrine-disrupting chemicals and some chemicals in a gaseous state (Yoo et al. 2020). Bacteriophages may also be used as indicators of pollution (Armon and Kott 1996). Many bacteriophages have the survival time related to the survival time of human viruses. Therefore they might be used to estimate the index of viral pollution in both air and water samples, e.g., to determine the drinking water quality.

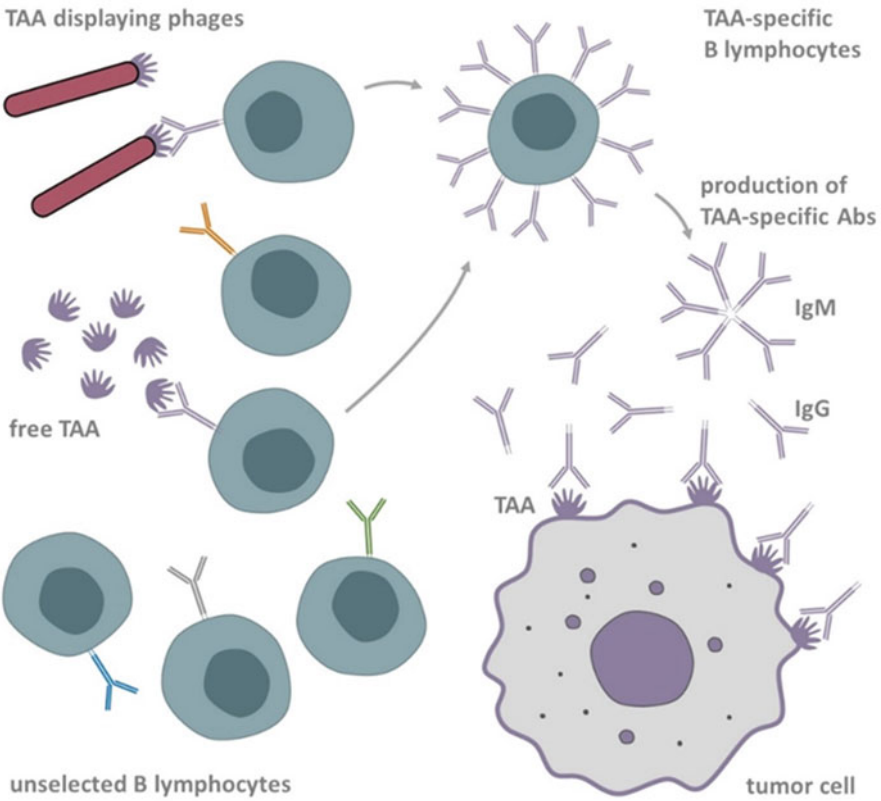

Fig. 20.3 Illustration of humoral response to tumor cells initiated by using tumor-associated antigens (TAA) -displaying bacteriophages. Phages displaying TAA are considered anti-cancer vaccines. The figure is inspired by (Goracci et al. 2020) on the Creative Common CC BY 4.0 license

20.11 Summary

Nowadays, there are a great many bacteria-sensing methods. The trick is to choose the one combining a short analysis time, high specificity, and detection limit as low as possible. Standard and the most often used detection methods require the isolation of the target bacteria, then culturing stage, usually followed by biochemical confirmation. They provide straightforward, cheap, and reliable protocols for bacteria identification. However, require trained operators, laboratory space, and up to a few days to obtain reliable results. Introducing new detection techniques to the standard diagnostics procedures may solve some of these problems. New molecular and instrumental techniques are advertised as extremely sensitive (e.g., PCR), label-free (e.g., SERS-based methods), or quick and easy to do (e.g., microscopic methods). But neither is universal, and each has some significant drawbacks,

limiting its applicability. Most of them generate costs because they require expensive and sophisticated equipment and skilled personnel. The risk of false-positive results caused by the presence of the dead bacterial cells accompanies the nucleic acid-based and MS methods. Moreover, the PCR protocol for detecting the target bacteria requires particular probes (primers). Some mutations would prevent the pairing of the probes to the target DNA sequence, causing the failure of the identification. The availability of specific antigens limits immune-based tests. Therefore, various types of biosensors are becoming a valid alternative for molecular methods (Chen et al. 2017a). Among them, bacteriophage-based methods seem one of the most promising.

The critical milestone to be achieved in bacteria detection is developing the method that works in complex samples while providing the LOD of about 10 CFU/mL or less and the time of the analysis of 1 h or less (Paczesny et al. 2020). This is because 10 CFU/mL in the blood is a mark of sepsis in neonates (Opota et al. 2015). Because this is a stage of severe sepsis that may rapidly transform into septic shock, the only one-hour analysis would be efficient before using the antibiotics (Alam et al. 2018). The following goal will be to reach the detection limit of below 1 CFU/100 mL within a one-shift period (around 8 h). This is crucial for online analysis of drinking water (Hinkley et al. 2020).

Phage-based method might provide such characteristics. There are already reports showing some important advancements. LOD of 1 CFU per 100 mL was already reported in 2003 (Neufeld et al. 2003). Protocol taking only 30 min and allowing for LOD of 3–5 CFU/mL was developed in 2020 (Farooq et al. 2020). However, the main challenge in front of researchers working in the field is to bring phage-based methods to the market. According to our best knowledge, the Sample6 DETECT HT System (Microbiologique) is the only commercially available product. Scientists need to focus also on other factors, including copiability, compactness, user-friendliness, acuteness, price, and movability. All these features are not necessary to produce scientific publications but are crucial from the application point of view to solve the socioeconomic problem of bacterial infections.

Acknowledgments The National Science Centre supported this work within the PRELUDIUM BIS grant according to decision number 2020/39/O/ST5/01017.

References

Ackermann H-W (2007) 5500 phages examined in the electron microscope. Arch Virol 152:227–243. https://doi.org/10.1007/s00705-006-0849-1

Ahmed A, Rushworth JV, Hirst NA, Millner PA (2014) Biosensors for whole-cell bacterial detection. Clin Microbiol Rev 27:631–646. https://doi.org/10.1128/CMR.00120-13

Alam N, Oskam E, Stassen PM et al (2018) Prehospital antibiotics in the ambulance for sepsis: a multicentre, open label, randomised trial. Lancet Respir Med 6:40–50. https://doi.org/10.1016/S2213-2600(17)30469-1

Alexandrakis D, Brunton NP, Downey G, Scannell AGM (2012) Identification of spoilage marker metabolites in Irish chicken breast muscle using HPLC, GC-MS coupled with SPME and

traditional chemical techniques. Food Bioprocess Technol 5:1917–1923. https://doi.org/10.1007/s11947-010-0500-8

Anany H, Brovko L, El Dougdoug NK et al (2018) Print to detect: a rapid and ultrasensitive phage-based dipstick assay for foodborne pathogens. Anal Bioanal Chem 410:1217–1230. https://doi.org/10.1007/s00216-017-0597-y

Armon R, Kott Y (1996) Bacteriophages as indicators of pollution. Crit Rev Environ Sci Technol 26:299–335. https://doi.org/10.1080/10643389609388494

Armstrong DW, Schulte G, Schneiderheinze JM, Westenberg DJ (1999) Separating microbes in the manner of molecules. 1. Capillary electrokinetic approaches. Anal Chem 71:5465–5469. https://doi.org/10.1021/ac990779z

Armstrong DW, Girod M, He L et al (2002) Mechanistic aspects in the generation of apparent ultrahigh efficiencies for colloidal (microbial) electrokinetic separations. Anal Chem 74:5523–5530. https://doi.org/10.1021/ac025726n

Arora P, Sindhu A, Dilbaghi N, Chaudhury A (2011) Biosensors as innovative tools for the detection of food borne pathogens. Biosens Bioelectron 28:1–12. https://doi.org/10.1016/j.bios.2011.06.002

Bárdy P, Pantůček R, Benešík M, Doškař J (2016) Genetically modified bacteriophages in applied microbiology. J Appl Microbiol 121:618–633. https://doi.org/10.1111/jam.13207

Bazan J, Całkosiński I, Gamian A (2012) Phage displaya powerful technique for immunotherapy: 1. Introduction and potential of therapeutic applications. Hum Vaccines Immunother 8:1817–1828. https://doi.org/10.4161/hv.21703

Beckmann BM, Grünweller A, Weber MHW, Hartmann RK (2010) Northern blot detection of endogenous small RNAs (~14 nt) in bacterial total RNA extracts. Nucleic Acids Res 38:2–11. https://doi.org/10.1093/nar/gkq437

Ben SM, Ben SM, Achouri F et al (2019) Detection of active pathogenic bacteria under stress conditions using lytic and specific phage. Water Sci Technol 80:282–289. https://doi.org/10.2166/wst.2019.271

Bhardwaj N, Bhardwaj SK, Mehta J et al (2016) Bacteriophage conjugated IRMOF-3 as a novel opto-sensor for: S. arlettae. New J Chem 40:8068–8073. https://doi.org/10.1039/c6nj00899b

Bhardwaj N, Bhardwaj SK, Mehta J et al (2017) MOF-bacteriophage biosensor for highly sensitive and specific detection of staphylococcus aureus. ACS Appl Mater Interfaces 9:33589–33598. https://doi.org/10.1021/acsami.7b07818

Bone U, Steller E, Warner JF, et al (2017) Chapter 14: Microsatellite analysis for identification of individuals, vol 1606, pp 205–217. https://doi.org/10.1007/978-1-4939-6990-6

Braun P, Rupprich N, Neif D, Grass G (2021) Enzyme-linked phage receptor binding protein assays (Elpra) enable identification of bacillus anthracis colonies. Viruses 13:1462. https://doi.org/10.3390/v13081462

Breaker RR, Banerji A, Joyce GF (1994) Continuous in vitro evolution of bacteriophage RNA polymerase promoters. Biochemistry 33:11980–11986. https://doi.org/10.1021/bi00205a037

Bremer E, Silhavy TJ, Weisemann JM, Weinstock GM (1984) λ placMu: a transposable derivative of bacteriophage lambda for creating lacZ protein fusions in a single step. J Bacteriol 158:1084–1093. https://doi.org/10.1128/jb.158.3.1084-1093.1984

Brindha J, Chanda K, Balamurali MM (2018) Biosensors for pathogen surveillance. Environ Chem Lett 16:1325–1337. https://doi.org/10.1007/s10311-018-0759-y

Burton DR (1995) Phage display. Immunotechnology 1:87–94. https://doi.org/10.1016/1380-2933(95)00013-5

Buszewski B, Rogowska A, Pomastowski P et al (2017) Identification of microorganisms by modern analytical techniques. J AOAC Int 100:1607–1623. https://doi.org/10.5740/jaoacint.17-0207

Buzby JC, Roberts T (2009) The economics of enteric infections: human foodborne disease costs. Gastroenterology 136:1851–1862. https://doi.org/10.1053/j.gastro.2009.01.074

Castañer O, Schröder H (2018) Response to: comment on "The gut microbiome profile in obesity: a systematic review". Int J Endocrinol 2018:9109451. https://doi.org/10.1155/2018/9109451

Cecchini F, Iacumin L, Fontanot M et al (2012) Identification of the unculturable bacteria Candidatus arthromitus in the intestinal content of trouts using dot blot and southern blot techniques. Vet Microbiol 156:389–394. https://doi.org/10.1016/j.vetmic.2011.11.020

Chen J, Andler SM, Goddard JM et al (2017a) Integrating recognition elements with nanomaterials for bacteria sensing. Chem Soc Rev 46:1272–1283. https://doi.org/10.1039/c6cs00313c

Chen I-H, Horikawa S, Bryant K et al (2017b) Bacterial assessment of phage magnetoelastic sensors for Salmonella enterica Typhimurium detection in chicken meat. Food Control 71: 273–278. https://doi.org/10.1016/j.foodcont.2016.07.003

Christian MD (2013) Biowarfare and bioterrorism. Crit Care Clin 29:717–756. https://doi.org/10.1016/j.ccc.2013.03.015

Cimiotti JP, Aiken LH, Sloane DM, Wu ES (2012) Nurse staffing, burnout, and health care-associated infection. Am J Infect Control 40:486–490. https://doi.org/10.1016/j.ajic.2012.02.029

Clausen CH, Dimaki M, Bertelsen CV et al (2018) Bacteria detection and differentiation using impedance flow cytometry. Sensors (Switzerland) 18:1–12. https://doi.org/10.3390/s18103496

Cross T, Schoff C, Chudoff D et al (2015) An optimized enrichment technique for the isolation of Arthrobacter bacteriophage species from soil sample isolates. J Vis Exp 2015:1–9. https://doi.org/10.3791/52781

Cunha AP, Henriques R, Cardoso S et al (2021) Rapid and multiplex detection of nosocomial pathogens on a phage-based magnetoresistive lab-on-chip platform. Biotechnol Bioeng 118: 3164–3174. https://doi.org/10.1002/bit.27841

de Jonge PA, Nobrega FL, Brouns SJJ, Dutilh BE (2019) Molecular and evolutionary determinants of bacteriophage host range. Trends Microbiol 27:51–63. https://doi.org/10.1016/j.tim.2018.08.006

Desai MJ, Armstrong DW (2003) Separation, identification, and characterization of microorganisms by capillary electrophoresis. Microbiol Mol Biol Rev 67:38–51. https://doi.org/10.1128/mmbr.67.1.38-51.2003

Dige I, Nilsson H, Kilian M, Nyvad B (2007) In situ identification of streptococci and other bacteria in initial dental biofilm by confocal laser scanning microscopy and fluorescence in situ hybridization. Eur J Oral Sci 115:459–467. https://doi.org/10.1111/j.1600-0722.2007.00494.x

Doern CD, Butler-Wu SM (2016) Emerging and future applications of matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry in the clinical microbiology laboratory: a report of the Association for Molecular Pathology. J Mol Diagnostics 18: 789–802. https://doi.org/10.1016/j.jmoldx.2016.07.007

Ebrahimizadeh W, Rajabibazl M (2014) Bacteriophage vehicles for phage display: biology, mechanism, and application, pp 109–120. https://doi.org/10.1007/s00284-014-0557-0

(2009) ECDC/EMEA joint technical report "The bacterial challenge: time to react"

Elsland D, Neefjes J (2018) Bacterial infections and cancer. EMBO Rep 19:1–11. https://doi.org/10.15252/embr.201846632

Farooq U, Ullah MW, Yang Q et al (2020) High-density phage particles immobilization in surface-modified bacterial cellulose for ultra-sensitive and selective electrochemical detection of Staphylococcus aureus. Biosens Bioelectron 157:112163. https://doi.org/10.1016/j.bios.2020.112163

Faure G, Shmakov SA, Yan WX et al (2019) CRISPR–Cas in mobile genetic elements: counter-defence and beyond. Nat Rev Microbiol 17:513–525. https://doi.org/10.1038/s41579-019-0204-7

Ferapontova EE (2020) Electrochemical assays for microbial analysis: how far they are from solving microbiota and microbiome challenges. Curr Opin Electrochem 19:153–161. https://doi.org/10.1016/j.coelec.2019.12.005

Ferone M, Gowen A, Fanning S, Scannell AGM (2020) Microbial detection and identification methods: bench top assays to omics approaches. Compr Rev Food Sci Food Saf 19:3106–3129. https://doi.org/10.1111/1541-4337.12618

Fox A (2006) Mass spectrometry for species or strain identification after culture or without culture: past, present, and future. J Clin Microbiol 44:2677–2680. https://doi.org/10.1128/JCM.00971-06

Franche N, Vinay M, Ansaldi M (2017) Substrate-independent luminescent phage-based biosensor to specifically detect enteric bacteria such as E. coli. Environ Sci Pollut Res 24:42–51. https://doi.org/10.1007/s11356-016-6288-y

Franco D, De Plano LM, Rizzo MG et al (2020) Bio-hybrid gold nanoparticles as SERS probe for rapid bacteria cell identification. Spectrochim Acta Part A Mol Biomol Spectrosc 224:117394. https://doi.org/10.1016/j.saa.2019.117394

Franco-Duarte R, Černáková L, Kadam S et al (2019) Advances in chemical and biological methods to identify microorganisms—from past to present. Microorganisms 7:130. https://doi.org/10.3390/microorganisms7050130

Frickmann H, Zautner AE, Moter A et al (2017) Fluorescence in situ hybridization (FISH) in the microbiological diagnostic routine laboratory: a review. Crit Rev Microbiol 43:263–293. https://doi.org/10.3109/1040841X.2016.1169990

Galluzzi L, Magnani M, Saunders N et al (2007) Current molecular techniques for the detection of microbial pathogens. Sci Prog 90:29–50. https://doi.org/10.3184/003685007780440521

Garrido-Maestu A, Fuciños P, Azinheiro S et al (2019) Specific detection of viable Salmonella Enteritidis by phage amplification combined with qPCR (PAA-qPCR) in spiked chicken meat samples. Food Control 99:79–83. https://doi.org/10.1016/j.foodcont.2018.12.038

Global Hospital-Acquired Infections (HAI) market size to grow at a CAGR of 3.6% 2021 to 2030 (2021) https://www.globenewswire.com/news-release/2021/07/12/2261025/0/en/Global-Hospital-acquired-Infections-HAI-Market-Sizeto-Grow-at-a-CAGR-of-3-6-2021-to-2030.html

Gómez-Torres N, Dunne M, Garde S et al (2018) Development of a specific fluorescent phage endolysin for in situ detection of Clostridium species associated with cheese spoilage. Microb Biotechnol 11:332–345. https://doi.org/10.1111/1751-7915.12883

Goodridge L, Griffiths M (2002) Reporter bacteriophage assays as a means to detect foodborne pathogenic bacteria. Food Res Int 35:863–870. https://doi.org/10.1016/S0963-9969(02)00094-7

Goracci M, Pignochino Y, Marchiò S (2020) Phage display-based nanotechnology applications in cancer immunotherapy. Molecules 25:1–28. https://doi.org/10.3390/molecules25040843

Gothandam KM (2018) Food security and water treatment

Halkare P, Punjabi N, Wangchuk J et al (2021) Label-free detection of Escherichia coli from mixed bacterial cultures using bacteriophage T4 on Plasmonic fiber-optic sensor. ACS Sensors 6:2720–2727. https://doi.org/10.1021/acssensors.1c00801

Harada LK, Silva EC, Campos WF et al (2018) Biotechnological applications of bacteriophages: state of the art. Microbiol Res 212–213:38–58. https://doi.org/10.1016/j.micres.2018.04.007

Harding E (2020) WHO global progress report on tuberculosis elimination. Lancet Respir Med 8:19. https://doi.org/10.1016/S2213-2600(19)30418-7

He Y, Wang M, Fan E et al (2017) Highly specific bacteriophage-affinity strategy for rapid separation and sensitive detection of viable Pseudomonas aeruginosa. Anal Chem 89:1916–1921. https://doi.org/10.1021/acs.analchem.6b04389

He Y, Shi Y, Liu M et al (2018) Nonlytic recombinant phage tail fiber protein for specific recognition of Pseudomonas aeruginosa. Anal Chem 90:14462–14468. https://doi.org/10.1021/acs.analchem.8b04160

Hinkley TC, Singh S, Garing S et al (2018a) A phage-based assay for the rapid, quantitative, and single CFU visualization of E. coli (ECOR #13) in drinking water. Sci Rep 8:1–8. https://doi.org/10.1038/s41598-018-33097-4

Hinkley TC, Garing S, Singh S et al (2018b) Reporter bacteriophage T7NLC utilizes a novel NanoLuc::CBM fusion for the ultrasensitive detection of: Escherichia coli in water. Analyst 143:4074–4082. https://doi.org/10.1039/c8an00781k

Hinkley TC, Garing S, Jain P et al (2020) A syringe-based biosensor to rapidly detect low levels of Escherichia Coli (ECOR13) in drinking water using engineered bacteriophages. Sensors 20: 1953. https://doi.org/10.3390/s20071953

Hiremath N, Guntupalli R, Vodyanoy V et al (2015) Detection of methicillin-resistant Staphylococcus aureus using novel lytic phage-based magnetoelastic biosensors. Sensors Actuators B Chem 210:129–136. https://doi.org/10.1016/j.snb.2014.12.083

Hiremath N, Chin BA, Park MK (2017) Effect of competing foodborne pathogens on the selectivity and binding kinetics of a lytic phage for methicillin-resistant Staphylococcus aureus detection. J Electrochem Soc 164:B142–B146. https://doi.org/10.1149/2.0901704jes

Holčapek M, Jirásko R, Lísa M (2012) Recent developments in liquid chromatography-mass spectrometry and related techniques. J Chromatogr A 1259:3–15. https://doi.org/10.1016/j.chroma.2012.08.072

Huang L, Sun DW, Wu Z et al (2021) Reproducible, shelf-stable, and bioaffinity SERS nanotags inspired by multivariate polyphenolic chemistry for bacterial identification. Anal Chim Acta 1167:338570. https://doi.org/10.1016/j.aca.2021.338570

Imai M, Mine K, Tomonari H et al (2019) Dark-field microscopic detection of bacteria using bacteriophage-immobilized SiO2@AuNP Core-Shell nanoparticles. Anal Chem 91:12352–12357. https://doi.org/10.1021/acs.analchem.9b02715

Irwin P, Gehring A, Tu S-I et al (2000) Minimum detectable level of salmonellae using a binomial - based bacterial ice nucleation detection assay (BIND ®). J AOAC Int 83:1087–1095

Jaffee S, Henson S, Unnevehr L, Grace D, Emilie Cassou (2019) The safe food imperative: accelerating progress in low- and middle-income countries. World Bank Group, Washington, DC

Janczuk M, Richter Ł, Hoser G et al (2017) Bacteriophage-based bioconjugates as a flow cytometry probe for fast bacteria detection. Bioconjug Chem 28:419–425. https://doi.org/10.1021/acs.bioconjchem.6b00596

Janczuk-Richter M, Marinović I, Niedziółka-Jönsson J, Szot-Karpińska K (2019) Recent applications of bacteriophage-based electrodes: a mini-review. Electrochem Commun 99:11–15. https://doi.org/10.1016/j.elecom.2018.12.011

Janda JM, Abbott SL (2002) Bacterial identification for publication: when is enough enough? J Clin Microbiol 40:1887–1891. https://doi.org/10.1128/JCM.40.6.1887-1891.2002

Jang KS, Kim YH (2018) Rapid and robust MALDI-TOF MS techniques for microbial identification: a brief overview of their diverse applications. J Microbiol 56:209–216. https://doi.org/10.1007/s12275-018-7457-0

Jassim SAA, Griffiths MW (2007) Evaluation of a rapid microbial detection method via phage lytic amplification assay coupled with live/dead fluorochromic stains. Lett Appl Microbiol 44:673–678. https://doi.org/10.1111/j.1472-765X.2007.02115.x

Jit M, Ng DHL, Luangasanatip N et al (2020) Quantifying the economic cost of antibiotic resistance and the impact of related interventions: rapid methodological review, conceptual framework and recommendations for future studies. BMC Med 18:38. https://doi.org/10.1186/s12916-020-1507-2

Jorgensen JH, Ferraro MJ (2009) Antimicrobial susceptibility testing: a review of general principles and contemporary practices. Clin Infect Dis 49:1749–1755. https://doi.org/10.1086/647952

Justé A, Thomma BPHJ, Lievens B (2008) Recent advances in molecular techniques to study microbial communities in food-associated matrices and processes. Food Microbiol 25:745–761. https://doi.org/10.1016/j.fm.2008.04.009

Kaufmann AF, Meltzer MI, Schmid GP (1997) The economic impact of a bioterrorist attack: are prevention and postattack intervention programs justifiable? Emerg Infect Dis 3:83–94. https://doi.org/10.3201/eid0302.970201

Kim J, Kim M, Kim S, Ryu S (2017) Sensitive detection of viable Escherichia coli O157:H7 from foods using a luciferase-reporter phage phiV10lux. Int J Food Microbiol 254:11–17. https://doi.org/10.1016/j.ijfoodmicro.2017.05.002

Kim C, Lee H, Devaraj V et al (2020) Hierarchical cluster analysis of medical chemicals detected by a bacteriophage-based colorimetric sensor array. Nanomaterials 10:121. https://doi.org/10.3390/nano10010121

King LA, Nogareda F, Weill FX et al (2012) Outbreak of Shiga toxin-producing Escherichia coli O104:H4 associated with organic fenugreek sprouts, France, June 2011. Clin Infect Dis 54:1588–1594. https://doi.org/10.1093/cid/cis255

Kneipp K, Kneipp H (2006) Single molecule Raman scattering. Appl Spectrosc 60:322A–334A. https://doi.org/10.1366/000370206779321418

Kortright KE, Chan BK, Koff JL, Turner PE (2019) Phage therapy: a renewed approach to combat antibiotic-resistant bacteria. Cell Host Microbe 25:219–232. https://doi.org/10.1016/j.chom.2019.01.014

Kosa G, Shapaval V, Kohler A, Zimmermann B (2017) FTIR spectroscopy as a unified method for simultaneous analysis of intra- and extracellular metabolites in high-throughput screening of microbial bioprocesses. Microb Cell Fact 16:1–11. https://doi.org/10.1186/s12934-017-0817-3

Koskella B, Meaden S (2013) Understanding bacteriophage specificity in natural microbial communities. Viruses 5:806–823. https://doi.org/10.3390/v5030806

Krueger AP (1931) The sorption of bacteriophage by living and dead susceptible bacteria. J Gen Physiol 14:493–516. https://doi.org/10.1085/jgp.14.4.493

Labrie SJ, Samson JE, Moineau S (2010) Bacteriophage resistance mechanisms. Nat Rev Microbiol 8:317–327. https://doi.org/10.1038/nrmicro2315

Lai A, Almaviva S, Spizzichino V et al (2017) Bacillus spp. cells captured selectively by phages and identified by surface enhanced Raman spectroscopy technique. Proceedings 1:519. https://doi.org/10.3390/proceedings1040519

Lankes HA, Zanghi CN, Santos K et al (2007) In vivo gene delivery and expression by bacteriophage lambda vectors. J Appl Microbiol 102:1337–1349. https://doi.org/10.1111/j.1365-2672.2006.03182.x

Law JWF, Mutalib NSA, Chan KG, Lee LH (2014) Rapid methods for the detection of foodborne bacterial pathogens: principles, applications, advantages and limitations. Front Microbiol 5:1–19. https://doi.org/10.3389/fmicb.2014.00770

Li ZP, Duan XR, Liu CH, Du BA (2006) Selective determination of cysteine by resonance light scattering technique based on self-assembly of gold nanoparticles. Anal Biochem 351:18–25. https://doi.org/10.1016/j.ab.2006.01.038

Li YY, Xie G, Qiu J et al (2018) A new biosensor based on the recognition of phages and the signal amplification of organic-inorganic hybrid nanoflowers for discriminating and quantitating live pathogenic bacteria in urine. Sensors Actuators B Chem 258:803–812. https://doi.org/10.1016/j.snb.2017.11.155

Loessner MJ, Rees CED, Stewart GSAB, Scherer S (1996) Construction of luciferase reporter bacteriophage A511::luxAB for rapid and sensitive detection of viable listeria cells. Appl Environ Microbiol 62:1133–1140. https://doi.org/10.1128/aem.62.4.1133-1140.1996

Luo J, Jiang M, Xiong J et al (2018) Exploring a phage-based real-time PCR assay for diagnosing Acinetobacter baumannii bloodstream infections with high sensitivity. Anal Chim Acta 1044:147–153. https://doi.org/10.1016/j.aca.2018.09.038

Luo J, Jiang M, Hiong J et al (2020) Rapid ultrasensitive diagnosis of pneumonia caused by Acinetobacter baumannii using a combination of enrichment and phage-based qPCR assay, pp 1–25. https://doi.org/10.21203/rs.3.rs-16845/v1

Mack JD, Yehualaeshet T, Park MK et al (2017) Phage-based biosensor and optimization of surface blocking agents to detect Salmonella Typhimurium on Romaine lettuce. J Food Saf 37:e12299. https://doi.org/10.1111/jfs.12299

Mahmood T, Yang PC (2012) Western blot: technique, theory, and trouble shooting. N Am J Med Sci 4:429–434. https://doi.org/10.4103/1947-2714.100998

Marcadet A, O'Connell P, Cohen D (1989) Standardized Southern blot workshop technique. Immunobiol HLA I:553–560. https://doi.org/10.1007/978-1-4612-3552-1_116

Mariey L, Signolle JP, Amiel C, Travert J (2001) Discrimination, classification, identification of microorganisms using FTIR spectroscopy and chemometrics. Vib Spectrosc 26:151–159. https://doi.org/10.1016/S0924-2031(01)00113-8

Marks JD, Ouwehand WH, Bye JM et al (1993) Human antibody fragments specific for human blood group antigens from a phage display library. Biotechnology 11:1145–1149. https://doi.org/10.1038/nbt1093-1145

Melendez JH, Frankel YM, An AT et al (2010) Real-time PCR assays compared to culture-based approaches for identification of aerobic bacteria in chronic wounds. Clin Microbiol Infect 16: 1762–1769. https://doi.org/10.1111/j.1469-0691.2010.03158.x

Mido T, Schaffer EM, Dorsey RW et al (2018) Sensitive detection of live Escherichia coli by bacteriophage amplification-coupled immunoassay on the Luminex® MAGPIX instrument. J Microbiol Methods 152:143–147. https://doi.org/10.1016/j.mimet.2018.07.022

Miller MB, Tang YW (2009) Basic concepts of microarrays and potential applications in clinical microbiology. Clin Microbiol Rev 22:611–633. https://doi.org/10.1128/CMR.00019-09

Miller-Petrie M, Pant S, Laxminarayan R (2017) Drug-resistant infections. Disease control priorities. In: Major infectious diseases, vol 6, 3rd edn, pp 433–448. https://doi.org/10.1596/978-1-4648-0524-0_ch18

Moon JS, Choi EJ, Jeong NN et al (2019) Research progress of M13 bacteriophage-based biosensors. Nanomaterials 9:1448. https://doi.org/10.3390/nano9101448

Moshirabadi A, Razi M, Arasteh P et al (2019) Polymerase chain reaction assay using the restriction fragment length polymorphism technique in the detection of prosthetic joint infections: a multi-centered study. J Arthroplasty 34:359–364. https://doi.org/10.1016/j.arth.2018.10.017

Neethirajan S, Ragavan V, Weng X, Chand R (2018) Biosensors for sustainable food engineering: challenges and perspectives. Biosensors 8:23. https://doi.org/10.3390/bios8010023

Nemudraya AA, Richter VA, Kuligina EV (2016) Phage peptide libraries as a source of targeted ligands. Acta Nat 8:48–57. https://doi.org/10.32607/20758251-2016-8-1-48-57

Neufeld T, Schwartz-Mittelmann A, Biran D et al (2003) Combined phage typing and amperometric detection of released enzymatic activity for the specific identification and quantification of bacteria. Anal Chem 75:580–585. https://doi.org/10.1021/ac026083e

Neufeld T, Mittelman AS, Buchner V, Rishpon J (2005) Electrochemical phagemid assay for the specific detection of bacteria using Escherichia coli TG-1 and the M13KO7 phagemid in a model system. Anal Chem 77:652–657. https://doi.org/10.1021/ac0488053

Niyomdecha S, Limbut W, Numnuam A et al (2018) Phage-based capacitive biosensor for Salmonella detection. Talanta 188:658–664. https://doi.org/10.1016/j.talanta.2018.06.033

Nobrega FL, Vlot M, de Jonge PA et al (2018) Targeting mechanisms of tailed bacteriophages. Nat Rev Microbiol 16:760–773. https://doi.org/10.1038/s41579-018-0070-8

Opota O, Jaton K, Greub G (2015) Microbial diagnosis of bloodstream infection: towards molecular diagnosis directly from blood. Clin Microbiol Infect 21:323–331. https://doi.org/10.1016/j.cmi.2015.02.005

Paczesny J, Bielec K (2020) Application of bacteriophages in nanotechnology. Nanomaterials 10: 1–25. https://doi.org/10.3390/nano10101944

Paczesny J, Richter Ł, Hołyst R (2020) Recent progress in the detection of bacteria using bacteriophages: a review. Viruses 12:845. https://doi.org/10.3390/v12080845

Peng H, Chen IA (2019) Rapid colorimetric detection of bacterial species through the capture of gold nanoparticles by chimeric phages. ACS Nano 13:1244–1252. https://doi.org/10.1021/acsnano.8b06395

Petrenko VA, Vodyanoy VJ (2003) Phage display for detection of biological threat agents. J Microbiol Methods 53:253–262. https://doi.org/10.1016/S0167-7012(03)00029-0

Petti CA (2007) Detection and identification of microorganisms by gene amplification and sequencing. Clin Infect Dis 44:1108–1114. https://doi.org/10.1086/512818

Pires DP, Cleto S, Sillankorva S et al (2016) Genetically engineered phages: a review of advances over the last decade. Microbiol Mol Biol Rev 80:523–543. https://doi.org/10.1128/mmbr.00069-15

Pizarro-Bauerle J, Ando H (2020) Engineered bacteriophages for practical applications. Biol Pharm Bull 43:240–249. https://doi.org/10.1248/bpb.b19-00914

Poul MA, Marks JD (1999) Targeted gene delivery to mammalian cells by filamentous bacteriophage. J Mol Biol 288:203–211. https://doi.org/10.1006/jmbi.1999.2678

Pulkkinen EM, Hinkley TC, Nugen SR (2019) Utilizing in vitro DNA assembly to engineer a synthetic T7 Nanoluc reporter phage for Escherichia coli detection. Integr Biol 11:63–68. https://doi.org/10.1093/intbio/zyz005

Punina NV, Makridakis NM, Remnev MA, Topunov AF (2015) Whole-genome sequencing targets drug-resistant bacterial infections. Hum Genomics 9:19. https://doi.org/10.1186/s40246-015-0037-z

Richter Ł, Matuła K, Leśniewski A et al (2016) Ordering of bacteriophages in the electric field: application for bacteria detection. Sensors Actuators B Chem 224:233–240. https://doi.org/10.1016/j.snb.2015.09.042

Richter Ł, Bielec K, Leśniewski A et al (2017) Dense layer of bacteriophages ordered in alternating electric field and immobilized by surface chemical modification as sensing element for bacteria detection. ACS Appl Mater Interfaces 9:19622–19629. https://doi.org/10.1021/acsami.7b03497

Richter Ł, Janczuk-Richter M, Niedziółka-Jönsson J et al (2018) Recent advances in bacteriophage-based methods for bacteria detection. Drug Discov Today 23:448–455. https://doi.org/10.1016/j.drudis.2017.11.007

Rippa M, Castagna R, Pannico M et al (2017) Octupolar metastructures for a highly sensitive, rapid, and reproducible phage-based detection of bacterial pathogens by surface-enhanced Raman scattering. ACS Sensors 2:947–954. https://doi.org/10.1021/acssensors.7b00195

Rippa M, Castagna R, Zhou J et al (2018) Dodecagonal plasmonic quasicrystals for phage-based biosensing. Nanotechnology 29:405501. https://doi.org/10.1088/1361-6528/aad2f5

Santos C, Fraga ME, Kozakiewicz Z, Lima N (2010) Fourier transform infrared as a powerful technique for the identification and characterization of filamentous fungi and yeasts. Res Microbiol 161:168–175. https://doi.org/10.1016/j.resmic.2009.12.007

Scallan E, Hoekstra RM, Angulo FJ et al (2011) Foodborne illness acquired in the United States-major pathogens. Emerg Infect Dis 17:7–15. https://doi.org/10.3201/eid1701.P11101

Sedki M, Chen X, Chen C et al (2020) Non-lytic M13 phage-based highly sensitive impedimetric cytosensor for detection of coliforms. Biosens Bioelectron 148:111794. https://doi.org/10.1016/j.bios.2019.111794

Sergueev KV, Filippov AA, Nikolich MP (2017) Highly sensitive bacteriophage-based detection of Brucella abortus in mixed culture and spiked blood. Viruses 9:144. https://doi.org/10.3390/v9060144

Sharma S, Chatterjee S, Datta S et al (2017) Bacteriophages and its applications: an overview. Folia Microbiol (Praha) 62:17–55. https://doi.org/10.1007/s12223-016-0471-x

Smith GP (1985) Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface. Science 228:1315–1317

Soga T, Ueno Y, Naraoka H et al (2002) Simultaneous determination of anionic intermediates for Bacillus subtilis metabolic pathways by capillary electrophoresis electrospray ionization mass spectrometry. Anal Chem 74:2233–2239. https://doi.org/10.1021/ac020064n

Srivastava SK, Ben HH, Kushmaro A et al (2015) Highly sensitive and specific detection of E. coli by a SERS nanobiosensor chip utilizing metallic nanosculptured thin films. Analyst 140:3201–3209. https://doi.org/10.1039/c5an00209e

Streit S, Michalski CW, Erkan M et al (2009) Northern blot analysis for detection and quantification of RNA in pancreatic cancer cells and tissues. Nat Protoc 4:37–43. https://doi.org/10.1038/nprot.2008.216

Tagini F, Greub G (2017) Bacterial genome sequencing in clinical microbiology: a pathogen-oriented review. Eur J Clin Microbiol Infect Dis 36:2007–2020. https://doi.org/10.1007/s10096-017-3024-6

Teutenberg T (2009) Potential of high temperature liquid chromatography for the improvement of separation efficiency—a review. Anal Chim Acta 643:1–12. https://doi.org/10.1016/j.aca.2009.04.008

Tian F, Li J, Nazir A, Tong Y (2021) Bacteriophage—a promising alternative measure for bacterial biofilm control. Infect Drug Resist 14:205–217. https://doi.org/10.2147/IDR.S290093

Tilton L, Das G, Yang X et al (2019) Nanophotonic device in combination with bacteriophages for enhancing detection sensitivity of Escherichia coli in simulated wash water. Anal Lett 52:2203–2213. https://doi.org/10.1080/00032719.2019.1604726

Touat M, Opatowski M, Brun-Buisson C et al (2019) A payer perspective of the hospital inpatient additional care costs of antimicrobial resistance in France: a matched case–control study. Appl Health Econ Health Policy 17:381–389. https://doi.org/10.1007/s40258-018-0451-1

Trasanidou D, Gerós AS, Mohanraju P et al (2019) Keeping crispr in check: diverse mechanisms of phage-encoded anti-crisprs. FEMS Microbiol Lett 366:1–14. https://doi.org/10.1093/femsle/fnz098

Ulitzur N, Ulitzur S (2006) New rapid and simple methods for detection of bacteria and determination of their antibiotic susceptibility by using phage mutants. Appl Environ Microbiol 72:7455–7459. https://doi.org/10.1128/AEM.00761-06

US Department of Health and Human Services, CDC (2019) Antibiotic resistance threats in the United States. Centers Dis Control Prevention, pp 1–113

Váradi L, Luo JL, Hibbs DE et al (2017) Methods for the detection and identification of pathogenic bacteria: past, present, and future. Chem Soc Rev 46:4818–4832. https://doi.org/10.1039/c6cs00693k

Velusamy V, Arshak K, Korostynska O et al (2010) An overview of foodborne pathogen detection: in the perspective of biosensors. Biotechnol Adv 28:232–254. https://doi.org/10.1016/j.biotechadv.2009.12.004

Vinay M, Franche N, Grégori G et al (2015) Phage-based fluorescent biosensor prototypes to specifically detect enteric bacteria such as E. coli and Salmonella enterica Typhimurium. PLoS One 10:1–17. https://doi.org/10.1371/journal.pone.0131466

Wan MLY, Forsythe SJ, El-Nezami H (2019) Probiotics interaction with foodborne pathogens: a potential alternative to antibiotics and future challenges. Crit Rev Food Sci Nutr 59:3320–3333. https://doi.org/10.1080/10408398.2018.1490885

Wang D, Chen J, Nugen SR (2017) Electrochemical detection of Escherichia coli from aqueous samples using engineered phages. Anal Chem 89:1650–1657. https://doi.org/10.1021/acs.analchem.6b03752

Wang Y, He Y, Bhattacharyya S et al (2020) Recombinant bacteriophage cell-binding domain proteins for broad-Spectrum recognition of methicillin-resistant Staphylococcus aureus strains. Anal Chem 92:3340–3345. https://doi.org/10.1021/acs.analchem.9b05295

Warren CR (2018) A liquid chromatography–mass spectrometry method for analysis of intact fatty-acid-based lipids extracted from soil. Eur J Soil Sci 69:791–803. https://doi.org/10.1111/ejss.12689

Wei C, Li M, Zhao X (2018) Surface-enhanced Raman scattering (SERS) with silver nano substrates synthesized by microwave for rapid detection of foodborne pathogens. Front Microbiol 9:2857. https://doi.org/10.3389/fmicb.2018.02857

Weiner-Lastinger LM, Pattabiraman V, Konnor RY et al (2022) The impact of coronavirus disease 2019 (COVID-19) on healthcare-associated infections in 2020: a summary of data reported to the National Healthcare Safety Network. Infect Control Hosp Epidemiol 43(1):12–25. https://doi.org/10.1017/ice.2021.362

Wisuthiphaet N, Yang X, Young GM, Nitin N (2019) Rapid detection of Escherichia coli in beverages using genetically engineered bacteriophage T7. AMB Express 9:55. https://doi.org/10.1186/s13568-019-0776-7

Wisuthiphaet N, Yang X, Young GM, Nitin N (2021) Application of engineered bacteriophage T7 in the detection of bacteria in food matrices. Front Microbiol 12:691003. https://doi.org/10.3389/fmicb.2021.691003

Witkowska E, Korsak D, Kowalska A et al (2017) Surface-enhanced Raman spectroscopy introduced into the International Standard Organization (ISO) regulations as an alternative method for detection and identification of pathogens in the food industry. Anal Bioanal Chem 409:1555–1567. https://doi.org/10.1007/s00216-016-0090-z

Witkowska E, Korsak D, Kowalska A et al (2018) Strain-level typing and identification of bacteria—a novel approach for SERS active plasmonic nanostructures. Anal Bioanal Chem 410:5019–5031. https://doi.org/10.1007/s00216-018-1153-0

World Health Organization (2011) Report on the burden of endemic health care-associated infection worldwide. In: WHO Libr Cat. Data

Wu L, Song Y, Luan T et al (2016) Specific detection of live Escherichia coli O157: H7 using tetracysteine-tagged PP01 bacteriophage. Biosens Bioelectron 86:102–108. https://doi.org/10.1016/j.bios.2016.06.041

Wu L, Hong X, Luan T et al (2021) Multiplexed detection of bacterial pathogens based on a cocktail of dual-modified phages. Anal Chim Acta 1166:338596. https://doi.org/10.1016/j.aca.2021.338596

Xu J, Chau Y, Lee YK (2019) Phage-based electrochemical sensors: a review. Micromachines 10:855. https://doi.org/10.3390/mi10120855

Xu J, Zhao C, Chau Y, Lee YK (2020) The synergy of chemical immobilization and electrical orientation of T4 bacteriophage on a micro electrochemical sensor for low-level viable bacteria detection via differential pulse voltammetry. Biosens Bioelectron 151:111914. https://doi.org/10.1016/j.bios.2019.111914

Yan C, Zhang Y, Yang H et al (2017) Combining phagomagnetic separation with immunoassay for specific, fast and sensitive detection of Staphylococcus aureus. Talanta 170:291–297. https://doi.org/10.1016/j.talanta.2017.04.007

Yang T, Zhang XX, Yang JY et al (2018) Screening arsenic(III)-binding peptide for colorimetric detection of arsenic(III) based on the peptide induced aggregation of gold nanoparticles. Talanta 177:212–216. https://doi.org/10.1016/j.talanta.2017.07.005

Yoo YJ, Kim WG, Ko JH et al (2020) Large-area virus coated ultrathin colorimetric sensors with a highly Lossy resonant promoter for enhanced chromaticity. Adv Sci 7:2000978. https://doi.org/10.1002/advs.202000978

Yue H, He Y, Fan E et al (2017) Label-free electrochemiluminescent biosensor for rapid and sensitive detection of pseudomonas aeruginosa using phage as highly specific recognition agent. Biosens Bioelectron 94:429–432. https://doi.org/10.1016/j.bios.2017.03.033

Yuen LKW, Ross BC, Jackson KM, Dwyer B (1993) Characterization of Mycobacterium tuberculosis strains from Vietnamese patients by Southern blot hybridization. J Clin Microbiol 31:1615–1618. https://doi.org/10.1128/jcm.31.6.1615-1618.1993

Zhang JI, Talaty N, Costa AB et al (2011) Rapid direct lipid profiling of bacteria using desorption electrospray ionization mass spectrometry. Int J Mass Spectrom 301:37–44. https://doi.org/10.1016/j.ijms.2010.06.014

Zhou Y, Marar A, Kner P, Ramasamy RP (2017) Charge-directed immobilization of bacteriophage on nanostructured electrode for whole-cell electrochemical biosensors. Anal Chem 89:5734–5741. https://doi.org/10.1021/acs.analchem.6b03751

Zourob M (2010) Recognition receptors in biosensors. Springer, New York

Nanobiosensors: A Promising Tool for the Determination of Pathogenic Bacteria

21

Ananya S. Agnihotri, Ann Maria Chungath George, and Nidhin Marimuthu

Abstract

Pathogenic bacterial detection is a significant concern for the well-being of all human beings. These tiny microbes are capable of causing numerous diseases, which can be nipped in the bud through proper monitoring and controlling at the early stages itself. Some common pathogenic bacteria include *Mycobacterium tuberculosis, Bacillus anthracis, Streptococcus pneumoniae, Escherichia coli, Salmonella* spp., etc. These microbes contaminate air, food, and water through different modes of transmission. The classical methods used for the identification of these bacteria are time-killing and backbreaking. Rapid pathogenic bacteria determination became possible through the intervention of biosensors. Biosensors are further modified with nanoparticles to build nanobiosensors that are tenfold efficient in bacterial detection. The optical and electrochemical nanobiosensors provide hassle-free detection of pathogenic bacteria, and point-of-care detection is also possible. This book chapter aims to give a brief idea about nanobiosensors starting from the principle to the advantages and disadvantages of bacterial detection. Relevant works of literature on different methods to detect bacteria, types of nanobiosensors, and their efficacy in pathogenic bacterial detection portray the current stand and the need for more innovations in the area of nanobiosensors.

Keywords

Pathogenic bacterial detection · Nanobiosensors · Point-of-care detection · Optical nano biosensing · Electrochemical nanobiosensors · Limit of detection · Linear range

A. S. Agnihotri · A. M. Chungath George · N. Marimuthu (✉)
Department of Chemistry, CHRIST (Deemed to be University), Bengaluru, India
e-mail: nidhin.m@christuniversity.in

S. Hameed, S. Rehman (eds.), *Nanotechnology for Infectious Diseases*,
https://doi.org/10.1007/978-981-16-9190-4_21

21.1 Introduction

Humans are haunted by bacterial infection from the early stages in the womb during pregnancy to the death bed. However, expectant mothers are tenfold more exposed to bacterial pestilence than healthy people, coupled with antimicrobial resistance due to antibiotics makes it more difficult to cure. Suppose pregnant women infected with some bacteria can cause preterm delivery, stillbirth, miscarriage, and ill effect on the offspring (Lee et al. 2020). Bacterial infection is life-threatening to humanity if early diagnosis is not possible. Thus, pathogenic bacteria detection is a hot topic of research in the medical field. High morbidity and mortality rate due to pathogenic bacteria marks it as a central public health problem. Increased cost inpatient management combats the existing traditional bacterial treatment, inefficient in dealing with antibiotic-resistant bacteria. Clinical microbiology lab tests are the first solace for infection treatment and antibiotic susceptibility testing (AST), which are often very time-consuming (Ghouri et al. 2020).

The invention of biosensors was a breakthrough in analytical chemistry. One can see the progress of innovations in biosensors by looking at the evolution of biosensors for glucose detection (Juska and Pemble 2020). Different biosensors with a biofunctionalized element (biochemical receptor like an enzyme, antibody, oligonucleotide) along with a transducer (shifts biological signal into electrical signals) have enabled bacterial detection hassle-free. Transducer design allows detection of the analyte concentration before converting it into an electrical signal. It is possible through using optical, electrochemical, acoustic, mechanical, calorimetrical, or electronic methods according to the analyte. Biosensors are analytical tools that can furnish exclusive quantitative or semi-quantitative analytical facts based on biological recognition (Perumal and Hashim 2014). One way to automate the endless efforts of clinical laboratories in complex AST, antibiotic resistance, and detection of bacteria is to promote research in the field of biosensors. In the 1970s, a nanoscale revolution emerged and changed all the conventional detection methods. Miniaturization and nano-level instrumentation paved the way for many innovative devices for detecting biological entities in seconds. Nanobiosensors are the foremost in the application of nanomaterials in day-to-day life. The transducer of a nanobiosensor is modified with nanoparticles or nanomaterials to facilitate rapid recognition and hassle-free analyte detection (Srivastava et al. 2018).

This chapter begins by describing traditional methods of bacterial detection followed by principles and types of nanobiosensors. Then, preceding by applying nanobiosensors in detecting human pathogenic bacteria, some relevant literature is also included to compare and contrast the efficacy of nanobiosensors. Overall, this chapter will give you clarity on the application and efficiency of nanobiosensors in bacterial detection.

21.2 Methods to Detect Pathogenic Bacteria

When an advanced and promising scientific method is established, biologists momentarily adapt it to the detection of pathogens. For instance, the use of polymerase chain reaction (PCR) as a DNA amplification tool has paved the way for the development of various methods that depend upon PCR for the determination of numerous harmful bacteria. New and promising techniques of detecting pathogens are always a ray of hope to the microbiologists as they help in resolving the existing and forthcoming problems and seal the knowledge gap. The emerging techniques of today become the keystones for the methods of tomorrow.

This strenuous field of detecting pathogenic bacteria has gained a great deal of interest in yesteryears. The conventional techniques of bacterial detection include biochemical, microscopic, immunological, culture, and genetic methods (Fig. 21.1). Besides these techniques, a variety of rapid microbiological methods facilitate the sensing and identification of virulent bacterial strains in complex matrices like food, water, air, etc. (Hameed et al. 2018; Ling and Hui 2019; Nguyen et al. 2017). Enzyme-linked immunoassay (Pang et al. 2018), polymerase chain reaction (Liu et al. 2019), biosensors and immunosensors (Kuss et al. 2018), concentric arcs of photovoltaic detectors with laser scanning (Bayguinov et al. 2018), DNA sequencing

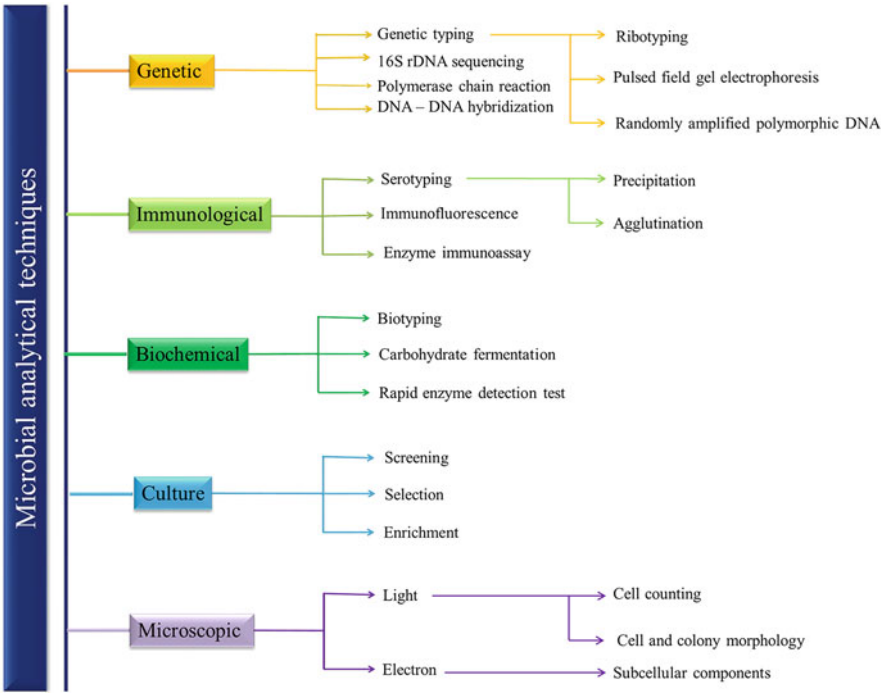

Fig. 21.1 Selected methods commonly used in the detection of pathogens

(Feigelman et al. 2017), direct epifluorescent filter technique (Chung et al. 2019), endospore detection (Vidic et al. 2020), flow cytometry (Meng et al. 2017), FTIR spectroscopy (Grewal et al. 2015), Raman spectroscopy (Ho et al. 2019) are a few of them. However, the efficiency of these techniques relies on post-collection sample processing. A blood sample or a nasal swab is the most used sample for pathogen detection. Further, the collected samples are subjected to enrichment processes before detection assay. In the forthcoming years, the development of direct methods for bacterial sensing would provide practical solutions to the in situ determination of bacteria in blood samples or exhaled air.

21.2.1 Bacterial Culture and Colony Counting Methods

These methods are the fundamental and the most widely used methods for isolation, culturing, and quantifying bacterial cells. The primary step involves the collection and uniform distribution of cells on a suitable medium that permits the cell growth of target bacteria. In the next step, the cultured cells are enriched for colony counting and other analyses. Though efficient, this method lacks point of care (POC) testing and requires manual expertise and labor. This type of analysis becomes very difficult when there are a large number of samples. To illustrate, Hsiao et al. have demonstrated the use of CHROM agar, chambers, and filters for detecting *Enterococcus* species. However, lack of self-operation and long assessment time are a few of the limitations. The relative humidity is a fundamental aspect of the efficiency of the culture technique (Hsiao et al. 2014). Rule et al. reported that a lowest of 15% of relative humidity is necessary for the culture technique to be efficient for aerosol collection samples. However, the whole process lasted for 16 h which is unappealing (Rule et al. 2009).

21.2.2 Immunological Assays in Bacterial Detection

Immunological detection techniques are one of the most commonly used methods for the detection of pathogenic bacteria. Despite their simplicity and acceptable detection performance, these techniques (E.g., Radioimmunoassay, ELISA, microfluidics, etc.) cannot be used without a biorecognition site or bioreceptor. Among the immunological assays, radioallergosorbent test (RAST) and enzyme-linked immunosorbent assay (ELISA) are the most practiced traditional methods to detect the presence of pathogens (Burge and Solomon 1987; Kwon et al. 2014; Mairhofer et al. 2009; Skládal et al. 2012). Besides their ability to accurately quantify the analyte, quick response, specificity, and sensitivity, immunological assays have inevitable limitations. For instance, ELISA requires multiple and tiresome pipetting and thorough rinsing (Fronczek and Yoon 2015), while RAST includes obligatory utilization of a radioactive label such as ^{125}I limits its relevance (Burge and Solomon 1987). Further, simplifying the complex procedures, direct

sensing of pathogenic bacteria, availability of mobile/portable sensing devices, and radioactive tags govern the real-time samples of bacteria in these techniques.

21.2.3 Polymerase Chain Reaction and Nucleic Acid-Based Techniques

Polymerase chain reaction (PCR) and associated detection techniques also provide selective and sensitive signals. These methods are fairly successful in detecting pathogen levels in the various samples. However, progressive developments have led to braving certain drawbacks, such as the need for laborious steps that can last up to 4 h (Fronczek and Yoon 2015). Despite the progress, conventional PCR is unappealing for the following reasons: involvement of complicated steps like DNA extraction/purification, thermocycling, and the need for separate imaging techniques like gel electrophoresis. Though PCR effectively detects pathogens with DNA in their genome, it fails to detect pathogens with RNA genetic material. To overcome this problem, reverse transcription-PCR (RT-PCR) can be implemented. RT-PCR involves the synthesis of cDNA, as an added step which is a much more cumbersome and complicated process.

Improvements in PCR technology have led to the development of primer kits, DNA extraction tools, real-time monitoring, compact, and handy devices. Nevertheless, PCR-based bacterial detection has still remained to be a time-consuming process. Many scientists have employed the PCR techniques for the detection of pathogens in the air (Agranovski 2007; Alvarez et al. 1994; Hospodsky et al. 2010; Sawyer et al. 1994; Stetzenbach et al. 2004). However, these methods include numerous processing steps such as sample collection, sample preparation, and PCR. The necessity of distinct sample collection and sensing units, multifold processing steps, and nonautomated operation has limited its relevance in real-time detections. Nonetheless, the inclusion of self-operation, minimizing and simplifying the complex processing steps, designing portable devices, and direct sensing of pathogens can make this technique better for real-time sensing.

On a whole, PCR and nucleic acid-based techniques require advancements in automation and real-time sensing of bacteria. Further, introducing a new sampling unit that can directly sense the target bacteria or direct it to the detection unit can tackle the shortcomings of the present PCR-based detection.

21.2.4 Biosensors and Nanobiosensors

In the previous decades, biosensors have turned out to be an efficient platform for the determination of pathogenic bacteria. Further, advances in bacterial sensing have led to the development of microfluidic bioassays that facilitates the rapid detection of pathogenic microbes. Regardless of these advancements, there is a lack of commercial devices with proven performance in real samples. The low concentration of bacteria in the ecological niche and the existence of interfering components have

sabotaged the diagnostic success. The progress in nanotechnology has enabled researchers to explore the remarkable properties of nanomaterials (like the large surface area to volume ratio, etc.) to build effective and sensitive detection techniques. Nanoscale materials facilitate the miniaturization of the sensing devices and thereby paves the way to the construction of portable, rapid, and sensitive diagnostic systems for pathogen detection. Hence it becomes necessary to understand the working principle of nanobiosensors.

21.3 Principles of Nanobiosensors

A combination of traditional biosensors and ever-growing nanotechnology gave rise to revolutionary nanobiosensors (Xu et al. 2020). One can define nanobiosensors as nanoscale devices that can detect biological molecules in nanoscale with the help of a biological recognition element and a transduction unit. The components of a nanobiosensor are a bioreceptor, physicochemical transducer, and detector as depicted in the figure (Fig. 21.2) (Christopher et al. 2020).

The basics of biosensors lie within the principle of molecular recognition. The biological receptors can detect the bacteria only when there is a specific molecular recognition between the bacteria and the receptor. The best example for molecular recognition is the lock and key model or antigen–antibody interaction. The outer part of the sensor which interacts with the target is the bio-receptor. Bio-receptors (enzymes, cells, aptamers, deoxyribonucleic acid (DNA), and antibodies) stable under different storage conditions are immovably fixed on the transducer surface to bind the target entity. Adsorption, cross-linking, entrapment, covalent bonding, and microencapsulation methods are employed to immobilize the biological recognition element. The immobilization of nano components is an uphill battle in the preparation of nanobiosensors. One can replace the biological part of the receptor with biologically originated molecules such as engineered artificial proteins, recombinant antibodies, imprinted polymers (biomimetic compounds) (Ertürk and Mattiasson 2017), a variety of ligands (De Paepe et al. 2019), and synthetical catalysts. Imprinted polymers are employed to increase the selectivity of the sensors

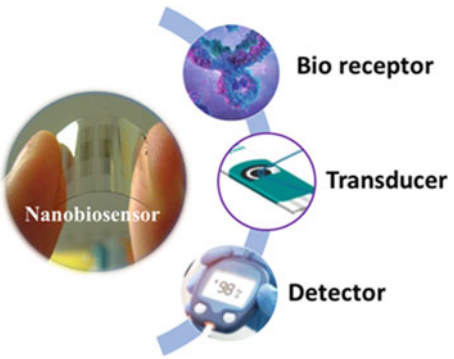

Fig. 21.2 Different components of a nanobiosensor

(Ann Maria et al. 2020). The selectiveness and sensitiveness of a biosensor solely depend on this bioreceptor performance (Bhattarai and Hameed 2020).

The transducer (electrodes, thermistors, semiconductor pH electrode, a piezoelectric device, photon counter, etc.) detects the effect (change in pH, mass, heat, light, or electroactivity of the analyte) caused by the molecular recognition. It acts as an interface and converts the energy of the physical change in the receptor into a measurable signal. The nanomodified transducers are the highlight in nanobiosensors which makes rapid detection possible within a short period. The presence and quantity of analytes can be detected effectively using nanobiosensors compared to simple biosensors.

Finally, a detector possesses a microprocessor that measures the electrical signal from the transducer and an electronic component that amplifies or analyses the electrical input. A variety of amplifiers and filters process the analogous signals from the transducer to digital form. The final data obtained in numeric, tabular, graphics, or an image is displayed in the device as concentration units or stored in the data bank. Recently smartphone-based detectors were introduced for lab on-chip or point-of-care detection of analytes in nanobiosensors (Seo et al. 2019).

Specific characteristics of nanobiosensors enhance the performance of the sensor indirectly. They are selectivity, sensitivity, reproducibility, linearity, and stability. Selectivity denotes the ability of the sensor to selectively detect the specific analyte from a pool of different analytes (Denmark et al. 2020). The sensitivity of a nanobiosensor is related to the lower detection limit possible with the sensor and corresponds to the device's robustness (Zhang et al. 2020a). The reliability of the nanobiosensor output is correlated with the reproducibility of the same result when done many times accurately and precisely. A working range or linear dynamic range where the concentration is directly proportional to the measured signal portrays the accuracy of the obtained output or linearity. The ability of the sensor to detect and quantify the analytes in different disturbances in measurement time without compromising accuracy and precision refers to the sensor stability. Based on the transduction and biological signaling, different types of nanobiosensors evolved.

21.4 Types of Nanobiosensors

Nanomaterials act as effective substrates for the fabrication of biosensors. Various nanomaterials that possess transduction properties are explored to construct piezoelectric bio- and chemo-sensors, optical, and electrochemical sensors. Effective use of nanomaterials like silver nanoparticles, graphene, gold nanoparticles, carbon nanotubes, quantum dots, etc. was explored to design various sensing scaffolds that detect a number of bacteria. However, optical and electrochemical sensors are more popular due to their ease of operation, applicability in real-time analysis, and portability due to their small size. For a comprehensive understanding, this section is split into two subsections: optical and electrochemical sensors.

21.4.1 Optical Biosensors

Optical biosensors can detect the sensitive optical signals that are produced as a result of interaction between the analytes and the probes. Based on different detection methods, various optical sensors such as colorimetry, fluorescence, energy transfer, and surface plasmon resonance have been used to detect bacterial pathogens. Among these methods, colorimetric detection method is the most accepted and widely used due to their ease of operation and simplicity. In the colorimetric biosensor, signals are produced in the form of colors, which are easily detected by the human naked eye. Furthermore, there is no need for the multiple and tiring intermediate steps and complex instrumentation as discussed in the previous sections. For example, colorimetric detection was employed for the sensing of four different bacteria: *Salmonella enteritidis, Campylobacter jejuni, Salmonella typhimurium,* and *Staphylococcus aureus,* using a cotton swab as the sample collector and detection platform. The immunoassays were carried out by immobilizing a specific antibody on a cotton swab for each bacterial strain. The cotton swabs were then used to develop sandwich immunoassay by dipping them in different colored nanobead-coupled antibody solutions that correspond to different bacteria. The colorimetric measurements for the immunoassays were recorded by noting a change in the color intensity of nanobeads owing to the genuine interaction with the bacteria. This colorimetric method of detecting bacteria is not only simple but also proved to be sensitive with 10 CFU/mL detection limit (LOD). The LOD of this method is comparable with the other similar reports, where the sensing was carried out using an immunofluorescent nanosphere and immunomagnetic nanospheres-based assays (Alamer et al. 2018; Wen et al. 2013).

A user-friendly one-step strategy was developed for the screening of *Salmonella typhimurium*. In this technique, immunomagnetic nanospheres (IMNS) and immunofluorescent nanospheres (IFNS) successfully captured and recognized *Salmonella typhimurium* simultaneously. Following the magnetic separation, a sandwich immunoassay was developed which was further detected using fluorescence microscopy. Fluorescence intensity varied linearly with the bacterial concentration in the range of 10^5–10^7 CFU/mL with LOD of 10 CFU/mL and $R^2 = 0.9994$. In comparison to the two-step detection method, where the bacteria are first captured by IMNS and then recognized by the IFNS, this method has simplified the intermediate steps and improved the sensitivity of the optical nanobiosensor. The strong anti-interference ability and selectivity towards *Salmonella typhimurium* have enabled the optical nanobiosensor to detect the bacteria in samples such as milk, urine, and fetal bovine serum (Wen et al. 2013). A schematic representation for the detection of bacteria using fluorescence is depicted in Fig. 21.3.

A fiber optic surface plasmon resonance (FOSPR) was used to detect *Escherichia coli* O157:H7 (E.coli O157:H7) in juice and water samples. The SPR-based biosensors have gained a quantum of interest in the research fraternity due to label-free detection. In SPR sensors, the charge density oscillations of free electrons are produced at the metal-dielectric surface called surface plasmons. The surface plasmons are excited by a beam of incident light and form an evanescent wave that

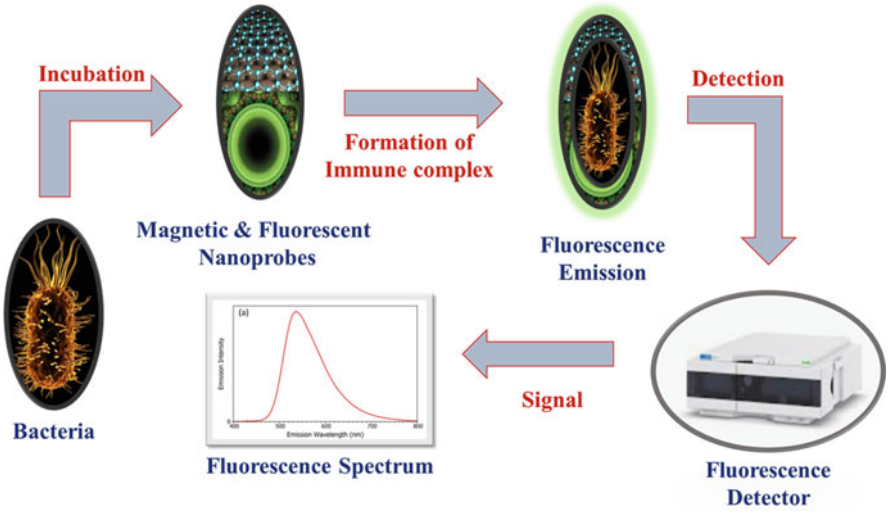

Fig. 21.3 Schematic representation for the detection of bacteria using fluorescence method

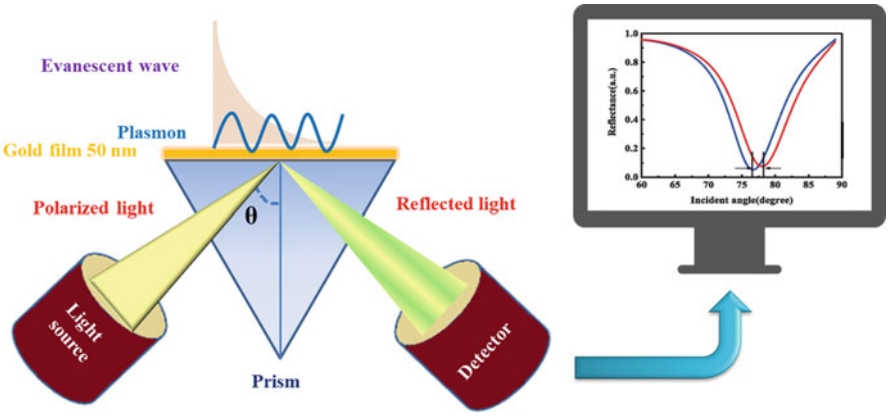

Fig. 21.4 Schematic representation of the principle of SPR-based optical sensors for cell-based clinical diagnosis

decays together with the metal–dielectric interface. The entire process is called surface plasmon resonance (Fig. 21.4). The FOSPR comprises antimicrobial peptides and magainin I as bioreceptors, and silver nanoparticles-reduced graphene oxide (AgNPs-rGO) nanocomposite serves as signal amplifiers. The AgNPs-rGO was uniformly spread over the surface of optic fiber and a gold film was covered over it. In the assay, the SPR wavelength showed a good linear relationship with the natural logarithm of the E.coli concentration within the range of 1.0×10^3 to 5.0×10^7 CFU/mL with the LOD of 5.0×10^2 CFU/mL. This FOSPR optical

sensor is highly sensitive to *E.coli* O157:H7 as it showed no obvious shift in wavelength of SPR peak towards the detection of *E.coli* K12, *Staphylococcus aureus,* and hemolytic streptococcus. The FOSPR showed good sensitivity, selectivity, stability, and reproducibility. It could also serve to detect foodborne pathogens and can be a potential tool in food analysis, and diagnostics (Yanase et al. 2014; Zhou et al. 2018).

21.4.2 Electrochemical Sensors

Though optical biosensors can be easily fabricated and used, their applicability in the real-time analysis may be hindered due to the limited sensitivity and unhandy devices. While electrochemical sensors can be easily tuned to be more sensitive, handy, they present a short detection time. Electrochemical sensors measure the electrical signals produced as a result of a change in the chemical environment due to the interaction of analytes with the transducer surface. Several electrochemical methods have been developed for the determination of analytes. Detection techniques include conductometry, linear sweep voltammetry (LSV), electrochemical impedance spectroscopy, amperometry, differential pulse voltammetry (DPV), to name a few. For example, gold nanoparticles (AuNPs) were nanopatterned on indium titanium oxide (ITO) electrodes. The Au-nanohole arrays were created on

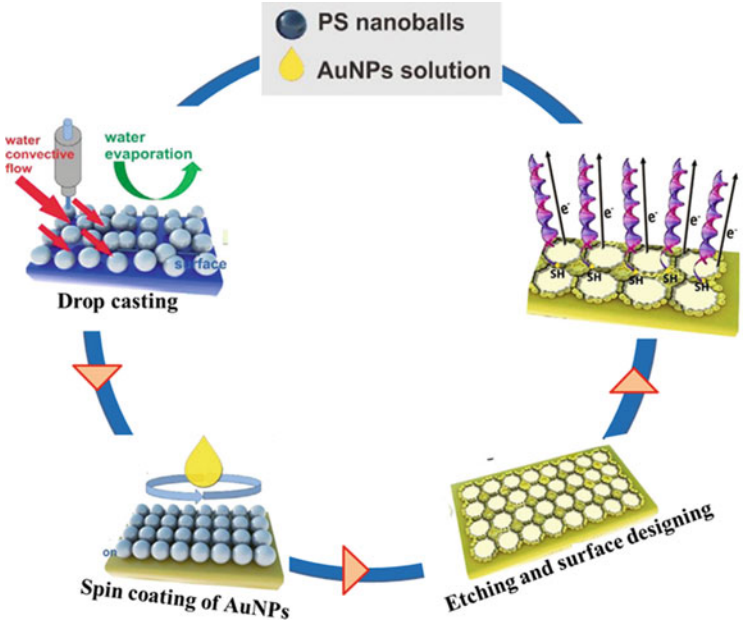

Fig. 21.5 Schematic overview of nanopatterning ITO substrate with gold nanoholes using NSL and electrochemical sensing of DNA Reprinted with permission from Purwidyantri et al. (2016)

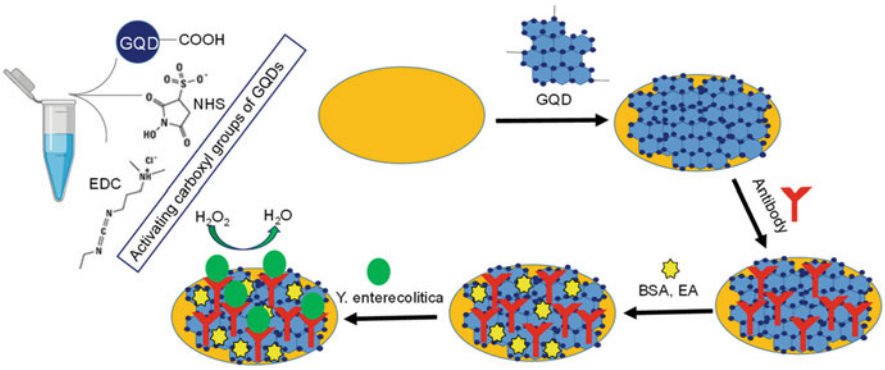

Fig. 21.6 Schematic overview of GQD as enzyme mimics for the quantification of *Y. enterocolitica* in milk and human blood serum samples. Reprinted with permission from Savas and Altintas (2019)

ITO substrates by etching polystyrene nanospheres (PS) using nanosphere lithography (NSL) (Fig. 21.5). The Au-nanohole arrays on ITO substrate act as signal amplifiers and enhance the peak currents of electrochemical evaluations by 82% when compared to bare ITO. This nanopatterned ITO substrate was used to detect *Staphylococcus aureus* 16S rRNA hybridization. On the contrary, Au-nanohole arrays showed an optimal sensitivity, enhanced DNA hybridization by 23% with the LOD value of 10 pM. The selectivity of this electrochemical sensor was probed by recording the electrochemical signals of 100 nM *Staphylococcus aureus* 16S rRNA in the presence of 1000 nM of *P. aeruginosa* and *E. coli.* The results suggested the high specificity and selectivity of the Au-nanohole arrays on ITO substrate towards the detection of *S. aureus* (Purwidyantri et al. 2016).

Another example demonstrates the use of a rapid POC on-chip electro-analytical device for the detection of cholera. The device is based on a dendritic gold framework functionalized with poly(2-cyanoethyl)pyrrole (PCEPy). The on-chip electrochemical immunosensor was used to detect cholera toxin subunit B (CTX). The high detection sensitivity of the on-chip electrochemical dendritic electrode is due to its 18 fold enhancement in the surface area against the planar gold surface. The LOD of 1 ng/mL and 100 ng/mL was observed for the on-chip electrochemical dendritic electrode and planar gold substrate, respectively. Further, these results are also in good agreement with the standard optical ELISA, but in a miniaturized diagnostic device with electrochemical readings. Thus this sensing device represents a promising route for POC disease detection for cholera in resource-limited regions (Valera et al. 2019).

Yersinia enterocolitica causes yersiniosis: a condition associated with nausea, joint pains, fever, diarrhea, abdominal pain and cramps, and symptoms that resemble appendicitis in teenagers and adults. Another recent finding reports the use of graphene quantum dots (GQDs)as nanozyme for the detection of *Y. enterocolitica* in human serum and milk samples (Fig. 21.6). Laser-cut patterned stainless steel electrodes were initially deposited with Au (400 nm) using an electron beam

evaporator. After washing, the electrodes were dried in nitrogen gas and treated with nitrogen plasma before the lamination of EDC-NHS activated GQDs as an enzyme mimic. The GQD-laminated Au sensing surface was covalently immobilized with monoclonal anti-*Y. enterocolitica* antibody and incubated with BSA for 1 h. The fabricated immunosensor was eventually used for the determination of *Y. enterocolitica* in milk and human blood serum samples. The GQD-based immunosensor successfully quantified *Y. enterocolitica* in a wide concentration range of 1–6.23 $\times$ 10^8 cfu/m with the LOD of 5 CFU/mL and 30 CFU/mL for milk and serum, respectively (Savas and Altintas 2019).

21.5 Pros and Cons of Nanobiosensors

The advantages of nanobiosensors over simple biosensors are remarkable. The nanobiosensors are tenfold more efficient than the biosensors. The main change is due to nanoparticles' presence, which alters the traditional properties of the material (quantum confinement effects, size, reactivity, electrical properties, magnetic properties, and optical properties) entirely in nano size. The enhanced surface-to-volume ratio of nanoparticles and size properties brings out so much efficiency in active interaction sites and adds uniqueness to the nanobiosensors (Sharma et al. 2020).

The repeated use of this rapid sensor saves money and effort for clinical detection, while the performance of the nanobiosensors is upheld by the advancement of the biorecognition unit in terms of reproducibility and sensitivity. The flexibility and simplicity of immobilization techniques make nanobiosensors a favorite of clinical microbiologists. The interfacial region of nanobiosensors ranges from 1 to 10 nm for the recognition of target analytes. When discussing the rear side of nanobiosensors, the complexity involved in the efficient immobilization of such nano components is noteworthy (Wahab et al. 2020).

Another exciting factor about nanobiosensors is that the reaction can occur at room temperature, unlike polymerase chain reaction (PCR), depending on temperature. This uniform room temperature enables field detection hassle-free (Bhatnagar et al. 2018). The simplicity of the nanobiosensors lies with the limited use of reaction mixture, which is cost-effective. The lowering of the detection limit is another added advantage resulting in effective detection of pathogens even in small traces. The speed of detection ensured through nanobiosensors is crucial for disease diagnosis and early treatment to avoid disease transmission. This miniaturization of biosensors into nanoscale enables personnel-free pathogen detection, reduces processing time, and uses minimal pieces of equipment. Multiplexity or the capability to selectively detect multiple analytes from the very same reaction mixture shortly is another plus point to some nanobiosensors.

When comparing different types of nanobiosensors each sensor comes with its advantages and disadvantages. The conventional methods used for bacterial detection lack varying sensitivity and precision in diverse clinical settings and conditions. These tedious methods make use of a high quantity of costly reagents and require

specific and expensive equipment (Kalali et al. 2015). Still, as it is readily available it is often used as the primary recognition method or routine diagnostic method. Optical nanosensors are popular due to label-free and amplification-free detection and the use of advanced instrumentation. Mostly the results are easy to read even with simple eyesight in a colorimetric assay. But it is not used clinically as the fabrication process is tedious, expensive, and possesses low portability (Jamali et al. 2014). Even though electrochemical sensors are having high portability and use fewer amounts of reagents they have restricted control over the surface of the working electrode at higher potentials and give fake positive results because of electrolytes from the samples (Zuo et al. 2007).

The major difficulty faced by microbiologists is the development of nanobiosensors when the pathogen is unknown. The bacteria identified or cultured in a lab are only a neglectable percentage of the actual number of bacteria in this universe. In that case, the need for foreseeing the structural and biochemical properties of various bacterial species exists. Another social concern regarding the exponential growth of research publications in nanobiosensors is applying academic publications and research into industries. It can be possible only when the methods adopted by the scientist are economical and less complicated. Ordinary people are benefitted from innovative technologies only when these ideas are industrialized, and point-of-care commercialization happens at affordable prices. The stumbling blocks of nanobiosensors in terms of commercializing nanobiosensors to ensure point-of-care diagnostics are biocompatibility and nanotoxicity. Nanobiosensors have more advantages compared to a few disadvantages, which is fixable through future research.

21.6 Human Pathogenic Bacteria and Their Detection Using Nanobiosensors

Bacteria are a class of micro-organisms that are responsible for various infections and diseases that humans encounter. These microbes are one of the most common reasons behind the outbreaks of numerous diseases via microbial contamination (e.g., Cholera). *Mycobacterium tuberculosis, Streptococcus pneumoniae, and Bacillus anthracis* are a few bacteria that are responsible for the spread of diseases through air contamination (Bhardwaj et al. 2021; Grisoli et al. 2009; Källenius et al. 2008). *M. tuberculosis* is an etiological agent of a highly contagious disease called tuberculosis (TB). It is a gram-positive bacteria that also causes pneumonia and meningitis. The doubling time of this bacteria is 4–24 h in vitro and has an incubation period of 4–6 weeks (Gill et al. 2009). TB is a highly transmissible disease that spreads through the air via saliva or phlegm droplets that are produced as a result of coughing (Lin et al. 2012; Millet et al. 2013). TB is one of the most common bacterial diseases across the globe infecting 14 million people and approximately 2 million deaths per annum (World Health Organization 2013). Though a great deal of effort has been put to eradicate TB, the antibiotic resistance developed by the bacteria has led to the rise of new disease upsurges.

Vibrio cholerae is the root cause for the rapid spread of cholera, a disease concerned with general public health in regions with poor hygiene and sanitation. *V. cholerae* is a gram-negative, flagellated, facultative anaerobe that is ubiquitous in salt and brackish waters. It has a doubling time of 1.1 h with an incubation period of 2 h–5 days (Gibson et al. 1880). Cholera is a highly communicable disease that is spread through water and has a characteristic "shooting star" appearance in the watery stools of cholera patients. It is also associated with outbreaks of cellulitis, gastroenteritis, or bacteremia.

Clostridium botulinum is an etiological agent of botulism disease in humans. The botulinum toxin is secreted by the spores of *C. botulinum* after their successful ingestion by humans and animals through food. The botulinum toxin produced inhibits the secretion of acetylcholine ester (neurotransmitter) and leads to muscle paralysis, double vision, breathing difficulties, and muscle weakness (Gupta et al. 2021). Apart from these, a large number of bacteria cause damage to human health and well-being. A few of them are listed along with the associated symptoms/diseases and mode of transmission in Table 21.1.

The foremost problem faced with pathogen detection is to differentiate between viral and bacterial infection. With the right choice of nanobiosensors, the treatment is started immediately without delay enhancing patient survival and efficient bedside treatment. Various optical and electrochemical nanobiosensors have been utilized to detect human pathogenic bacteria. Numerous nanoparticles, nanocomposites, etc. have been employed to improve the sensitivity and selectivity of nanobiosensors for a plethora of bacteria over a wide concentration range. Based on the type of nanomaterial used, sensor employed, and the bacteria of interest, the performance of the nanobiosensor may vary. To provide clarity to the readers, we have compared a few key factors that affect the overall performance of the nanobiosensors in Table 21.2.

The optical and electrochemical properties of the nanoparticles and nanocomposites are well exploited to construct selective and efficient sensing platforms for the detection of virulent bacterial strains. However, an immediate collation of the functioning of these two sensors cannot be parallelly made due to the difference in the methodologies adopted for the determination and quantification of bacterial pathogens. Nevertheless, both optical and electrochemical nanobiosensors have shown large differences in their sensitivities based on the target bacteria and nanomaterial used. For instance, electrochemical determination of *Escherichia coli* O157:H7 employing SG–PEDOT–AuNPs@GCE is far more sensitive than its optical detection using AgNPs-rGO nanocomposite. The electrochemical nanobiosensor achieved the LOD of 3.4×10 CFU/mL for the sensing of *Escherichia coli* O157:H7 while an optical nanobiosensor was able to detect only 5.0×10^2 CFU/mL for the same target bacteria (Zhou et al. 2018; Guo et al. 2015). Similarly, DNA-based nanobiosensor achieved a LOD of 10^4 CFU/mL and 0.15 nM for the detection of *Helicobacter pylori* optically and electrochemically, respectively. Though the performance of these nanobiosensors cannot be compared parallelly their performance is way too better than the conventional detection strategies in terms of real-time analysis, portability, ease of use, lack of laborious

Table 21.1 Different types of pathogenic bacteria and their characteristics

Air/food/ waterborne pathogens	Pathogenic bacteria	Diseases/symptoms	Mode of transmission
Air (Chen et al. 2020)	*Mycobacterium tuberculosis*	TB	Infected sputum, saliva droplets in the air
	Bacillus anthracis	Anthrax	Spread of bacterial spores
	Streptococcus pneumoniae	Pneumonia, sinus and ear infections, adult meningitis, and septicemia present in immunodeficient patients	Disseminated through sneezing coughs, and intimate contact with affected persons
Food (Zhang et al. 2020b)	*Escherichia coli*	Hemorrhagic colitis, hemolytic uremic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP), and bloody diarrhea	Consumption of water or food polluted with excreta of infected humans or animals
	Salmonella spp.	Abdominal pain, diarrhea, high temperature, and vomiting	Intake of polluted water or food and immediate contacting with effected animals
	Staphylococcus aureus	Vomiting, perspiration, nausea, chills, headache, dizziness, muscular cramping, abdominal cramps, and general weakness	Poor personal hygiene, insufficient refrigeration, inadequate cooking and heating
	Listeria monocytogenes	Meningitis, septicemia, and gastroenteritis	Uptake of eatables from effected animals, intake of polluted food or water, and personal interactions
	Shigella spp.	Gastrointestinal infections, malaise, watery diarrhea, fever, abdominal cramps, and fatigue	Intake of focally polluted food or water
Water (Magana-Arachchi and Wanigatunge 2020)	*Serovarieties* O1 and O139 and *vibrio cholera*	Gastroenteritis and cholera	Fecal-oral pathway
	Shigella sp.	Shigellosis	Fecal-oral way
	Salmonella sp.	Gastroenteritis, Salmonellosis	Fecal-oral pathway
	Serotype O157: H7, *Escherichia coli*	Hemorrhagic colitis, diarrhea, hemolytic uremic syndrome	Fecal-oral way
	Francisella tularensis	Tularemia	Arthropod bites, intimate contact with affected animals, and breathing or intake of polluted water

Table 21.2 Different types and trends of fabrication of nanobiosensors for the human pathogenic bacterial detection

Type of nanobiosensor and references	Nano modification	Target	Transmission mode	Linear dynamic range	Limit of detection	Real sample
Optical sensors (Ali et al. 2019)	DNAzyme immobilized on agarose beads. Urease is attached at the 5′-end of the DNAzyme through a sequence tag	*Helicobacter pylori*	Food	–	10^4 cfu/mL	Human stool
Wen et al. (2013)	Immunomagnetic nanospheres and immunofluorescent nanospheres	*Salmonella typhimurium*	Food	10^5–10^7 CFU/mL	10 CFU/mL	Milk, fetal bovine serum, urine
Zhou et al. (2018)	Antimicrobial peptides and magainin I bioreceptors, and silver nanoparticles-reduced graphene oxide nanocomposite as signal amplifiers	*Escherichia coli* O157:H7	Food	1.0×10^3 to 5.0×10^7 CFU/mL	5.0×10^2 CFU/mL	Juice, water samples
Electrochemical Sensors (Peng et al. 2017)	ß-CD/dsDNA/AuE	*Helicobacter pylori*	Food	0.3–240 nM	0.15 nM	Beef specimen
Guo et al. (2015)	SG–PEDOT–AuNPs@GCE	*Escherichia coli* O157:H7	Food	7.8 x 10–7.8 x10^6 CFU/mL	3.4×10 CFU/mL	Springwater and milk
Zou et al. (2019)	PPy-rGO/AuNPs nanocomposite	*E.coli K12*	Food	1.0×10^1 to 1.0×10^7 CFU/mL	10 CFU/mL	Tap water and milk
Chen et al. (2017)	AuNPs and PANI-rGO	*M. tuberculosis*	Food	0.1–104 pM	50 fM	Sputum

Abbreviations: *ß-CD/dsDNA/AuE* ß-Cyclodextrin/double stranded DNA/Gold electrode, *PEDOT* Poly(3,4-Ethylenedioxythiophene), *SG* sulfonated graphene, *PPy* Polypyrrole, *PANI* Polyaniline, *rGO* reduced graphene oxide, *CFU* Colony forming unit, *GCE* Glassy carbon electrode

intermediate steps, and most importantly the development of POC devices for clinical diagnostics.

21.7 Conclusion and Prospects

The spread of pathogenic bacteria through the air, water, and food has been a major issue of concern across the world. The transmission of pathogens may occur in several forms such as dust particles, endospores, water droplets, fecal residues, sneezing, coughing, or even by communicating to an infected person. Getting exposed to pathogenic bacteria leads to numerous health complications such as disorders, allergies, or diseases. Plenty of conventional methods are available for the determination of pathogenic bacteria including colony counting technique, immunoassays, polymerase chain reaction, etc. as discussed in the chapter. Nonetheless, these methods detect bacteria by indirect sampling methods and involve lengthy, tedious, and time-consuming processes.

Biosensors have recently turned out to be an outstanding platform for the detection of pathogenic bacteria. Exploiting the remarkable properties of nanomaterials like the large surface area to volume ratio has enhanced the efficacy of biosensors to the next level. Nanobiosensors: both optical and electrochemical methods have contributed to the enhanced sensitivity and selective detection of pathogenic bacteria. They have also paved the way for developing portable, miniaturized, POC diagnostic devices for numerous pathogens. Nanobiosensors have addressed the limitations of conventional detection methods such as the need for highly sensitive sample collectors and detectors. Future works should be focused on developing automated, portable, reliable, and reproducible nanobiosensors on a large scale. Also, the biocompatibility and toxic impact of the nanomaterials used to construct the nanobiosensors should be considered in the near future. Thus, further, improvements are required to construct productive, and efficient pathogen detection flatforms with computerized and rapid testing, excellent sensitivity, stability, inexpensive, and in situ sensing of target bacteria.

References

Agranovski IE (2007) Personal sampler for viable airborne microorganisms: main development stages. Clean—Soil, Air, Water 35(1):111–117. https://doi.org/10.1002/clen.200600020

Alamer S, Eissa S, Chinnappan R, Zourob M (2018) A rapid colorimetric immunoassay for the detection of pathogenic bacteria on poultry processing plants using cotton swabs and nanobeads. Mikrochim Acta 185(3):164

Ali MM et al (2019) A DNAzyme-based colorimetric paper sensor for Helicobacter pylori. Angew Chem Int Ed Engl 58(29):9907–9911

Alvarez AJ et al (1994) Use of solid-phase PCR for enhanced detection of airborne microorganisms. Appl Environ Microbiol 60(1):374–376

Ann Maria CG, Akshaya KB, Rison S, Varghese A, George L (2020) Molecularly imprinted PEDOT on carbon fiber paper electrode for the electrochemical determination of 2,4-dichlorophenol. Synth Met 261:116309. https://doi.org/10.1016/j.synthmet.2020.116309

Bayguinov PO, Oakley DM, Shih C-C, Geanon DJ, Joens MS, Fitzpatrick JAJ (2018) Modern laser scanning confocal microscopy. Curr Protoc Cytom 85(1):e39

Bhardwaj SK et al (2021) Recent progress in nanomaterial-based sensing of airborne viral and bacterial pathogens. Environ Int 146:106183

Bhatnagar I, Mahato K, Ealla KKR, Asthana A, Chandra P (2018) Chitosan stabilized gold nanoparticle mediated self-assembled gliP nanobiosensor for diagnosis of Invasive Aspergillosis. Int J Biol Macromol 110:449–456

Bhattarai P, Hameed S (2020) Basics of biosensors and nanobiosensors. Nanobiosensors. https://doi.org/10.1002/9783527345137.ch1

Burge HA, Solomon WR (1987) Sampling and analysis of biological aerosols. Atmos Environ (1967) 21(2):451–456. https://doi.org/10.1016/0004-6981(87)90026-6

Chen Y, Li Y, Yang Y, Wu F, Cao J, Bai L (2017) A polyaniline-reduced graphene oxide nanocomposite as a redox nanoprobe in a voltammetric DNA biosensor for Mycobacterium tuberculosis. Microchim Acta 184(6):1801–1808. https://doi.org/10.1007/s00604-017-2184-5

Chen X, Kumari D, Achal V (2020) A review on airborne microbes: the characteristics of sources, pathogenicity and geography. Atmosphere 11(9):919. https://doi.org/10.3390/atmos11090919

Christopher FC, Kumar PS, Christopher FJ, Joshiba GJ, Madhesh P (2020) Recent advancements in rapid analysis of pesticides using nano biosensors: a present and future perspective. J Clean Prod 269:122356. https://doi.org/10.1016/j.jclepro.2020.122356

Chung N, Ramakrishnan SR, Kwon J-H (2019) Experimental validation and evaluation of electronic sensing techniques for rapid discrimination of electron-beam, γ-ray, and X-ray irradiated dried green onions (*Allium fistulosum*). J Food Sci Technol 56(12):5454–5464

De Paepe B, Maertens J, Vanholme B, De Mey M (2019) Chimeric LysR-type transcriptional biosensors for customizing ligand specificity profiles toward flavonoids. ACS Synth Biol 8(2):318–331

Denmark DJ, Mohapatra S, Mohapatra SS (2020) Point-of-care diagnostics: molecularly imprinted polymers and nanomaterials for enhanced biosensor selectivity and transduction. EuroBiotech J 4(4):184–206. https://doi.org/10.2478/ebtj-2020-0023

Ertürk G, Mattiasson B (2017) Molecular imprinting techniques used for the preparation of biosensors. Sensors 17(2):288. https://doi.org/10.3390/s17020288

Feigelman R et al (2017) Sputum DNA sequencing in cystic fibrosis: non-invasive access to the lung microbiome and to pathogen details. Microbiome 5(1):20

Fronczek CF, Yoon J-Y (2015) Biosensors for monitoring airborne pathogens. J Lab Autom 20(4):390–410

Ghouri F, Hollywood A, Ryan K (2020) 'There is no choice apart from antibiotics. . .': qualitative analysis of views on urinary infections in pregnancy and antimicrobial resistance. Health Expect 23(3):644–650. https://doi.org/10.1111/hex.13044

Gibson B, Wilson DJ, Feil E, Eyre-Walker A (1880) The distribution of bacterial doubling times in the wild. Proc Biol Sci 285:2018. https://doi.org/10.1098/rspb.2018.0789

Gill WP, Harik NS, Whiddon MR, Liao RP, Mittler JE, Sherman DR (2009) A replication clock for Mycobacterium tuberculosis. Nat Med 15(2):211–214

Grewal MK, Jaiswal P, Jha SN (2015) Detection of poultry meat specific bacteria using FTIR spectroscopy and chemometrics. J Food Sci Technol 52(6):3859–3869

Grisoli P et al (2009) Assessment of airborne microorganism contamination in an industrial area characterized by an open composting facility and a wastewater treatment plant. Environ Res 109(2):135–142. https://doi.org/10.1016/j.envres.2008.11.001

Guo Y et al (2015) Electrochemical immunosensor assay (EIA) for sensitive detection of E. coli O157:H7 with signal amplification on a SG–PEDOT–AuNPs electrode interface. Analyst 140(2):551–559. https://doi.org/10.1039/c4an01463d

Gupta R, Raza N, Bhardwaj SK, Vikrant K, Kim K-H, Bhardwaj N (2021) Advances in nanomaterial-based electrochemical biosensors for the detection of microbial toxins, pathogenic bacteria in food matrices. J Hazard Mater 401:123379

Hameed S, Xie L, Ying Y (2018) Conventional and emerging detection techniques for pathogenic bacteria in food science: a review. Trends Food Sci Technol 81:61–73. https://doi.org/10.1016/j.tifs.2018.05.020

Ho C-S et al (2019) Rapid identification of pathogenic bacteria using Raman spectroscopy and deep learning. Nat Commun 10(1):4927

Hospodsky D, Yamamoto N, Peccia J (2010) Accuracy, precision, and method detection limits of quantitative PCR for airborne bacteria and fungi. Appl Environ Microbiol 76(21):7004–7012

Hsiao P-K, Cheng C-C, Chang K-C, Yiin L-M, Hsieh C-J, Tseng C-C (2014) Performance of CHROMagar VRE medium for the detection of airborne vancomycin-resistant/sensitive *Enterococcus* species. Aerosol Sci Technol 48(2):173–183. https://doi.org/10.1080/02786826.2013.865833

Jamali AA, Pourhassan-Moghaddam M, Dolatabadi JEN, Omidi Y (2014) Nanomaterials on the road to microRNA detection with optical and electrochemical nanobiosensors. TrAC Trends Anal Chem 55:24–42. https://doi.org/10.1016/j.trac.2013.10.008

Juska VB, Pemble ME (2020) A critical review of electrochemical glucose sensing: evolution of biosensor platforms based on advanced nanosystems. Sensors 20(21). https://doi.org/10.3390/s20216013

Kalali B, Formichella L, Gerhard M (2015) Diagnosis of Helicobacter pylori: changes towards the future. Diseases 3(3):122–135. https://doi.org/10.3390/diseases3030122

Källenius G, Pawlowski A, Hamasur B, Svenson SB (2008) Mycobacterial glycoconjugates as vaccine candidates against tuberculosis. Trends Microbiol 16(10):456–462

Kuss S, Amin HMA, Compton RG (2018) Electrochemical detection of pathogenic bacteria-recent strategies, advances and challenges. Chem Asian J 13(19):2758–2769

Kwon H-J, Fronczek CF, Angus SV, Nicolini AM, Yoon J-Y (2014) Rapid and sensitive detection of H1N1/2009 virus from aerosol samples with a microfluidic immunosensor. J Lab Autom 19(3):322–331

Lee YH et al (2020) Maternal bacterial infection during pregnancy and offspring risk of psychotic disorders: variation by severity of infection and offspring sex. Am J Psychiatry 177(1):66–75

Lin H-H, Dowdy D, Dye C, Murray M, Cohen T (2012) The impact of new tuberculosis diagnostics on transmission: why context matters. Bull World Health Organ 90(10):739–747A

Ling S, Hui L (2019) Evaluation of the complexity of indoor air in hospital wards based on PM2.5, real-time PCR, adenosine triphosphate bioluminescence assay, microbial culture and mass spectrometry. BMC Infect Dis 19(1):646. https://doi.org/10.1186/s12879-019-4249-z

Liu Y, Cao Y, Wang T, Dong Q, Li J, Niu C (2019) Detection of 12 common food-borne bacterial pathogens by TaqMan real-time PCR using a single set of reaction conditions. Front Microbiol 10:222

Magana-Arachchi DN, Wanigatunge RP (2020) Ubiquitous waterborne pathogens. In: Waterborne pathogens. pp 15–42. https://doi.org/10.1016/b978-0-12-818783-8.00002-5

Mairhofer J, Roppert K, Ertl P (2009) Microfluidic systems for pathogen sensing: a review. Sensors 9(6):4804–4823

Meng X, Yang G, Li F, Liang T, Lai W, Xu H (2017) Sensitive detection of Staphylococcus aureus with vancomycin-conjugated magnetic beads as enrichment carriers combined with flow cytometry. ACS Appl Mater Interfaces 9(25):21464–21472

Millet J-P et al (2013) Factors that influence current tuberculosis epidemiology. Eur Spine J 22(S4):539–548. https://doi.org/10.1007/s00586-012-2334-8

Nguyen TT, Trinh KTL, Yoon WJ, Lee NY, Ju H (2017) Integration of a microfluidic polymerase chain reaction device and surface plasmon resonance fiber sensor into an inline all-in-one platform for pathogenic bacteria detection. Sensors Actuators B Chem 242:1–8. https://doi.org/10.1016/j.snb.2016.10.137

Pang B et al (2018) Development of a low-cost paper-based ELISA method for rapid Escherichia coli O157:H7 detection. Anal Biochem 542:58–62

Peng P et al (2017) Fabrication of an electrochemical sensor for Helicobacter pylori in excrement based on a gold electrode. Int J Electrochem Sci 12:9478–9487. https://doi.org/10.20964/2017.10.19

Perumal V, Hashim U (2014) Advances in biosensors: principle, architecture and applications. J Appl Biomed 12(1):1–15. https://doi.org/10.1016/j.jab.2013.02.001

Purwidyantri A et al (2016) Spin-coated Au-nanohole arrays engineered by nanosphere lithography for a Staphylococcus aureus 16S rRNA electrochemical sensor. Biosens Bioelectron 77:1086–1094

Rule AM, Schwab KJ, Kesavan J, Buckley TJ (2009) Assessment of bioaerosol generation and sampling efficiency based on Pantoea agglomerans. Aerosol Sci Technol 43(6):620–628. https://doi.org/10.1080/02786820902806709

Savas S, Altintas Z (2019) Graphene quantum dots as nanozymes for electrochemical sensing of in milk and human serum. Materials 12(13). https://doi.org/10.3390/ma12132189

Sawyer MH, Chamberlin CJ, Wu YN, Aintablian N, Wallace MR (1994) Detection of varicella-zoster virus DNA in air samples from hospital rooms. J Infect Dis 169(1):91–94

Seo SE et al (2019) Smartphone with optical, physical, and electrochemical nanobiosensors. J Ind Eng Chem 77:1–11. https://doi.org/10.1016/j.jiec.2019.04.037

Sharma A, Sharma N, Kumari A, Lee H-J, Kim T, Tripathi KM (2020) Nano-carbon based sensors for bacterial detection and discrimination in clinical diagnosis: a junction between material science and biology. Appl Mater Today 18:100467. https://doi.org/10.1016/j.apmt.2019.100467

Skládal P et al (2012) Electrochemical immunosensor coupled to cyclone air sampler for detection of Escherichia coli DH5α in bioaerosols. Electroanalysis 24(3):539–546. https://doi.org/10.1002/elan.201100448

Srivastava AK, Dev A, Karmakar S (2018) Nanosensors and nanobiosensors in food and agriculture. Environ Chem Lett 16(1):161–182. https://doi.org/10.1007/s10311-017-0674-7

Stetzenbach LD, Buttner MP, Cruz P (2004) Detection and enumeration of airborne biocontaminants. Curr Opin Biotechnol 15(3):170–174

Valera AE, Nesbitt NT, Archibald MM, Naughton MJ, Chiles TC (2019) On-chip electrochemical detection of cholera using a polypyrrole-functionalized dendritic gold sensor. ACS Sens 4(3):654–659

Vidic J, Chaix C, Manzano M, Heyndrickx M (2020) Food sensing: detection of spores in dairy products. Biosensors 10(3). https://doi.org/10.3390/bios10030015

Wahab RA et al (2020) On the taught new tricks of enzymes immobilization: an all-inclusive overview. React Funct Polym 152:104613

Wen C-Y et al (2013) One-step sensitive detection of Salmonella typhimurium by coupling magnetic capture and fluorescence identification with functional nanospheres. Anal Chem 85(2):1223–1230

World Health Organization (2013) Global tuberculosis report 2013. World Health Organization

Xu L, Shoaie N, Jahanpeyma F, Zhao J, Azimzadeh M, Al Jamal KT (2020) Optical, electrochemical and electrical (nano)biosensors for detection of exosomes: a comprehensive overview. Biosens Bioelectron 161:112222

Yanase Y et al (2014) Surface plasmon resonance for cell-based clinical diagnosis. Sensors 14(3):4948–4959

Zhang Y et al (2020a) Aptamer-modified sensitive nanobiosensors for the specific detection of antibiotics. J Mater Chem B Mater Biol Med 8(37):8607–8613

Zhang R, Belwal T, Li L, Lin X, Xu Y, Luo Z (2020b) Nanomaterial-based biosensors for sensing key foodborne pathogens: advances from recent decades. Compr Rev Food Sci Food Saf 19(4): 1465–1487

Zhou C, Zou H, Li M, Sun C, Ren D, Li Y (2018) Fiber optic surface plasmon resonance sensor for detection of E. coli O157:H7 based on antimicrobial peptides and AgNPs-rGO. Biosens Bioelectron 117:347–353

Zou Y, Liang J, She Z, Kraatz H-B (2019) Gold nanoparticles-based multifunctional nanoconjugates for highly sensitive and enzyme-free detection of E.coli K12. Talanta 193:15–22

Zuo X, Song S, Zhang J, Pan D, Wang L, Fan C (2007) A target-responsive electrochemical aptamer switch (TREAS) for reagentless detection of nanomolar ATP. J Am Chem Soc 129(5): 1042–1043

Nanosensors for Detection of Human Fungal Pathogens

22

Vandana Ghormade

Abstract

Worldwide, infections caused by human fungal pathogens are being reported increasingly and have inflicted large losses in terms human lives ~1.6 million annually. The rise in fungal pathogen infections is ascribed to the increased usage of antifungals, immunosuppressive drugs, and climate change which is conducive to fungal proliferation. Rapid detection of fungal pathogens plays an important role in mitigating their threat to public health. Currently, conventional practices of fungal disease detection are based on detection of symptoms and identification of causal organism using laboratory techniques. Conventional culture-based methods are time consuming and the methods like PCR, ELISA or HPLC, etc. require expensive equipment and skilled personnel in laboratory settings, leading to a delayed diagnosis. In the hospitals, radiological imaging of human subjects is used for detection, however this method lacks specificity. There is a lack of on-site detection devices for rapid and specific detection of fungal pathogens. Nanotechnology can contribute to diagnostics by downsizing of detection platforms and increasing the portability of devices. Incorporation of "nanoscale" aspect at the physical and material levels can significantly contribute to the development of nano-tools for improved sample processing, signal detection, and ease of handling. Portable devices such as lateral flow assays, nanopore devices, nanoarrays, lab-on-a-chip, nanobarcodes, and nano-detection kits will advance rapid on-site detection of human fungal pathogens and their subsequent management in the healthcare sectors.

V. Ghormade (✉)
Nanobioscience Group, Agharkar Research Institute, Pune, India
e-mail: vandanaghormade@aripune.org

S. Hameed, S. Rehman (eds.), *Nanotechnology for Infectious Diseases*,
https://doi.org/10.1007/978-981-16-9190-4_22

Keywords

Fungal pathogens · Human · Nanotechnology · Detection

22.1 Introduction

Globally human fungal infections have become more prevalent due to increase in numbers of immunocompromised patients. Mainly, immunocompromised patients suffering from cancer, AIDS, diabetes, and those undergoing surgery or organ transplant are prone to fungal infections (Nweze et al. 2012; de Pauw 2011). Human pathogens cause infections mostly among the immunocompromised patients that lead to serious mortalities. More than billion people are infected by human fungal pathogens that lead to ~1.6 million deaths annually (Almeida et al. 2019). Opportunistic fungal pathogens can attack the human body leading to either local or systemic infections. Dimorphic fungi *Candida, Histoplasma, Blastomyces,* or *Wangiella* spp. grow and thrive at body temperature and show the morphological transition from yeast to mycelium forms to cause infections. Filamentous fungi like *Aspergillus flavus* and *A. fumigatus* cause Aspergillosis due to lodging of spores in lung tissues.

Traditionally fungal pathogens are detected by culture-based methods which are tedious and time consuming. Other methods for confirmation of the pathogen are identification by ELISA, HPLC, or LCMS which are expensive and require skilled personnel and are not suitable for on-site detection.

Molecular methods for identification of the pathogen are available for a few pathogens and are not optimized for a wide range of pathogens. Currently, few fungal pathogenic organisms like *Candida, Aspergillus* can be diagnosed on molecular diagnostic based tools with high degree of sensitivity and specificity. However, PCR or ELISA methods require sophisticated instruments and cannot be applied in the field for rapid on-site detection. Moreover, reagents like enzymes for molecular biology and antibodies for serology, etc., are expensive and labile which limit the application of such methods in developing countries.

Nanotechnology can contribute to the rapid diagnostics through utilization of nano-scaled materials for miniaturization of the sampling and testing platforms, sample preparation, reducing the sample volumes, signal detection and amplification, ease of handling, and portability. Portable diagnostic systems such nanosensors, nanobarcodes, nano-detection kits can contribute immensely to the quick on-site detection of human fungal pathogens and towards the better management of human diseases. Here, we describe the concepts and current state of art of the nanotechnology applications in detection of plant and human fungal pathogens.

22.2 Fungal Pathogens and Current Ways of Their Detection

Systemic mycoses spreads throughout the body and is often caused by dimorphic fungi. These pathogens are represented by *Candida* spp., *Cryptococcus neoformans, Blastomyces dermatitidis, Histoplasma capsulatum, Sporothrix schenckii, Paracoccoidioides brasiliensis,* etc. Dimorphic fungi display the ability of reversible morphological change from yeast to hyphae forms. Usually the hyphal form permits penetration into body tissues while yeast form spreads to other tissue via the circulatory system. Filamentous fungi like *Aspergillus fumigatus*, *A. flavus*, *A. terreus,* etc. cause infection due to lodging of the spores in the lung tissues. These fungi form mycelial pellets due to filamentous growth in the lung and can cause invasive aspergillosis by dissemination.

Skin mycosis is another category of fungal infections caused by dermatophytic fungi at the cutaneous or subcutaneous levels. Cutaneous fungal infections are caused by invasion of the superficial layers of the skin, hair, and nail. Cutaneous mycoses termed as tineas on the body foot or groin are caused by three genera of dermatophytes like *Trichophyton, Epidermophyton, Microsporum* and cause infections on different body sites such as tineas pedis (foot), tinea cruris (groin), tinea corporis (whole body).

Penetration of epidermis and dermis by fungus to infect the underlying skin layers causes subcutaneous mycoses. Sporotrichosis, mycetoma, chromoblastomycosis, rhinosporidiosis, zygomycosis, pheohyphomycosis, and lobomycosis are the most common subcutaneous mycoses.

Malassezia spp. are basidiomycetous yeasts and the causal agent of head and neck dermatitis, seborrheic dermatitis, folliculitis, and pityriasis versicolor. These fungi are a part of the normal body flora and cause infection on lowering of the host immunity status.

Apart from these pathogenic fungi, several genera of fungi account for 1–2% of all fungemia cases. These include hyaline filamentous fungi such as *Fusarium*, *Acremonium* and *Scedosporium*, *Paecilomyces*, dematiaceous fungi like *Alternaria*, *Bipolaris,* and *Exophiala,* rare yeast like *Rhodotorula*, *Malassezia,* and *Geotrichum* and zygomycetes like *Rhizopus*, *Mucor,* and *Lichtheimia* (Meletiadis and Roilides 2013).

Mucormycosis and aspergillosis cases are highly reported during the current COVID-19 pandemic. Several of these cases were reported from the Indian subcontinent and were linked with the administration of steroids. Cryptic fungal pathogens are difficult to identify and treat. Recently, an outbreak of the rare pathogen, *Exserohilum rostratum* was reported to cause fungal meningitis infections and 61 deaths in patients due to contaminated steroid preparations (Katragkou et al. 2014; Larone and Walsh 2013). Similarly, in 2011 several cases of necrotizing mucormycosis were caused by a soil fungus called *Apophysomyces trapeziformis* when contaminated debris were air-borne when a tornado hit Joplin, Missouri (Fanfair et al. 2012). Identification of this fungus was only possible by a coordinated response from the Centers for Disease Control and Prevention. These problems elucidate a prevalent issue in medical mycology, which is the lack of trained

personnel and sophisticated equipment for correct fungal identification (Steinbach et al. 2003).

Occurrences of human fungal pathogens are being reported more frequently which are usually intensified by reduced host immunity, increasing fungal resistance and the climate changes. Often, rare fungal genera, that may account for ~10% of all opportunistic fungal infections, display clinical manifestations similar to that of the common fungal pathogens which makes diagnosis difficult. Due to these challenges, rare fungal pathogens lead to increased mortality rates (60–100% mortality) despite antifungal therapy (Walsh et al. 2004; Fleming et al. 2002).

Mycological diagnosis is carried out in clinical samples by culturing for presence of fungal pathogen, molecular detection of related markers, and serological analysis for immunoreactive components. Pathologists employ the prevailing practice of symptomatic detection of fungal infections to detect the pathogens. Lung infections are screened with the help of radiological or high resolution computed tomography scans.

Traditionally, diagnosis of fungal pathogens is carried out by culturing the fungus from clinical specimens from serum, oral lavage, skin or membrane scraping, or broncho-alveolar lavage (BAL) for presence of fungal pathogen. In case of lung infections such as *Aspergillosis, Blastomycosis, Histoplasmosis,* the diagnosis of BAL requires a tedious procedure of bronchoscopy for sample collection from the patients which displays low sensitivity (Barton 2013; Guarner and Brandt 2011; Klont et al. 2001). Detection of the organism by blood culture is slow and insensitive (Barton 2013; Brown et al. 2012; Kousha et al. 2011). Most frequently, the causative organism is isolated from sputum or serum samples.

Culture-based techniques are cost-effective and are easy to perform, however, they are time consuming and often require minimum of 2–7 days for growth on appropriate media and temperature for positive confirmation of infection (Mortensen et al. 2011). Further antifungal-susceptibility testing may be initiated on isolation of the positive culture of causative organism. Automated BACTEC blood culture systems (Becton Dickinson, NJ, USA) are utilized for efficient isolation and detection of human fungal pathogens at some centers. However, these systems lack multi-center validation (Rosa et al. 2011). Mostly, radiographic and clinical tests support the culture-based diagnostic methods for human fungal pathogens as it is not sufficient to distinguish between invasive infections and colonization (Horvath and Dummer 1996).

It is difficult to distinguish between different fungal infections by microscopic examination of clinical specimens due to the close similarities in hyphal growth in tissues (Barton 2013; Denning 1998; Kousha et al. 2011; Meersseman et al. 2008; Paugam et al. 2010). Further, employing specific dyes and stains during microscopic examination of clinical samples has improved the diagnosis of culture-based detection by 15–20% (Denning 1998). Detection of *Aspergillus* is highly improved by staining the clinical specimens with fluorescent stains such as Calcofluor white or Blankophor mixed with potassium hydroxide (KOH, 10–20%). Morphological characters of cultures in conjunction with taxonomic keys are used for identification of the pathogenic fungi (Guarner and Brandt 2011).

Histopathological examination of biopsy samples is often employed to establish the infection. Fungal hyphae often show angioinvasion that causes hemorrhage or necrotic patches in the surrounding tissue (Guarner and Brandt 2011). Histopathological diagnosis for presence of fungal pathogens is non-specific and faces the limitations such as variations in stain quality, sampling error, and inconsistent interpretations (Hayden et al. 2002). Specific diagnosis during histopathological examination is challenging as most invasive fungal infections present similar indications with lesions and invading hyphae (Merz et al. 1988). Specific diagnosis could differentiate *Aspergillus* hyphae from other fungal pathogens by immunohistochemistry based staining of biopsy tissue with a specific monoclonal/polyclonal antibody labeled with fluorescent probe or peroxidase (Fukuzawa et al. 1995; Jensen et al. 1997; Kaufman et al. 1997; Phillips and Weiner 1987; Piérard et al. 1991).

The fungal cell wall component 1,3-β-D-glucan (BDG) has great importance in fungal pathogenesis (Wright et al. 2011). It is the most abundant fungal cell wall polysaccharide and fungi release BDG during their growth, a feature that is useful in its early non-specific detection. BDG is detectable from medically important fungi such as *Aspergillus* spp., *Fusarium* spp., *Candida* spp., *Pneumocystis jiroveci,* and *Acremonium* spp. (Hope et al. 2005; Tran and Beal 2016). However, *Cryptococcus*, *Blastomyces,* and zygomycetous fungi are not detected by this test. Presently, the US FDA approved Fungitell BDG determination assay is available for non-invasive diagnosis of fungal infections. Fungitell assay is widely used clinically and has a limit of detection (LOD) of 1 pg mL^{-1} (Hope et al. 2005; Wright et al. 2011). Due to the ambiguous nature of this test, it is usually used in conjunction with other tests for early diagnosis of fungal infections (Pazos et al. 2005; Theel and Doern 2013).

Another cell wall component, galactomannan (GM) is the major constituent of *Aspergillus* cell walls and is released through the growing hyphal tip during early infection. GM contains the immunoreactive β-(1-5)-galactofuranosyl (gal*f*) moiety that is useful in serological detection of aspergillosis infections (Mennink-Kersten et al. 2004, 2006). Presently, GM detection is performed with a commercial Platelia™ *Aspergillus* Ag ELISA kit (Bio-Rad, Marnes-la-Coquette, France) with serum, BAL, urine, and cerebrospinal fluid samples (Chong et al. 2016; Duettmann et al. 2014; Wheat 2003).

The Platelia assay includes a monoclonal antibody EB-A2 (IgM) specific towards the gal*f* side chains of GM and displays the limit of detection of 1 ng mL^{-1} of GM with serum (Mennink-Kersten et al. 2006; Stynen et al. 1992; Verdaguer et al. 2007). Currently, early diagnosis of invasive aspergillosis is confirmed by the Platelia assay, in conjunction with chest CT scans, due to the reported assay heterogeneity in sensitivity and specificity (Dixon et al. 2011; Busca et al. 2006; Maertens et al. 2001). HR-CT scans of lungs reveal typical radiological abnormalities such as well-circumscribed lung lesions with/without a halo sign or a reversed halo sign, air-crescent sign, cavity or ground-glass attenuation in patients afflicted with Aspergillosis, Coccidioidomycosis, Cryptococcosis (Blum et al. 1994; Caillot et al. 2001; Georgiadou et al. 2011; Jin et al. 2017; Orlowski et al. 2017; Kuhlman et al. 1985). However, radiological HR-CT chest scans are unable to differentiate between the

different invasive pulmonary mold infections and are non-specific (Chamilos and Kontoyiannis 2006; Lee et al. 2005).

Recently, the highly sensitive and discriminative molecular detection using 5S/18S ribosomal RNA displayed accurate identification and differentiation of distinct fungal infections. The in situ hybridization with sequence-specific DNA probes for rRNA demonstrated 93% sensitivity and 100% specificity (Hayden et al. 2002).

Another diagnostic method involving the molecular approach for detection of nucleic acid by polymerase chain reaction (PCR) is a sensitive and specific test for fungal pathogens. Several PCR kits such as *Asp*ID® multiplex PCR test, MagicPlex™ assays, Light cycler® Septi*Fast,* and MycAssay *Aspergillus* PCR assay having the detection limit of 1–10 fg DNA are available commercially for detection of invasive aspergillosis (Hope et al. 2005).

Rapid identification of the pathogenic fungus like *Cryptococcus*, *Blastomyces,* and *Histoplasma* can be performed with AccuProbe (Hologic) test having a single stranded DNA probe with a chemiluminescent label. Identification of the fungi could be performed with from blood cultures with >98% sensitivity and specificity (Wickes and Wiederhold 2018). However, this kit showed false positives with other fungi. *Candida* spp. was detected with >96% sensitivity from blood cultures on basis of nested multiplex PCR and DNA melt curve analysis with BioMerieux BioFire FilmArray (Wickes and Wiederhold 2018). Although molecular methods are sensitive, they are unable to discriminate between colonization and invasive infection (Hayette et al. 2001; Kousha et al. 2011). In addition, the method is costly due to molecular reagents and equipment required. When combined with antigen detection, the nucleic acid detection by PCR has the potential for early diagnosis of fungal infections. Furthermore, detection of genetic markers associated with antifungal resistance may help in active treatment (Barnes and White 2016).

A culture free method based on Single MOLecule Tethering or SMOLT has been described by Cheng et al. (2020). SMOLT is an amplification-free and purification-free molecular assay that can detect microorganisms in body fluids with high sensitivity using a robust and rapid optical approach without the need of culturing. Micron-size beads are tethered by DNA probes that are between 1 and 7 microns long and their displacement generates the signal of SMOLT. This method is being preferred as it allows the rapid diagnosis of infectious diseases by analysis of body fluids (blood, urine, and sputum) for presence of microbial nucleic acids. SMOLT has the ability to detect nucleic acids threefold faster in body fluids at sub-femtomolar concentrations and microorganisms in blood at 1 CFU mL^{-1} (colony forming unit per milliliter). The clinical utility of SMOLT was demonstrated by multiplex and simultaneous detection of sepsis-causing *Candida* species directly in whole blood.

22.3 Nanotechnology for Detection of Human Pathogenic Fungi

Nanotechnology can contribute to human healthcare by improving the detection of human fungal pathogens. Nanoparticles can aid the development of various detection platforms based on colorimetric, fluorometric enzymatic, and electrochemical assays based on their unique size and shape dependent physical and chemical properties (Ghormade et al. 2011). Different nanoparticles such as semiconductors, noble metals, and metal oxides can be harnessed for diverse sensing and imaging application. Development of specific detection methods by signal amplification involves the conjugation of nanoparticles as labels to biomolecular recognition elements such as antibodies, peptides, aptamers, DNA, or RNA (Fig. 22.1). Gold nanoparticles display colorimetric change due to aggregation of the gold nanoparticles and the associated color change from red to blue can be used as an indicator for presence of the target molecules (Fig. 22.1b). Several assay platforms can be developed depending on the mode of signal amplification and detection. Nanobiosensors such as array biosensors and lab-on-a chip devices can be coupled with surface plasmon resonance, fluorescence, or optical detection (Fig. 22.1e, f). These devices can compress the entire work flow from sample preparation, signal detection, and amplification onto a single platform allowing rapid detection**.** These platforms can handle detection of multiple targets by multiplexing. Lateral flow assays have the ease of visual detection and can be easily used without the need of any sophisticated instruments.

22.4 Bioimaging

Bioimaging for presence of the pathogenic fungi in the tissues or patient samples is a visual method for detection of the causal organism. A variety of nanoparticles labels such as gold nanoparticles, carbon dots, or quantum dots are employed for bioimaging for their colorimetric or fluorescent signals. These nanoparticles can be functionalized with ligands for recognition of the pathogenic fungi (Fig. 22.1h).

Aspergillus niger spores cause allergy and their detection is of interest due to their potentially negative impacts on personal and public health. A rapid and sensitive detection of allergenic *Aspergillus niger* fungal spores was demonstrated by a colorimetric strategy based on interactions between fungal spores and gold nanoparticles (AuNPs) (Lee et al. 2021). An *A. niger* specific spore-binding peptide ligand was immobilized on the AuNPs surfaces. Functionalization of AuNPs with peptide ligands promoted their rapid binding to *A. niger* spores that led to a visible color change of the supernatant after sedimentation of the spores. The color change was detected using a smartphone-based image analysis application that resulted in rapid detection of *A. niger* spores in less than 10 min. The simple colorimetric approach had a high sensitivity of ∼50 spores.

In another study, Pandey et al. (2020) developed carbon dots (C-Dots) and demonstrated their application for bioimaging. These C-Dots exhibited negligible

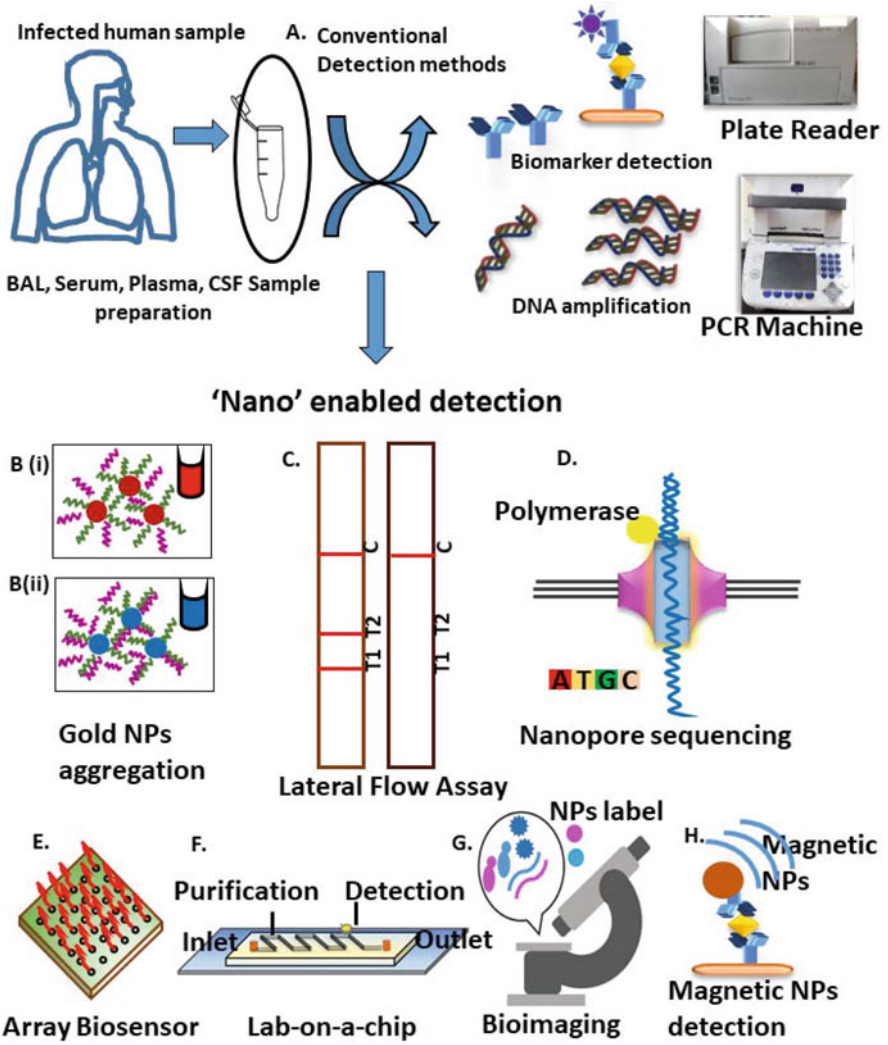

Fig. 22.1 Application of nanotechnology for detection of human fungal pathogens. Schematic to compare the traditional method for detection with nano enabled detection methods. (**a**) The infected human samples are processed for extraction of pathogenic protein or DNA detection and detected with ELISA reader and PCR machine. (**b**) The extracted samples can be directly detected by colorimetric assay for detection change in color from **b**(i) Red, due to colloidal NPs and **b**(ii). Purple, due to aggregation of gold nanoparticles due to binding of capture molecules with the target. (**c**) LFA for rapid detection, (**d**) Detection at the nanoscale in nanopore DNA sequencer where a strand of DNA passes through a nanopore which is gated by polymerase enzyme to unravel the double strand to a single strand. Further, the ionic current is measured when the molecule exits the nanopore and translated into the DNA sequence, (**e**) Array Biosensor and (**f**) Lab-on-a-chip devices where the signal is transduced by different biological molecules which can be immobilized on the sensor platform, amplified, and then detected by different methods, i.e., Fluorescence, SPR, Spectrophotometry, Voltammetry, (**g**) (**h**) Detection with Magnetic NPs Bioimaging with NPs as labels to identify the fungal organism by microscopy

cytotoxicity and were efficiently internalized by the fungal pathogen, *Candida albicans,* cells. The C-Dots were robust and biocompatible and can be employed as fluorescent probes for bioimaging. The C-Dots can be decorated with appropriate cell recognition molecules making them more applicable for targeted cellular imaging and live imaging applications.

22.5 Lateral Flow Assays

The advent of "nano" enabled lateral flow assays has reduced the time and dependence on expensive equipment required for the detection of human pathogenic fungi (Fig. 22.1c). Direct and rapid detection can enable the appropriate control of the diseases by application of appropriate antifungal treatments. Paper based assays are user friendly and can be carried out in the lateral assay or the dot-blot assay formats (Kolosova et al. 2007; Ye et al. 2010). These assays are rapid, cost-effective and permit easy visual detection aided by gold nanoparticles. Gold nanoparticles (AuNPs) are used extensively as a colorimetric label as they are easy to synthesize, functionalize, and exhibit good stability (Nolan et al. 2019; Zhao et al. 2018).

The human fungal pathogen *Aspergillus fumigatus* and other species such as *A. flavus, A. terreus* cause aspergillosis in immunocompromised patients. The infections are manifested in a varying range of clinical symptoms from allergic reactions to systemic invasive infection depending on host immunity. Rapid diagnostics of aspergillosis using Platelia ELISA for *Aspergillus* antigen is currently being used as a non-culture-based testing for identification and treatment of patients (Jenks et al. 2020). However, this test faces significant drawbacks such as variable performance, high cost, and requirement of sophisticated instrumentation. Recently, "sōna *Aspergillus* Galactomannan LFA (GM-LFA)," a lateral flow assay was commercialized by IMMY, Norman, OK, USA for *Aspergillus* GM detection in serum and BAL samples (Jenks et al. 2019a, b). The LFA uses a proprietary mix of two different mAbs: the ME-A5 13 279 human IgG monoclonal and an undisclosed proprietary mAb with an unknown target. The limit of detection for GM-LFA was 1.7 ng mL^{-1} and 2.25 ng mL^{-1} in serum and BAL, respectively. Testing of respiratory BAL samples with GM-LFA generated 83–92% sensitivity and 91–92% specificity (Mercier et al. 2019). The evaluation of GM-LFA showed good performance for hematological malignancy and non-neutropenic patients where the ELISA GM testing was not feasible (Jenks et al. 2019a, b). Point-of-care testing with GM-LFA reduced the time required for diagnosis for invasive pulmonary aspergillosis, a recognized complication among ICU patients (Mercier et al. 2020). The GM-LFA yielded a good test performance for 178 ICU patients with sensitivity of 0.88–0.94 and specificity of 0.81 (Mercier et al. 2020).

Recently, another nano-immunodiagnostic dot-blot assay for invasive aspergillosis was reported for rapid detection of galactomannan from serum and BAL samples. The sensitive, rapid dot-blot assay employed gold nanoparticles conjugated polyclonal antibodies against galactomannan and had a LOD of 1 pg/mL. The dot-blot assay was more sensitive in comparison to the Platelia ELISA for *Aspergillus* having

a cut-off value of 0.5 associated with 1 ng/mL. Evaluation of the assay with 109 clinical samples showed overall assay accuracy of high sensitivity of 80%, specificity of 93%, and an overall assay accuracy of 89%. Such diagnostic assays have good potential for use in rapid, specific, sensitive, on-site diagnosis of invasive aspergillosis under resource poor settings (Rawal et al. 2019; Mercier et al. 2020).

Dufresne et al. (2012) developed another lateral flow device (LFD) using monoclonal antibodies (MAb476) against the galactofuranose antigen of *Aspergillus* reported the detection of GM in urine. The circulating *Aspergillus* GM was detected up to 100 ng mL^{-1} by renal excretion in urine from IA patients (Bennet et al. 1985; Dupont et al. 1987; Haynes et al. 1990).

An immunochromatographic LFD for invasive aspergillosis (IA) was developed by Thornton (2008) which is now marketed by OLM Diagnostics, Newcastle upon Tyne, UK as the "*Aspergillus* Lateral-Flow Device (*Asp*LFD)." The *Asp*LFD employs a mouse monoclonal antibody (mAb JF5, IgG3) specific to a cell wall-associated *N*-linked glycoprotein antigen of *Aspergillus.* A limit of detection of ~35 ng mL^{-1} in serum and BAL samples has been described (Thornton 2008; Thornton et al. 2012). The performance of *Asp*LFD for immunocompromised patients reported a moderate to high (38–100%) sensitivity and (63–100%) specificity. The on-going antifungal treatment affected the detection as was reported in case of Platelia ELISA earlier (Prattes et al. 2014). The cross-reactivity of both *Asp*LFD and Platelia ELISA to a few *Penicillium* species remains an issue (Thornton 2010).

*Asp*LFD detected most *Aspergillus* species and a few other closely related species like *Emericella nidulans*, *Eurotium amstelodami*, and *Neosartorya fischeri*. The *Asp*LFD displayed weak cross-reactivity towards *Paecilomyces variotii* antigens (Thornton 2008; Thornton 2013). The *Asp*LFD JF5 mAbs did not react with other invasive fungal pathogens such as *Candida albicans*, *Cryptococcus neoformans*, *Fusarium solani*, *Rhizopus oryzae*, *Pseudallescheria boydii, Trichosporon,* and *Scedosporium*.

A lateral flow immunoassay (LFIA) was developed by He et al. (2016) for the detection of Candidiasis. *Candida albicans* is the predominant cause of invasive candidiasis and is of common nosocomial occurrence. The enolase metallozyme, present in the *Candida* cell walls, converts 2-phosphoglycerate into phosphoenolpyruvate and is reported to elicit an antibody response in infected hosts. The antibody towards the recombinant enolase was utilized as the capture agent and detection was performed with gold nanoparticles labeled with anti-human IgG.

The LFIA was tested with sera from 38 clinically proven cases and 50 healthy control subjects. The LFIA was in agreement with the standard ELISA test and specificity and sensitivity of the LFIA were reported to be 98.2 and 84.8%, respectively. The LFIA test was proposed for serological surveillance of invasive candidiasis in resource poor settings.

Cyrptococcosis is caused by breathing of *Cryptococcus neoformans* or *Cryptococcus gatti* capsules into the lungs from debris, litter, or excreta of birds. Cryptococcal meningitis is caused by the dissemination of the yeast *C. neoformans* or its spore from the lung to the central nervous system. Cryptococcal cases account for a million cases per year globally and cause 13–14% mortality among

immunocompromised patients (Park et al. 2009). The CrgAg lateral flow assay (CrAgLFA) employs a cocktail of two monoclonal antibodies to detect the four capsular serotypes of cell wall antigen glucuronoxylomannan rapidly in 10 min using a small, lightweight, dipstick format. The CrAgLFA manufactured by Immy, Norman, OK, USA received approval from US FDA in 2011 and detects the cryptococcal antigen with qualitative or semiquantitative results. The assay sensitivity was greater than the commercially available LA and EIA assays for the CrAg of A, B, C, and D serotypes.

LFA meets the ASSURED (affordable, sensitive, specific, user friendly, rapid, deliverable) criteria of WHO for rapid diagnostic tests. The assay is user friendly and recommended for use with serum or urine samples (Kozel and Bauman 2012). Vidal and Boulware (2015) employed the CrgLFA to detect asymptomatic patients requiring pre-emptive antifungal treatment thus preventing symptomatic infection and contributing in better disease management.

Another LFA for cryptococcosis detection was developed by Biosynex CryptoPS LFA (Biosynex, Paris, France) and is also marketed by Bio-Rad (Hercules, CA). CryptoPS LFA is validated with a study involving 186 serum/plasma and 23 CSF samples from Cameroon and yielded 78% sensitivity in serum, 92% in plasma, and 100% in CSF in comparison to the Immy CrAg LFA (Temfack et al. 2018). Good specificity was (100%) observed for all sample types. However, further validation is required for CSF specimens and serum specimens with low titers (Temfack et al. 2018).

StrongStep, another cryptococcosis detection LFA is developed by Liming Bio, China and was validated by a two-site study in Uganda where 143 CSF and 167 plasma samples were tested in comparison to the Immy CrAg LFA and CSF culture (Mpoza et al. 2018). The StrongStep LFA showed good performed with CSF samples showing 100% sensitivity and 98% specificity (Mpoza et al. 2018). However, the performance faced substantial challenges regarding specificity with plasma samples (90%).

The Dynamiker CrAg LFA and FungiXpert Cryptococcal Capsular Polysaccharide K-Set developed in China have not received approvals in Europe or the USA. The diagnostic performance of the Dynamiker CrAg LFA compared to the IMMY CrAg LFA as the reference standard (Kwizera et al. 2021). The sensitivity of Dynamiker CrAg LFA was 98% in serum, 100% in plasma, 100% in CSF from 450 symptomatic patients (150 serum, 115 plasma, 100 cerebrospinal fluid), and 96% in serum from 113 serum samples from asymptomatic patients. Although the Dynamiker CrAg LFA displayed excellent sensitivity, the specificity varied from 61 to 86% particularly when tested on serum and plasma. FungiXpert Cryptococcal Capsular Polysaccharide K-Set is manufactured in China and does not have published validation studies.

Progressive disseminative histoplasmosis (PDH) is caused by *Histoplasma capsulatum* among immunocompromised patients. Histoplasmosis causes substantial mortality among HIV patients especially in regions where access to antiretroviral therapies and diagnostic testing are limited (Cáceres et al. 2019). The commercial Miravista Diagnostics *Histoplasma* antigen LFA was evaluated for diagnosis of

PDH with 75 serum samples (Cáceres et al. 2019). The test displayed a high sensitivity (92%) and specificity (94%) and required minimal laboratory equipment. Cross reactivity was reported with patients diagnosed with paracoccidioidomycosis.

Fungal melanonychia or nail infection is caused by the fungal pathogen *T. rubrum* or sometimes by the non-dematiaceous *Aspergillus niger,* and a gold nanoparticles based rapid detection system was developed for rapid detection (Sojinrin et al. 2017). The aggregation of colloidal gold nano particles leads to color change from red to purple (Fig. 22.1b). The color change correlated to the presence of the *A. niger* fungal cells that was confirmed by Raman spectroscopy (Sojinrin et al. 2017).

Dermatophytosis is a common transmissible disease showing development of skin lesions and is caused by filamentous fungi known as dermatophytes, including several genera and various species, as described earlier. The accurate diagnosis of skin lesions for presence of dermatophytes requires lengthy and tedious conventional laboratory diagnostics. A recently developed monoclonal antibody-based lateral flow was analyzed in 222 samples for onychomycosis diagnosis in comparison to the traditional microscopic examination and PCR method. The accuracy obtained from the three compared methods was 92.5%, 90.5%, and 76.6%, respectively (Tsunemi and Hiruma 2016; Aboul-Ella et al. 2020). In case of dermatophytes the local skin flora has to be taken in account while collection of the sample for testing may yield false positives.

22.6 Rapid Isothermal Amplification Assay

Molecular identification of fungal pathogens is specific and sensitive. However, these techniques often involve the use of expensive equipment for polymerase chain reaction amplification of the genetic sequences. Recently, isothermal amplification technologies are developed and sought after for their cost-effectiveness as they avoid sophisticated thermal cycling equipment. The recombinase polymerase amplification (RPA) shows improved specificity as compared to the loop-mediated isothermal amplification (LAMP) and can be performed with minimal training (Daher et al. 2016; Dai et al. 2019). RPA technology depends on the recombinase activity of the enzyme to open the double strands of DNA molecules and the strand-displacing activity to amplify DNA targets. RPA can be carried within 20–30 min at the temperature range of 37°–42 °C (Daher et al. 2016). The end-point of the isothermally amplified DNA target can be analyzed by gel electrophoresis or fluorescent nucleic acid staining. Recently RPA reaction has been combined with lateral flow assay (LFA) to make “instrument-free” signal readouts possible for pathogen detection (Qi et al. 2018). The signals are detected visually by a colorimetric signal due to the specific interaction of gold nanoparticles (AuNPs) with the labeled isothermal amplification products, colored signals are observed with the naked eye. Such on-site detection of fungal pathogens is important for the timely implementation of disease management and treatment strategies.

Definitive diagnosis of cryptococcal meningitis is carried out by identification of causal organism from patient cerebral spinal fluid. Conventional methods for identification of *Cryptococcus neoformans* or *C. gattii* such as India ink staining and microbial culture are time consuming and tedious. A lateral flow assay was developed in tandem with recombinase polymerase amplification (LF-RPA) to detect the specific DNA sequences of *Cryptococcus* spp. (Ma et al. 2019). The assay was specific, detected 1 pg of pathogen DNA and did not show any cross-reactivity with other pathogens. The diagnostic performance of the LF-RPA assay was evaluation with 114 clinical specimens in comparison to CrAg Lateral Flow Assay. The LF-RPA assay displayed a high sensitivity (95%) and specificity (95%). The rapid, visual, and accurate detection of *Cryptococcus* spp. in cerebral spinal fluid by sensitive and specific LF-PRPA assay may prove to be a handy diagnostic method for clinical preliminary screening of cryptococcal meningitis. Further analysis of the method is being carried out by the authors for plasma, serum, and whole blood samples.

Recently, Zhao et al. (2019) employed the multiple cross displacement amplification (MCDA) method with gold nanoparticles based lateral flow biosensor (LFB) to detect the organism sensitively. MCDA is an isothermal amplification technology similar to LAMP but with higher sensitivity and the internal transcribed space II regions of *C. albicans* were amplified. The amplification reaction products were detected visually on the LFB without the need of sophisticated equipment. The simple, specific MCDA-LFB assay was highly sensitive with detection as low as 200 pg of pathogen DNA and has potential for the rapid detection of Candidiasis.

These approaches are very promising for the clinical diagnosis and have potential for the simultaneous detection of different fungal pathogens.

22.7 Portable Genome Sequencer (Nanopore Sequencing System)

Davis Deamer at University of California and George Church and Daniel Branton of Harvard University pioneered the Nanopore technology which is commercialized from Oxford Technologies. This innovative sequencing technology is based on the direct electronic analysis of DNA while it moves through the protein nanopore (Fig. 22.1d). The α hemolysis protein, from the bacterial channel protein family, serves as a nanopore to permit a single strand of DNA, anchored by the DNA polymerase, to move through it (Bayley 2015). A polymer bilayer membrane, holding the protein nanopores, is stretched over a microwell and is held in a sensor chip for measurement of the ionic current during the passage of DNA bases through the nanopore. The commercial real time DNA and RNA sequencer, MinION is available as a handy, portable sequencer. This application of this technology was reported to confirm the outbreak of nosocomial hospital borne infections caused by human fungal pathogen *Candida auris* by the rapid genome analysis (Rhodes et al. 2018). Nanopore sequencing was instrumental in identification of the etiological diagnosis of lower respiratory tract infections by fungal strains namely *C. albicans*

and *Candida cheilosis* (Chan et al. 2020). Further, the Minion nanopore technology could detect the resistance alleles and the Asian origin of the resistant *Candida auris* strain during the recent outbreak in the UK.

This technology is gaining acceptance, however the main challenges faced by nanopore sequencing are the control of the speed with which the DNA strand passes through the nanopore, as this is related with the read length and the quality of the data generated (Clarke et al. 2009). Though nanopore sequencing platform faces unique challenges this platform is simple and straightforward and holds potential.

During the outbreak of an unexplained disease in Guyana in March 2019, the evidence for *Histoplasma* infection was first provided by the nanopore platform with lung, brain, and blood serum samples (Wang et al. 2019). In comparison to the NGS systems, MinION nanopore technology is portable, handy, simple to use, rapid and thus is highly applicable for real-time sequence analysis. Although the NGS platforms like MiniSeq and MGI2000 systems worked slower (24 h) than nanopore sequencing (13 h), they show a higher sensitivity and higher detection of *Histoplasma* (Wang et al. 2019).

Nanomaterials are crucial for development of fast and accurate biosensors. New hybrid bioorganic-inorganic materials combine the potential of nanomaterials with the recognition, selectivity, and sensitivity of biomolecules (Castillo et al. 2017). Nanoporous anodic alumina (NAA) is the material of choice based on its highly suitable features like biocompatibility, surface tunability, and high loading capacity. Further, easy functionalization of NAA surface is possible to create "molecular gates" with biomolecules with the aluminum oxide chemistry. Pla et al. (2021) proposed the nanoporous anodic alumina scaffold for specifically recognition of *C. auris* genomic DNA. The fluorescent indicator rhodamine B was added to NAA and its pores were blocked with different oligonucleotides capable of recognizing the fungus. DNA recognition controlled the gate opening and cargo delivery. The NAA platform could detect *C. auris* at a concentration as low as 6 CFU/mL in clinical samples in 1 h without any prior DNA extraction or amplification steps.

22.8 Array Biosensors

Array biosensors are detection platforms that can detect multiple targets or organisms simultaneously and usually integrated with surface enhanced RAMAN spectroscopy (SERS) for improved signal detection (Fig. 22.1e). The detection relies on the enhancement of signals from biomolecules in close proximity of nanostructured surfaces based on their electromagnetic and chemical enhancement.

Identification of the fungi human pathogenic fungi *Aspergillus flavus* causing invasive aspergillosis, *Scopulariopsis brumptii* causing phaeohyphomycosis/onychomycosis, *Candida krusei* causing candidiasis and the dermatophytic fungus *Trichophyton rubrum* was based on their specific SERS signals rapidly A silver nanoparticles coated waveguide surface was used for detection of cell constituents like protein, nucleotides, and cell wall components like chitin, β 1-3 glucans, and galactomannan by analyzing the SERS signals (Witkowska et al. 2016).

Simultaneous, label free, rapid identification of the fungi was successfully carried out with the SERS based array biosensor and the multiple detection was possible in conjunction with the principal component analyses.

Often clinical samples show the presence of multiple pathogens and array biosensors are useful for the practical diagnosis of infectious diseases. The pathogenic fungi *Aspergillus fumigatus*, *Candida glabrata*, *Candida krusei,* and *Cryptococcus neoformans* account for >10% of septicaemia occurrences and cause serious opportunistic infections in immunocompromised patients (Richardson 2005). To identify these pathogenic fungal DNAs, four kinds of capture probe DNAs (Asper03, Cgla01, Ckru001, and Cne12) were labeled with Cy5, a RAMAN dye showing resonant vibration at 633 nm (Yoo et al. 2011; Kang et al. 2011). The nanowire sensor was fabricated by placing 4 Au NWs, each immobilized with a different capture DNAs, on a Au film. The SERS platform was assisted with an exonuclease III for target DNA recycling. After addition of multiple probe DNAs to the target DNA solution, only the complementary probe DNA is selectively digested by exonuclease III, leading to a decrease in its concentration. The digestion process is repeated by recycling of target DNAs. The DNA detection was significantly improved to a limit of 100 fM by uniting the NW-on-film sensor with the target recycling reaction. The SERS platform permitted identification of multiple pathogen DNAs in a single step for improved disease diagnosis.

The silver nanoparticles enhanced SERS platform was integrated on a chip with the dielectrophoretic (DEP) method for cell separation for rapid detection multiplex *Candida* detection. Cheng et al. (2007) fabricated a microfluidic chip with 3D DEP gates for deflecting and separating fungal *C. albicans*, and bacterial *E. coli*, *Lactobacillus* pathogen cells into channels based on their negative DEP mobility. Further identification was based on collection of the silver nanoparticles enhanced SERS signals.

22.9 Lab-on-a-Chip

Lab-on-a-chip is an attractive diagnostic devices as it compresses the pathogen isolation and identification workflow on a single platform. As a detection device, lab-on-a-chip incorporates sample preparation, signal amplification, and detection on a single chip (Fig. 22.1f). The human pathogenic fungus, *Candida* was detected by a platform combining the single wall carbon nanotube (SWCNT) and field effect transistors for signal transduction. One dimensional SWNTs are suitable as biosensors for molecular recognition as they display an improved signal transduction ability and are easy to functionalize with a variety of biomolecules. A SWCNT-field effect transistor was fabricated by chemical vapor deposition on a silicon chip and screen-printing of the source and drain electrodes (Villamizar et al. 2009). The SWCNTs surfaces were functionalized using anti-*Candida* monoclonal antibodies which resulted in selective detection of *Candida albicans* with a limit of detection (LOD) of 50 CFU/mL. The assay did not show any cross-reactivity with other yeasts such as *Cryptococcus albidus* and *Saccharomyces cerevisiae*.

Schumacher et al. (2012) reported a lab-on-chip (LOC) platform for the in vitro diagnosis of *Candida albicans* and other bacterial pathogens. A single microfluidic platform was fabricated by spotting nanoliter volumes of the capture molecules on the array chip for sensitive electrochemical sensing for optical detection by total internal reflected fluorescence (TIRF). The highly integrated microfluidic LOC workflow combined on-chip DNA isolation from samples with DNA amplification by PCR and fragmentation of amplicon by DNAse. Further the hybridization compartment was heated to ensure binding of target DNA to the probes on the pre-spotted cyclo olefin polymer slides followed by detection by the optical sensing using TIRF.

Microfluidic digital PCR platform is recently introduced as a robust platform for fungal detection. The droplet digital PCR (ddPCR) permits sample partitioning into thousands of water in oil droplet "nanolitre" reactors within microchannels on a disposable chip. These nano reactors contain single molecules that are evaluated by end-point PCR. The sophisticated liquid handling operations involve magnetic force and electro-wetting to manipulate droplet merging and movement to carry out various steps essential for PCR, namely DNA extraction, purification, heating, and cooling. Employing this technique, Schell et al. (2012) used the commercial Advanced Liquid Logic, Inc. microfluidic chip to detect *C. albicans* from blood samples of candidemia patients. DNA was extracted from the samples off-chip and applied to the biochip for detection. The ddPCR method achieved a sensitivity of 56% within 45 min as compared to the conventional PCR real-time analysis (69%, 70 min).

Tian et al. (2020) developed a LAMP-based microfluidic chip for rapid detection of pathogen in cryptococcal meningitis. The microfluidic chip integrated sample *Cryptococcus* enrichment, nucleic acid extraction, and LAMP amplification on-board. The LOC streamlined the process and reduced the exposure risk of direct handling of cryptococcal samples.

The microfluidic chip incorporated four duplicate filtration membrane structures for enhanced target capture and simplified enrichment process with lyticase digestion as well as thermal alkaline lysis for optimal *Cryptococcus* spp. nucleic acid extraction. A portable UVA flashlight was employed for visual detection of the LAMP products. The LOC showed rapid, reliable, and highly efficient approach with the use of other equipment that has immense potential for clinical prediagnosis cryptococcosis.

22.10 Magnetic Nanoparticles for Detection of Fungal Pathogens

Magnetic nanoparticles are applied for the separation of different components and for detection on basis of their magnetic resonance signals (Fig. 22.1h). Nucleic magnetic resonance (NMR) is a characteristic release of electromagnetic radiation by nuclei in response to a magnetic field for the sensitive detection of biomolecules. NMR allows the analysis of biological samples such as urine, blood, serum samples without the requirement of a filtration or purification with high sensitivity.

Downscaling of NMR can be beneficial to target and quantify bioanalytes during pathogen detection. The sensitivity of NMR for detection of species specific DNA was utilized by Neely et al. (2013) for pathogen identification. Initially, pan-*Candida* PCR primers targeted the inter-transcribed spacer region for amplification which was followed by hybridization of amplicons with recognition probes tagged to superparamagnetic nanoparticles. DNA hybridization caused the dispersed superparamagnetic nanoparticles to cluster and the resultant T2 magnetic resonance showed an alteration in the relaxation time that was used for detection of pathogen.

The DNA bio-barcode assay employs magnetic nanoparticles functionalized with probes for target DNA separation which is followed by recognition and signal amplification by oligonucleotide-modified gold nanoparticles (Nam et al. 2004). The gold-magnetic nanoparticles (AuMNPs) bio-barcode test provides a highly sensitive method for rapid detection of protein and nucleic acids targets at low-concentrations (Goluch et al. 2006). The bio-barcode assay is unique for the quick detection of pathogen DNA or protein.

The US FDA approved commercial T2X platform, T2 Biosystems employs aggregation of superparamagnetic nanoparticles and the T2MR measurements for detection of Candidiasis (Mylonakis et al. 2015). Samples are processed automatically by the machine to extract pathogen DNA by bead beating followed by DNA amplification with pan-*Candida* inter-transcribed spacer primers towards RNA gene. Detection of pathogen occurs by DNA amplicon induced aggregation of superparamagnetic nanoparticles followed by T2MR measurements. The system displayed a sensitive detection 1 CFU/mL of *Candida* in 3–5 h.

A magneto-nanosensor biochip was fabricated with arrays of giant magnetoresistive spin-valve sensors for detection of *A. fumigatus* (Kim and Wang 2012). The chip comprised of antibodies coating to capture the protein biomarkers and magnetic nanoparticles labeled secondary antibodies as the recognition element. The chip could detect low concentrations in solutions with a detection limit of 100 pg/mL. The magnetic nanoparticles generate a stray magnetic field in real time to perturb the oscillating external magnetic field which is recorded during the measurement. As a result of the GMR effect, spin-valve resistance is altered when the magnetic nanoparticles bind to the sensor surface leading to the specific biological signal measured. Such magneto-nanosensor biochips are promising as sensitive diagnostic devices for fungal pathogens.

22.11 Conclusion

Fungi are generally harmless but can assume threatening due to reduced host immunity and increased drug resistance. The detection of human pathogenic fungi assumes importance for timely application of antifungal treatment for their proper management and disease control. One of the main limitations for rapid detection of fungal pathogens is the lack of on-site diagnosis. Nanotechnology can not only contribute to miniaturization of pathogen detection platforms for their on-site applicability but also improve the diagnostic sensitivity of the detection platforms. Lateral

flow assays using gold nanoparticles are handy and have potential for rapid detection of human pathogenic fungi. Array Biosensors, Lab-on-a-chip, nanopore technology, and magnetic nanoparticles are devices that can improve detection and integrated sample processing on a single platform. Development of robust, portable sensors for detection would allow for appropriate interventions for the management of pathogenic fungi. Detection of human fungal pathogens will allow timely treatment and therapy of several diseases/ailments thus reducing hospitalization costs and mortality.

Acknowledgements VG acknowledges the funding from DBT.

References

Aboul-Ella H, Hamed R, Abo-Elyazeed H (2020) Recent trends in rapid diagnostic techniques for dermatophytosis. Int J Vet Sci Med 8:115–123

Almeida F, Rodrigues ML, Coelho C (2019) The still underestimated problem of fungal diseases worldwide. Front Microbiol 10:214

Barnes RA, White PL (2016) PCR Technology for detection of invasive aspergillosis. J Fungi 2:1–9

Barton RC (2013) Laboratory diagnosis of invasive aspergillosis: from diagnosis to prediction of outcome. Scientifica 2013:459405

Bayley H (2015) Nanopore sequencing: from imagination to reality. Clin Chem 61:25–31. https://doi.org/10.1373/clinchem.2014.223016

Bennet JE, Bhattacharjee AK, Glaudemans CPJ (1985) Galactofuranosyl groups are immunodominant in *Aspergillus fumigatus* galactomannan. Mol Immunol 22:251–254

Blum U, Windfuhr M, Buitrago-Tellez C, Sigmund G, Herbst EW, Langer M (1994) Invasive pulmonary aspergillosis. MRI, CT, and plain radiographic findings and their contribution for early diagnosis. Chest 106:1156–1161

Brown GD, Denning DW, Gow NA, Levitz SM, Netea MG, White TC (2012) Hidden killers: human fungal infections. Sci Transl Med 4:1–9

Busca A, Locatelli F, Barbui A, Limerutti G, Serra R, Libertucci D et al (2006) Usefulness of sequential *Aspergillus* galactomannan antigen detection combined with early radiologic evaluation for diagnosis of invasive pulmonary aspergillosis in patients undergoing allogeneic stem cell transplantation. Transplant Proc 38:1610–1613

Cáceres DH, Gómez BL, Tobón AM, Chiller TM, Lindsley MD (2019) Evaluation of a *Histoplasma* antigen lateral flow assay for the rapid diagnosis of progressive disseminated histoplasmosis in Colombian patients with AIDS. Mycoses 00:1–6

Caillot D, Couaillier JF, Bernard A, Casasnovas O, Denning DW, Mannone L, Lopez J, Couillault G, Piard F, Vagner O, Guy H (2001) Increasing volume and changing characteristics of invasive pulmonary aspergillosis on sequential thoracic computed tomography scans in patients with neutropenia. J Clin Oncol 19:253–259

Castillo RR, Baeza A, Vallet-Regí M (2017) Recent applications of the combination of mesoporous silica nanoparticles with nucleic acids: development of bioresponsive devices, carriers and sensors. Biomater Sci 5:353–377

Chamilos G, Kontoyiannis DP (2006) Defining the diagnosis of invasive aspergillosis. Med Mycol 44:163–S173

Chan WS, Au CH, Leung SM, Ho DN, Wong EYL, To MY, Ng MK, Chan TL, Ma ESK, Tang BSF (2020) Potential utility of targeted Nanopore sequencing for improving etiologic diagnosis of bacterial and fungal respiratory infection. Diagn Pathol 15:41

Cheng IF, Chang HC, Hou D, Chang HC (2007) An integrated dielectrophoretic chip for continuous bioparticle filtering, focusing, sorting, trapping, and detecting. Biomicrofluidics 1:021503

Cheng WH, Horn T, Zayats M, Rizk G, Major S, Zhu H, Russell J, Xu Z, Rothman RE, Celedon A (2020) Ultra-sensitive and rapid detection of nucleic acids and microorganisms in body fluids using single-molecule tethering. Nat Commun 11:4774

Chong GM, Maertens JA, Lagrou K, Driessen GJ, Cornelissen JJ, Rijnders BJ (2016) Diagnostic performance of galactomannan antigen testing in cerebrospinal fluid. J Clin Microbiol 54:428–431

Clarke J, Wu H-C, Jayasinghe L, Patel A, Reid S, Bayley H (2009) Continuous base identification for single-molecule nanopore DNA sequencing. Nat Nanotechnol 4:265270

Daher RK, Stewart G, Boissinot M, Bergeron MG (2016) Recombinase polymerase amplification for diagnostic applications. Clin Chem 62(7):947–958

Dai T, Yang X, Hu T, Jiao B, Xu Y, Zheng X, Shen D (2019) Comparative evaluation of a novel recombinase polymerase amplification-lateral flow dipstick (RPA-LFD) assay, LAMP, conventional PCR, and leaf-disc baiting methods for detection of *Phytophthora sojae*. Front Microbiol 10:1884

de Pauw BE (2011) What are fungal infections? Mediterr J Hematol Infect Dis 3(1):e2011001

Denning DW (1998) Invasive aspergillosis. Clin Infect Dis 26:781–805

Dixon C, Leader MT, Bala S (2011) Biomarker qualification microbiology review. pp 1–94. https://www.fda.gov/media/90001/download

Duettmann W, Koidl C, Troppan K, Seeber K, Buzina W, Wölfler A, Wagner J, Krause R, Hoenigl M (2014) Serum and urine galactomannan testing for screening in patients with hematological malignancies. Med Mycol 52:647–652

Dufresne SF, Datta K, Li X, Dadachova E, Staab JF, Patterson TF, Feldmesser M, Marr KA (2012) Detection of urinary excreted fungal galactomannan-like antigens for diagnosis of invasive aspergillosis. PLoS One 7:1–10

Dupont B, Huber M, Kim SJ, Bennett JE (1987) Galactomannan antigenemia and antigenuria in aspergillosis: studies in patients and experimentally infected rabbits. J Infect Dis 155:1–11

Fanfair RN, Benedict K, Bos J, Bennett SD, Lo YC, Adebanjo T, Etienne K, Deak E, Derado G, Shieh WJ, Drew C, Zaki S (2012) Necrotizing cutaneous mucormycosis after a tornado in Joplin, Missouri, in 2011. N Engl J Med 367:2214–2225

Fleming RV, Walsh TJ et al (2002) Emerging and less common fungal pathogens. Infect Dis Clin North Am 16(4):915–933

Fukuzawa M, Inaba H, Hayama M, Sakaguchi N, Sano K, Ito M, Hotchi M (1995) Improved detection of medically important fungi by immunoperoxidase staining with polyclonal antibodies. Virchows Arch 427:407–414

Georgiadou SP, Sipsas NV, Marom EM, Kontoyiannis DP (2011) The diagnostic value of halo and reversed halo signs for invasive mold infections in compromised hosts. Clin Infect Dis 52:1144–1155

Ghormade G, Deshpande MV, Paknikar KM (2011) Perspectives for nano-biotechnology in plant protection and nutrition. Biotechnol Adv 29:792–803

Goluch ED, Nam JM, Georganopoulou DG, Chiesl TN, Shaikh KA, Ryu KS, Barron AE, Mirkin CA, Liu C (2006) A biobarcode assay for on-chip attomolar-sensitivity protein detection. Lab Chip 6:12931299

Guarner J, Brandt ME (2011) Histopathologic diagnosis of fungal infections in the 21st century. Clin Microbiol Rev 24:247–280

Hayden RT, Qian X, Procop GW, Roberts GD, Lloyd RV (2002) *In situ* hybridization for the identification of filamentous fungi in tissue section. Diagn Mol Pathol 11:119–126

Hayette MP, Vaira D, Susin F, Boland P, Christiaens G, Melin P, De Mol P (2001) Detection of *Aspergillus* Species DNA by PCR in bronchoalveolar lavage fluid. J Clin Microbiol 39:2338–2340

Haynes KA, Latge JP, Rogers TR (1990) Detection of *Aspergillus* antigens associated with invasive infection. J Clin Microbiol 28:2040–2044

He ZX, Shi LC, Ran XY, Li W, Wang XL, Wang FK (2016) Development of a lateral flow immunoassay for the rapid diagnosis of invasive candidiasis. Front Microbiol 7:1451

Hope WW, Walsh TJ, Denning DW (2005) Laboratory diagnosis of invasive aspergillosis. Lancet Infect Dis 5:609–622

Horvath JA, Dummer S (1996) The use of respiratory-tract cultures in the diagnosis of invasive pulmonary aspergillosis. Am J Med 100:171–178

Jenks JD, Mehta SR, Taplitz R, Law N, Reed SL, Hoenigl M (2019a) Bronchoalveolar lavage *Aspergillus* galactomannan lateral flow assay versus *Aspergillus*-specific lateral flow device test for diagnosis of invasive pulmonary aspergillosis in patients with hematological malignancies. J Infect 78:249–259

Jenks JD, Mehta SR, Taplitz R, Aslam S, Reed SL, Hoenigl M (2019b) Point-of-care diagnosis of invasive aspergillosis in non-neutropenic patients: *Aspergillus* galactomannan Lateral Flow assay versus *Aspergillus*-specific Lateral Flow Device test in bronchoalveolar lavage. Mycoses 62:230–236

Jenks JD, Miceli MH, Prattes J, Mercier T, Hoenigi M (2020) The aspergillosis lateral flow assay for the diagnosis of invasive aspergillosis: an update. Curr Fungal Infect Rep 14:378–383

Jensen HE, Salonen J, Ekfors TO (1997) The use of immunohistochemistry to improve sensitivity and specificity in the diagnosis of systemic mycoses in patients with haematological malignancies. J Pathol 181:100–105

Jin J, Depei W, Liu Y, Pan SS, Yan JL, Aram JA, Lou Y, Meng H, Chen X, Zhang X, Schwartz IS, Patterson TF (2017) Utility of computed tomography for the diagnosis of invasive aspergillosis in hematological malignancy. Blood 130:1–6

Kang T, Yoo SM, Yoon I, Lee S, Choo J, Lee SY, Kim B (2011) Au nanowire-on-film SERRS sensor for ultrasensitive Hg^{2+} detection. Chem Eur J 17:2211

Katragkou A, Pana ZD, Perlin DS, Kontoyiannis DP, Walsh TJ, Roilides E (2014) *Exserohilum* infections: review of 48 cases before the 2012 United States outbreak. Med Mycol 52:376–386

Kaufman L, Standard PG, Jalbert M, Kraft DE (1997) Immunohistologic identification of *Aspergillus* spp. and other hyaline fungi by using polyclonal fluorescent antibodies. J Clin Microbiol 35:2206–2209

Kim D, Wang SX (2012) A magneto-nanosensor immunoassay for sensitive detection of *Aspergillus fumigatus* allergen Asp f 1. IEEE Trans Magn 48:3266–3268

Klont RR, Meis JFGM, Verweij PE (2001) Critical assessment of issues in the diagnosis of invasive aspergillosis. Clin Microbiol Infect Dis 7:32–37

Kolosova AY, De Saeger S, Sibanda L, Verheijen R, Van Peteghem C (2007) Development of a colloidal gold-based lateral-flow immunoassay for the rapid simultaneous detection of zearalenone and deoxynivalenol. Anal Bioanal Chem 389:2103–2107

Kousha M, Tadi R, Soubani AO (2011) Pulmonary aspergillosis: a clinical review. Eur Respir Rev 20:156–174

Kozel TR, Bauman SK (2012) CrAg lateral flow assay for cryptococcosis. Expert Opin Med Diagn 6:245–251

Kuhlman JE, Fishman EK, Siegelman SS (1985) Invasive pulmonary aspergillosis in acute leukemia: characteristic findings on CT, the CT halo sign, and the role of CT in early diagnosis. Radiology 157:611–614

Kwizera R, Omali D, Tadeo K, Kasibante J, Rutakingirwa MK, Kagimu E, Ssebambulidde K, Williams DA, Rhein J, Boulware D, Meya DB (2021) Evaluation of the Dynamiker cryptococcal antigen lateral flow assay for the diagnosis of HIV-associated cryptococcosis. J Clin Microbiol 59:e02421–e02420

Larone DH, Walsh TJ (2013) *Exserohilum rostratum*: anatomy of a national outbreak of fungal meningitis. Clin Microbiol News 35:185–193

Lee YR, Choi YW, Lee KJ, Jeon SC, Park CK, Heo JN (2005) CT halo sign: the spectrum of pulmonary diseases. Br J Radiol 78:862–865

Lee JI, Cheon Jang S, Chung J, Choi WK, Hong C, Ahn GR, Hwan Kim S, Yang Lee B, Chung WJ (2021) Colorimetric allergenic fungal spore detection using peptide-modified gold nanoparticles. Sensors Actuators B Chem 327:128894

Ma Q, Yao J, Yuan S, Liu H, Wei N, Zhang J, Shan W (2019) Development of a lateral flow recombinase polymerase amplification assay for rapid and visual detection of *Cryptococcus neoformans*/*C. gattii* in cerebral spinal fluid. BMC Infect Dis 19:108

Maertens J, Verhaegen J, Lagrou K, Van Eldere J, Boogaerts M (2001) Screening for circulating galactomannan as a noninvasive diagnostic tool for invasive aspergillosis in prolonged neutropenic patients and stem cell transplantation recipients: a prospective validation. Blood 97:1604–1610

Meersseman W, Lagrou K, Maertens J, Wilmer A, Hermans G, Vanderschueren S et al (2008) Galactomannan in bronchoalveolar lavage fluid: a tool for diagnosing aspergillosis in intensive care unit patients. Am J Respir Crit Care Med 177:27–34

Meletiadis J, Roilides E (2013) Rare invasive fungal infections: epidemiology, diagnosis and management. Curr Fungal Infect Rep 7:351–360

Mennink-Kersten MA, Donnelly JP, Verweij PE (2004) Detection of circulating galactomannan for the diagnosis and management of aspergillosis. Lancet Infect Dis 4:349–357

Mennink-Kersten MA, Ruegebrink D, Wasei N, Melchers WJ, Verweij PE (2006) In vitro release by *Aspergillus fumigatus* of galactofuranose antigens, 1,3-β-D-glucan, and DNA, surrogate markers used for diagnosis of invasive aspergillosis. J Clin Microbiol 44:1711–1718

Mercier T, Dunbar A, de Kort E, Schauwvlieghe A, Reynders M, Guldentops E, Blijlevens NMA, Vonk AG, Rijnders B, Verweij PE, Lagrou K, Maertens J (2019) Lateral flow assays for diagnosing invasive pulmonary aspergillosis in adult hematology patients: a comparative multicenter study. Med Mycol 58(4):444–452. https://doi.org/10.1093/mmy/myz079

Mercier T, Dunbar A, Veldhuizen V, Holtappels M, Schauwvlieghe A, Maertens J, Rijnders B, Wauters J (2020) Point of care aspergillus testing in intensive care patients. Crit Care 24:642

Merz WG, Karp JE, Hoagland M, Jett-Goheen M, Junkins JM, Hood AF (1988) Diagnosis and successful treatment of fusariosis in the compromised host. J Infect Dis 158:1046–1055

Mortensen KL, Johansen HK, Fuursted K, Knudsen JD, Gahrn-Hansen B, Jensen RH, Howard SJ, Arendrup MC (2011) A prospective survey of *Aspergillus* spp. in respiratory tract samples: prevalence, clinical impact and antifungal susceptibility. Eur J Clin Microbiol Infect Dis 30:1355–1363

Mpoza E, Mukaremera L, Kundura DA, Akampurira A, Luggya T, Tadeo KK, Pastick KA, Bridge SC, Tugume L, Kiggundu R, Musubire AK, Williams DA, Muzoora C, Nalintya E, Rajasingham R, Rhein J, Boulware DR, Meya DB, Abassi M (2018) Evaluation of a point-of-care immunoassay test kit 'StrongStep' for cryptococcal antigen detection. PLoS One 13:e0190652

Mylonakis E, Clancy CJ, Ostrosky-Zeichner L, Garey KW, Alangaden GJ, Vazquez JA et al (2015) T2 magnetic resonance assay for the rapid diagnosis of candidemia in whole blood: a clinical trial. Clin Infect Dis 60:892–899

Nam JM, Stoeva SI, Mirkin CA (2004) Bio-bar-code-based DNA detection with PCR-like sensitivity. J Am Chem Soc 126:59325933

Neely LA, Audeh M, Phung NA, Min M, Suchocki A, Plourde D et al (2013) T2 magnetic resonance enables nanoparticle-mediated rapid detection of Candidemia in whole blood. Sci Transl Med 5:182–154

Nolan P, Auer S, Spehar A, Elliott CT, Campbell K (2019) Current trends in rapid tests for mycotoxins. Food Addit Contam Part A 36:800–814

Nweze EI, Ghannoum A, Chandra J, Ghannoum MA, Mukherjee PK (2012) Development of a 96-well catheter-based microdilution method to test antifungal susceptibility of *Candida* biofilms. J Antimicrob Chemother 67:149–153

Orlowski HLP, McWilliams S, Mellnick VM, Bhalla S, Lubner MG, Pickhardt PJ et al (2017) Imaging spectrum of invasive fungal and fungal-like infections. Radiographics 37:1119–1134

Pandey PK, Preeti, Rawat K, Prasad T, Bohidar HB (2020) Multifunctional, fluorescent DNA-derived carbon dots for biomedical applications: bioimaging, luminescent DNA hydrogels, and dopamine detection. J Mater Chem B 8:1277

Park BJ, Wannemuehler KA, Marston BJ (2009) Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. AIDS 23:525–530

Paugam A, Baixench MT, Lebuisson A, Dupouy-Camet J (2010) Diagnosis of invasive pulmonary aspergillosis: value of bronchoalveolar lavage galactomannan for immunocompromised patients. Pathol Biol 58:100–103

Pazos C, Pontón J, Del PA (2005) Contribution of (1→3)-β-D-glucan chromogenic assay to diagnosis and therapeutic monitoring of invasive aspergillosis in neutropenic adult patients: a comparison with serial screening for circulating galactomannan. J Clin Microbiol 43:299–305

Phillips P, Weiner MH (1987) Invasive aspergillosis diagnosed by immunohistochemistry with monoclonal and polyclonal reagents. Hum Pathol 18:1015–1024

Piérard GE, Arrese EJ, Piérard FC, Thiry A, Stynen D (1991) Immunohistochemical expression of galactomannan in the cytoplasm of phagocytic cells during invasive aspergillosis. Am J Clin Pathol 96:373–376

Pla L, Santiago-Felipe S, Tormo-Mas MA, Ruiz-Gaitán A, Pemán J, Valentín E, Sancenón F, Aznar E, Martínez-Máñez R (2021) Oligonucleotide-capped nanoporous anodic alumina biosensor as diagnostic tool for rapid and accurate detection of *Candida auris* in clinical samples. Emerg Microbes Infect 10:407–415. https://doi.org/10.1080/22221751.2020.1870411

Prattes J, Flick H, Prüller F, Koidl C, Raggam RB, Palfner M et al (2014) Novel tests for diagnosis of invasive aspergillosis in patients with underlying respiratory diseases. Am J Respir Crit Care Med 190:922–929

Qi Y, Yin Q, Shao Y, Cao M, Li S, Chen H, Shen W, Rao J, Li J, Li X, Sun Y, Lin Y, Deng Y, Zeng W, Zheng S, Liu S, Li Y (2018) Development of a rapid and visual nucleotide detection method for a Chinese epidemic strain of Orientia tsutsugamushi based on recombinase polymerase amplification assay and lateral flow test. Int J Infect Dis 70:42–50

Rawal KM, Ghormade V, Rajamohanan PR, Choudhary H, Rudramurthy SM, Chakrabarti A et al (2019) Development of a nano-gold immunodiagnostic assay for rapid in-site detection of invasive aspergillosis. J Med Microbiol 68:1341–1352

Rhodes J, Abdolrasouli A, Farrer RA, Cuomo CA, Aanensen DM, Armstrong-James D et al (2018) Genomic epidemiology of the UK outbreak of the emerging human fungal pathogen *Candida auris*. Emerg Microbes Infect 7:1–12

Richardson MD (2005) Changing patterns and trends in systemic fungal infections. J Antimicrob Chemother 56:5

Rosa C, Araujo R, Rodrigues AG, Pinto-de-Sousa MI, Pina-Vaz C (2011) Detection of *Aspergillus* species in BACTEC blood cultures. J Med Microbiol 60:1467–1471

Schell WA, Benton JL, Smith PB, Poore M, Rouse JL, Boles DJ et al (2012) Evaluation of a digital microfluidic real-time PCR platform to detect DNA of *Candida albicans* in blood. Eur J Clin Microbiol Infect Dis 31:2237–2245

Schumacher S, Nestler J, Otto T, Wegener M, Ehrentreich-Förster E, Michel D et al (2012) Highly-integrated lab-on-chip system for point-of-care multiparameter analysis. Lab Chip 12:464–473

Sojinrin T, Conde J, Liu K, Curtin J, Byrne HJ, Cui D, Tian F (2017) Plasmonic gold nanoparticles for detection of fungi and human cutaneous fungal infections. Anal Bioanal Chem 409:4647–4658

Steinbach WJ, Mitchell TG, Schell WA, Espinel-Ingroff A, Coico RF, Walsh TJ et al (2003) Status of medical mycology education. Med Mycol 41:457–467

Stynen D, Sarfati J, Goris A, Prevost MC, Lesourd M, Kamphuis H et al (1992) Rat monoclonal antibodies against *Aspergillus* galactomannan. Infect Immun 60:2237–2245

Temfack E, Kouanfack C, Mossiang L, Loyse A, Fonkoua MC, Molloy SF, Koulla-Shiro S, Delaporte E, Dromer F, Harrison T, Lortholary O (2018) Cryptococcal antigen screening in asymptomatic HIV-infected antiretroviral naive patients in Cameroon and evaluation of the new semi-quantitative Biosynex CryptoPS test. Front Microbiol 9:409

Theel ES, Doern CD (2013) β-D-glucan testing is important for diagnosis of invasive fungal infections. Journal of Clinical Microbiology 51(11): 3478–3483

Thornton CR (2008) Development of an Immunochromatographic Lateral-Flow device for rapid serodiagnosis of invasive aspergillosis. Clinical and Vaccine Immunology 15(7): 1095-1105
Thornton CR (2010) Chapter 6—Detection of invasive aspergillosis. Adv Appl Microbiol 70:187–216
Thornton CR (2013) Lateral-flow device for diagnosis of fungal infection. Curr Fungal Infect Rep 7:244–251
Thornton CR, Johnson G, Agrawal S (2012) Detection of invasive pulmonary aspergillosis in haematological malignancy patients by using lateral-flow technology. J Vis Exp 61:1–5
Tian Y, Zhang T, Guo J, Lu H, Yao Y, Chen X, Zhang X, Sui G, Guan M (2020) A LAMP-based microfluidic chitp for rapid detection of pathogen in Cryptococcal meningitis. BioRxiv preprint. https://doi.org/10.1101/2020.11.16.386045
Tran T, Beal SG (2016) Application of the 1,3-β-D-glucan (Fungitell) assay in the diagnosis of invasive fungal infections. Arch Pathol Lab Med 140:181–185
Tsunemi Y, Hiruma M (2016) Clinical study of dermatophyte test strip, an immunochromatographic method, to detect tinea unguium dermatophytes. J Dermatol 43(12): 1417–1423
Verdaguer V, Walsh TJ, Hope W, Cortez KJ (2007) Galactomannan antigen detection in the diagnosis of invasive aspergillosis. Expert Rev Mol Diagn 7:21–32
Vidal JE, Boulware DR (2015) Lateral flow assay for cryptococcal antigen: an important advance to improve the continuum of HIV care and reduce cryptococcal meningitis-related mortality. Rev Inst Med Trop Sao Paulo 57:38–45
Villamizar RA, Maroto A, Rius FX (2009) Improved detection of Candida albicans with carbon nanotube field-effect transistors. Sensor Actuators B 136:451–457
Walsh TJ, Groll A, Hiemenz J, Fleming R, Roilides E, Anaissie E (2004) Infections due to emerging and uncommon medically important fungal pathogens. Clin Microbiol Infect 10:48–66
Wang J, Zhou W, Ling H, Dong X, Zhang Y, Li J, Zhang Y, Song J, Liu WJ, Li W, Zhang R, Zhen W, Cai K, Zhu S, Wang D, Xiao J, Tong Y, Liu W, Song L, Wu W, Liu W, Zhao X, Wang R, Ye S, Wang J, Lu R, Huang B, Ye F, Lei W, Gao R (2019) Identification of *Histoplasma* causing an unexplained disease cluster in Matthews Ridge, Guyana. Biosaf Health 1:150–154
Wheat LJ (2003) Rapid diagnosis of invasive aspergillosis by antigen detection. Transpl Infect Dis 5:158–166
Wickes BL, Wiederhold NP (2018) Molecular diagnostics in medical mycology. Nat Commun 9: 5135
Witkowska E, Jagielski T, Kaminska A, Kowalska A, Hryncewicz-Gwozdz A, Wa J (2016) Detection and identification of human fungal pathogens using surface-enhanced Raman spectroscopy and principal component analysis. Anal Methods 8:8427
Wright WF, Overman SB, Ribes JA (2011) (1-3)-β-D-Glucan assay: a review of its laboratory and clinical application. Lab Med 42:679–685
Ye Y, Zhou Y, Mo Z, Cheng W, Yang S, Wang X, Chen F (2010) Rapid detection of aflatoxin B1 on membrane by dot-immunogold filtration assay. Talanta 81:792–798
Yoo SM, Kang T, Kang H, Lee H, Kang M, Lee SY, Kim B (2011) Combining a nanowire SERRS sensor and a target recycling reaction for ultrasensitive and multiplex identification of pathogenic fungi. Small 2011(7):3371–3376
Zhao S, Wang S, Zhang S, Liu J, Dong Y (2018) State of the art: lateral flow assay (LFA) biosensor for on-site rapid detection. Chin Chem Lett 29:1567–1577
Zhao F, Niu L, Yan L, Nong J, Wang C, Wang J, Gao N, Zhu X, Wu L, Zheng F, Hu S (2019) Establishment and application of multiple cross displacement amplification coupled with lateral flow biosensor (MCDA-LFB) for visual and rapid detection of candida albicans in clinical samples. Front Cell Infect Microbiol 9:102

Improvement of COVID-19 Diagnostic Tools: Nanobiosensors Challenges and Perspectives

23

Heba S. Abbas, Abeer E. Aly, Hossam M. Mohamed, Manal A. Nabil, Reem M. Mohamed El Sapagh, and Doha H. Abou Baker

Abstract

To date, no vaccine or specific drug has been developed to treat CoV-2 infection, also early diagnosis is critical in dealing with the CoV-2 pandemic. Existing tests have some limitations including long analysis time, poor performance, insufficient sensitivity, and less than optimal portability. The development of biosensor technology promises development of fast and highly sensitive tests, and is suitable for on-site testing, which can make testing for CoV-2 much easier. However, the practical application of such a biosensor in a pandemic remains to be achieved. This review can serve as a guide for the development of modern nanomaterial techniques capable of applying biosensors to meet today's demand

H. S. Abbas (✉)
Microbiology Department, National Organization for Drug Control and Research (NODCAR), Egyptian Drug Authority, Giza, Egypt

Microbiology Department, Faculty of Pharmacy, Misr University for Science and Technology, Giza, Egypt

A. E. Aly
Basic Science Department at High Institute of Engineering and Technology, Alexandria, Egypt

H. M. Mohamed
Faculty of Pharmacy, Al-Azhar University, Assiut Branch, Cairo, Egypt

M. A. Nabil
Department of Immunology and Allergy, Medical Research Institute, Alexandria University, Alexandria, Egypt

R. M. Mohamed El Sapagh
Faculty of Pharmacy, Cairo University, Cairo, Egypt

D. H. Abou Baker
Medicinal and Aromatic Plants Department, Pharmaceutical and Drug Industries Division, National Research Centre, Cairo, Egypt

S. Hameed, S. Rehman (eds.), *Nanotechnology for Infectious Diseases*,
https://doi.org/10.1007/978-981-16-9190-4_23

for inexpensive, rapid, and early diagnosis of COV-2 infection and fungal post-COVID infection. Further efforts will be needed to track and avoid further pandemic outbreaks of viral infectious diseases.

Keywords

Nanobiosensors · SARS-CoV-2 · Virus detection · Biosensors · Fungi associated COVID

23.1 Introduction

Diagnosis of viruses aims to identify agents which most likely responsible for the disease. For doctors, ideal virus identification methods in an infected patient should be specific, sensitive, and rapid, as once patient has recovered or died diagnosis has less practical value. From another side epidemiologic researches may have thousands of samples which require use of low cost, and high throughput virus modalities. Virus has its own detection challenges due to their simple biology, small size, and also obligates intracellular life cycle. Since 1940, there have been three general approaches on detecting virus: Analysis the response of viruses on the host organism, especially antibody serology, detection of a virus's molecular fingerprints, including viral nucleic acid and protein, and direct recognition of whole viral particle (Payne 2017). The traditional viral diagnostic techniques, especially culture, are expensive, slow, and often the diagnostic choice to make decision when no therapeutic agents are available (Storch 2000). Besides, there is high risk for human to expose to viruses in environment matrix. Additionally, detection of viral pathogen needs identification of the presence and/or quantity of virus (Julian and Schwab 2012).

The current episode of the extreme intense respiratory condition SARS-CoV-2 has given disease transmission experts around the world challenge: the capacity to dependably foresee the spread of this novel profoundly infectious Covid 19 and, in result, apply fitting isolate measures to forestall the transmission of the disease. In result, there is a critical need for symptomatic apparatuses capable not exclusively to dependably distinguish tainted individuals (Li et al. 2020). Moreover, an irreplaceable objective for the control of the COVID-19 pandemic is the limit with respect to mass populace screening, a condition that requests quick and cost-efficient measure draws near. Accordingly, various Point-of-Care (POC) quick and moderately affordable tests for SARS-CoV-2 have been as of late created (Sheridan 2020).

Currently, the real-time reverse-transcriptase polymerase chain reaction (RT-PCR) procedure is the most widely recognized and solid research facility testing technique for subjective/quantitative SARS-CoV-2 identification followed by serum infection balance examine (SVNA) for the assurance of neutralizer balance and compound connected immunoassays (ELISA) for the location of antibodies against SARS-CoV-2 (Chowell et al. 2015; Kang et al. 2017; Huang et al. 2020; Cesewski and Johnson 2020). Besides that, the significant restrictions of these research facility

based symptomatic tests are the obtrusive idea of the tests that regularly require prepared individual for nasopharyngeal example assortment, alongside the prerequisite of exceptionally complex machines, cross-reactivity with other infections, and longer span of testing. Therefore, there is an urgent need to find simple, inexpensive appropriate method for viral detection (Tahamtan and Ardebili 2020).

Biosensors are extremely precise, sensitive, and specific systems for calculating very low analytical sample concentrations. A biosensor is an analytical instrument composed of biological components, such as micro-organisms, organelles, receptors, enzymes, nucleic acids, etc., and an electric parameter transducer that transforms these signals. A high degree of specialty based on unique binding sites is essential for biosensor components. Various medical and therapeutic uses are planned to be used for biosensors, such as: (1) quick diagnosis and treatment of diseases such as cancer or diabetes; (2) pathogens detection; (3) measurement of the drugs, and the metabolites thereof; (4) new medicines development; (5) drug evaluation; and (6) evaluation of analyte and early detection of diseases by means of quick testing of biological samples (Pashazadeh et al. 2017; Hasanzadeh et al. 2016, 2017).

Recently, biomedical nanomaterials attract attention because of materials, their numerous exceptional optical, electronic, attractive, and mechanical properties. Until now, nanoparticles, such as metal, metal oxide, quantum spots, carbon nanotubes, graphene nanotubes, and polymeric nanomaterials have been utilized in viral identification, typically formed on their surface with biomolecules got from infections (DNA, RNA, antibodies, etc.) (Kang et al. 2017; Suo et al. 2020).

It is significant that the use of nanomaterials in the production of biosensor has diminished the size of biosensor, making them more appropriate for in-field detection (Suo et al. 2020). In this review, we discuss nano-based sensor as effective method for viral detection including Sars-CoV-2 and the fungal infection as post-covid symptoms.

23.2 Biosensors for Viral Detection

Compound or organic receptors and transducers form the sensors. The receptor directly links with an objective analyte, and the transducer transforms into a quantitative sign over the recognition period (Ozer et al. 2020). Biosensors are analytical instruments in which organic particles, such as compounds, antibodies, or nucleic acids, paired with a transducer and an identifier that identifies the associated analyte and gives a computerized yield (Fig. 23.1). In light of innovation brought about, there are four kinds of biosensors via, optical biosensors, electrochemical biosensors, piezoelectric biosensors, and thermal biosensors (Saylan et al. 2019).

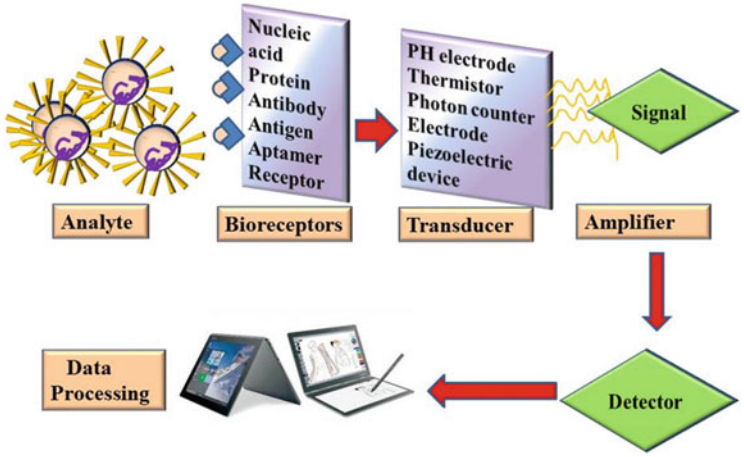

Fig. 23.1 Graphic diagram of biosensor for detection of viruses

23.3 Nanomaterials Based Sensor

Recent nanoscience and nanotechnology advances, together with the possibility of small-scale electrodes, allow nanoscale sensors, leading to a new range of diagnostic biosensors known as nanobiosensors. In this consequence, we should note that the arrangement of the nanomaterials and biological materials is essential to design hybrid nanostructured analytical instruments (Pinheiro et al. 2011; Gerrard 2013; Bellan et al. 2011). Due to the extremely large surface to volume ratios of nanosize devices, the surface interaction between the sensors and the analyte become extremely powerful (Nguyen et al. 2009). Nanoscale materials therefore exhibit specific characteristics, functionality, and effects. Nanotechnology currently focuses on removing the drawbacks of existing virus detection methods in order to reduce costs and time usage (Krishna et al. 2018). In addition, nanomaterial use in the biosensors' construction led to improved performance and sensitivity (Krishna et al. 2018) (Fig. 23.2). Due to the electrical and mechanical characteristics of nanomaterials for biomedical applications, many nanomaterials, such as nanorods, nanotubes, nanowires, thin films, and nanoparticles, have been studied for biomedical purposes. We will investigate the use of various nanomaterials for the manufacture of pathogenic viruses' biosensor such as quantum dots, carbon nanotubes, and metallic nanoparticles.

23.3.1 Magnetic Biosensors

Magnetic nanoparticles have provided significant interest to biosensing methods because they provide unrivaled benefits over other techniques. The development of

Fig. 23.2 Different nanomaterials that used as biosensor for viral detection

magnetic nanoparticles, for example, is cost-effective, mechanically and chemically stable, and biocompatible. Moreover, biological samples have practically no magnetic history and thus extremely sensitive experiments can be conducted without extra processing. However, optical methods are often influenced inside the sample by dispersion, absorption, and/or autofluorescence (Haun et al. 2010). A number of methods for sensing biomolecules with magnetic labels have been developed. These include magnetic sensors such as SQUID, and Hall sensors that sense magnetic particles directly (Kotitz et al. 1997; Chemla et al. 2000; Li et al. 2006; Osterfeld et al. 2008; Wang et al. 2005; Aytur et al. 2006). Another approach which has been considerably effective is a process that uses magnetic nanoparticles to speed up the relaxation rate of neighboring water molecules, which is based on magnetic resonance (MRI/NMR). This device is similar to magnetic resonance visualization used to see into the human body and is considered magnetic resonance diagnostic (DMR). DMR experiments use affinity molecule-conjugated magnetic nanoparticles to bind molecular goals and adjust the relaxation rate of protons using either of two separate types of proceedings. Firstly, it is important to tag large objects, such as whole cells, and then wash away unbound nanoparticle sensors. Secondly, the uses of the magnetic resonance switching phenomenon, where molecular targets are used to

Table 23.1 DMR biosensing methods for viruses

Viruses	Magnetic nanoparticles sensors	References
Herpes simplex virus	Anti-gpD (HSV-1)-CLIO; Anti-HSV1-CLIO	Perez et al. (2003)
Adenovirus-5	Anti-Adenovirus-5-CLIO	Perez et al. (2003)
Influenza viruses	Magnetic nanoparticles with H5N1 viral antibodies	Chou et al. (2011)
SARS-CoV-2	Giant magnetoresistive (GMR) biosensor and magnetic nanoparticles	Aminul Islam and Ziaul Ahsan (2020)

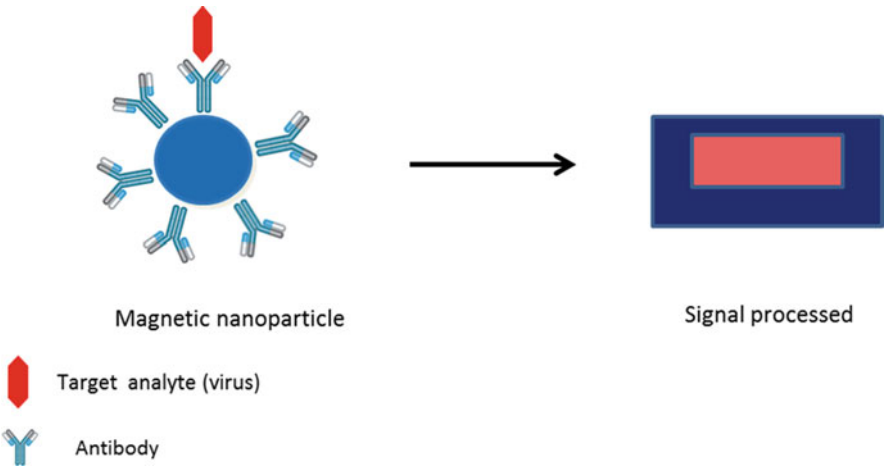

Fig. 23.3 Graphic diagram of magnetic nanoparticle for detection of viruses

assemble magnetic nanoparticles in clusters and thus affecting a corresponding shift in the change of bulk relaxation (Fig. 23.1) (Perez et al. 2002; Josephson et al. 2001). Magnetic nanoparticles (MNPs) are functionalized with antibodies or DNA/RNA probes that can precisely bind to target analytes. The target analyte concentration is then translated into the magnetic signal produced by the magnetic nanoparticles (Wu et al. 2020; Storch 2000). A wide range of high sensitivity and unique molecular targets viral pathogens were identified successfully by DMR as shown in Table 23.1.

Antibody-conjugated MRSw sensor is used to detect viruses. This is exciting matter since these targets are of the same or even greater order than magnetic nanoparticles. For example, identification of adenovirus-5 and herpes simplex virus1 by polyclonal antibody conjugated cross linked iron oxide nanoparticles in serum (Fig. 23.3) (Perez et al. 2003). Also, Chou et al. (2011) fabricated magnetic nanoparticles with H5N1 viral antibodies, which show high specific binding to H5N1 viruses.

Recent literature studied the detection of viral genome like +ssRNA, and S (spike)—protein containing into SARS-CoV-2 by magnetic nanoparticles.

Attachments of viruses with magnetic nanoparticles are necessary to detect the viral genome mechanism by magnetic biosensors. As supramolecular architecture with special building blocks, viruses and nanoparticles are attached. These supramolecular assemblies with the optimum virus ratios change pure magnetic nanoparticles optical and magnetic properties (Aminul Islam and Ziaul Ahsan 2020). These supermolecular structures are very vulnerable to the virus, enabling magnanimous nanosensors to be built to distinguish targets like nucleic acid (DNA, RNA) and proteins, particularly SARS-CoV-2, and thus finally to rapidly identify SARS-CoV-2 protein (+ssRNA) (Wang et al. 2002; Schotter et al. 2004).

The giant magnetoresistive (GMR) biosensor along with magnetic nanoparticles is a potent device for rapid biomolecule detection. The basic concept of GMR-based immunoassay is magnetization alternation with electrical transition. As a consequence, when the spin collision increases, the electrical resistance decreases, which eventually increases the magnetization of the interface between the magnetic nanoparticles and proteins, and thus, through proper calibration of the uninfected body, the magnetic signal may be the measurement parameters of the detector in its operation. In the overall debate, detecting respiratory viral pathogens such as SARS-CoV-2, magnetic nanoparticles play a crucial role in GMR biosensing technology. The GMR biosensor-based platform is more responsive, time-consuming, and low cost than other traditional testing systems (Wang et al. 2015; Schotter et al. 2004; Krishna et al. 2016; Nabaei et al. 2018).

23.3.2 Gold Biosensors

Gold nanostructures were used particularly to produce biosensors for virus detection in terms of optical signal amplifiers, current amplifier, and light diffusion (Draz and Shafiee 2018). They have excellent optical/electric characteristics, fantastic biocompatibility, catalytic properties, and relatively easy production processes (Bollella et al. 2017). Qiu et al. (2020) have developed a novel plasmone-based double-function biosensing platform for sensing SARS-CoV-2. In the course of auto-assembly, the Au-Film was first prepared by magnetron sputtering using a two-dimensional gold nanoisland (AuNIs) chip [2D-AuNIs]. On the BK7 glass surface, the au-film was formed by magnetron sputtering. Furthermore, 2D-AuNI were worked with the complementary DNA receptors and were enabled by a nucleic acid hybridization to detect SARS-CoV-2 sensitively (Fig. 23.4). In order to improve laser beam sensing properties, the plasmonic resonances of the plasmonic photothermal and localized surface plasmon resonance were permitted to drop at two different wavelengths. The in situ enhancement in plasmonic photothermal was furthermore reported to significantly improve the kinetic hybridization and thus the specific detection of nucleic acid. With present COVID-19 detection methods, there are several false positives or false negatives registered. The plasmonic photothermal heating inhibits the falsified binding of non-compatible sequences and thus prevents the erroneous diagnosis (Qiu et al. 2020).

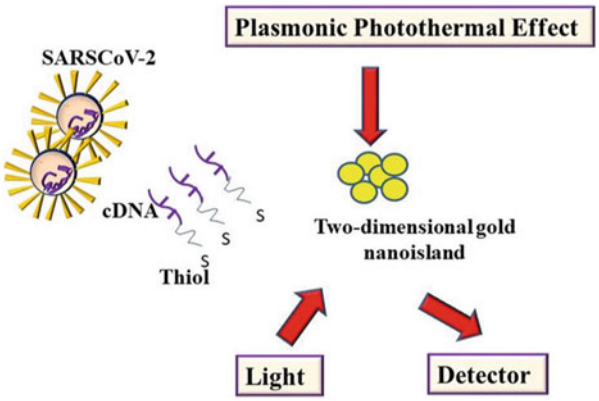

Fig. 23.4 Schematic diagram of two-dimensional gold nanoisland-plasmonic photothermal enhanced localized surface plasmon resonance DNA sensor. (Antiochia 2020)

23.3.3 Graphene-Based Biosensors

A graph sheet is the sensing region for the biosensor based on field effect transistor (FET), which is transmitted to a substratum SiO_2/Si and subsequently modified by the spike antibody SARS-CoV-2, which is disseminated correctly to the graphene sheet surface by casting. The system allowed detection at levels of 1 fg/mL in the phosphate buffer of a SARS-CoV-2 antigen spike protein, which is much smaller than the ELISA and PCR-methods shown (Qiu et al. 2020; Chu et al. 2020). Moreover, there was no substantial response to MERS-CoV spike protein from this sensor, ensuring that the antigen protein was highly selective and unique for the Spike SARS-CoV-2. Without sample preparation or preprocessing, this nanobiosensor permitted the discrimination between patient and normal samples with detection limits lower than those recorded with other current methods as denoted in Fig. 23.5 (Seo et al. 2020). In another recently published study, *Vibrio parahaemolyticus* can be translated for COVID-19 detection using a portable electrochemical biosensor based on graphene for highly sensitive point-of-care diagnostic tests. Detection was achieved with a loop-mediated isothermal amplification (LAMP) (SPE). The interaction between a graphene-based screen-printed electrode and amplicons leads to a change from the interplay of the redox sensor with ds-DNA in cathodic current (Kampeera et al. 2019).

23.3.4 Black Phosphorous Nanobiosensor

Infection with SARS-CoV-2 allows IgM, IgA, and IgG antibodies to form, like infections with other pathogens (Ma et al. 2020). A new research has shown that the electrochemical biosensor was formed with an aptamer-functionalized nanostructured black phosphorus. After poly-L-Lysine coating, the black phosphorus nanosheets are functionalized with anti-antibody-aptamers. Compared to the lower graphene oxide biosensors, biosensors on the black phosphorus nanostructure

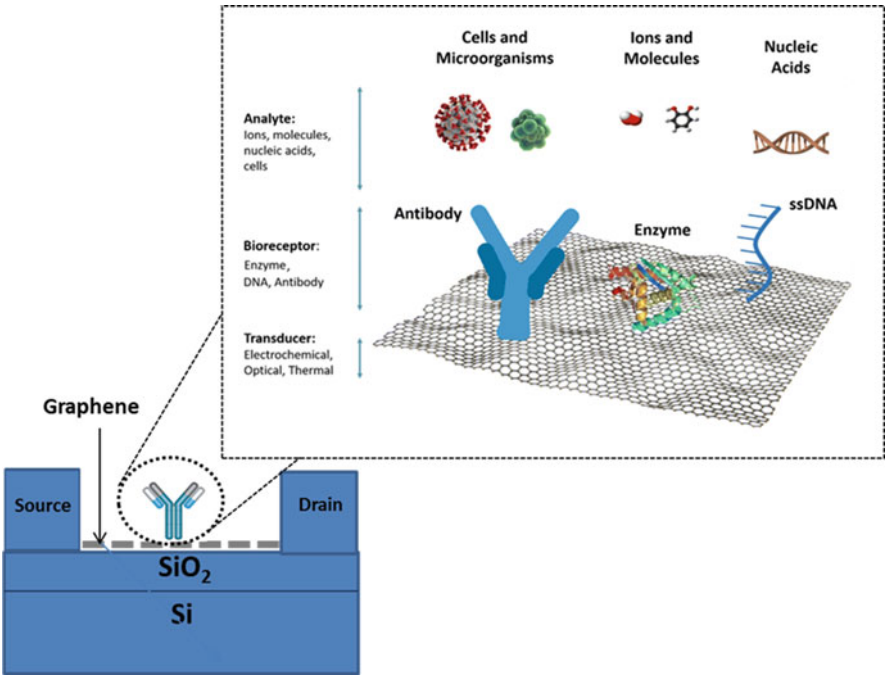

Fig. 23.5 Graphic diagram of graphene-based biosensors

base demonstrate higher sensitivity and specificity to detect, respectively, down to pg and ng level (Kumar et al. 2016).

23.3.5 Carbon Nanotubes Sensor

Carbon nanotubes have offered a wide range of scientific research according to particular characteristics and activities, such as thermal, electrical, chemical, and mechanical behavior. In the field of biosensors, the biomedical usefulness of these carbon-based nanomaterials is of particular interest. In the preparation of biosensors that can detect target molecules in trace quantities, carbon nanotubes play a significant role (Yang et al. 2015). In order to generate a modified AuNP-containing carbon nanotube electrode, Oh et al. (2009) and Duc Chinh et al. (2019) used electrochemical impedances to deposit AuNPs on single in situ wall carbohybrid nanotubes (SWCNTs). The DNA of hepatitis B and papilloma virus were restrained on SWCNTs/Au and catched by DNA probe as shown in Fig. 23.6 (Duc Chinh et al. 2019; Oh et al. 2009). Also, carbon nanotubes/Pt/Cr sensor used for detecting the DNA of influenza type A and catched by using DNA probe (Gopinath et al. 2018). The new generation of carbon nanotube biosensors showed great sensitivity because of their high surface area, simple preparation, and a good retention for nanoparticle (Oh et al. 2009).

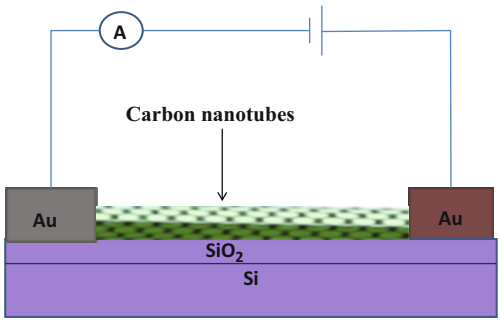

Fig. 23.6 Graphic diagram of carbon nanotubes based biosensors

23.3.6 Silica Nano-Based Sensor

The visible benefits of silica nanoparticles concern their ability to design special, biologically compliant nanolayer structures. It promotes the use of bio- and immunosensors in various forms. Chen et al. (2010) have studied the identification of Epstein-Barr virus-derived latent membrane protein 1 (LMP-1) using a multi-layered structure to improve the detection sensitivity. With silica nanospheres and QDs of CdTe/QDs on gold layer, a biosensor was designed to amplify the signals with a limit of LMP-1 detection of 1 pg/mL and 0.001–10 ng/mL of linear range.

23.3.7 Silver Nano-Based Sensor

The fluorescence features of silver nanostructures provided high sensitivity for optical biosensors. Cao et al. (2015) prepared biosensors based upon fluorescence activity of the silver nanoclusters with the detection of target DNA sequence (HIV), (HBV), and (HTLV-I) genes. The fluorescence behavior of the silver nanoclusters is strong and bright before the conjugation of the hairpin probe with the target DNA viruses. However, the fluorescence of nanoclusters become weak and the structure of the hairpin probe is thus disorders after binding between the strand of the probe and the target DNA. High sensitivity and low LOD of 4.4, 6.8, and 8.5 nM for the detection of HIV, HBV, and HTLV-I were the advantages of this type of biosensor (Fig. 23.7).

23.3.8 Copper Nano-Based Sensor

New nanotechnology offers new ways to investigate the viral impact of copper nanoparticles. Copper nanoparticles can interact closely with the virus and identify it easily (Magdassi et al. 2010). In recent study, they used copper nanoclusters to create colorimetric biosensing technique. A naked eye may make it possible to identify the DNA hepatitis B virus. This method has significant potential compared to traditional methods. The high sensitivity and selectivity, correct diagnosis, and a

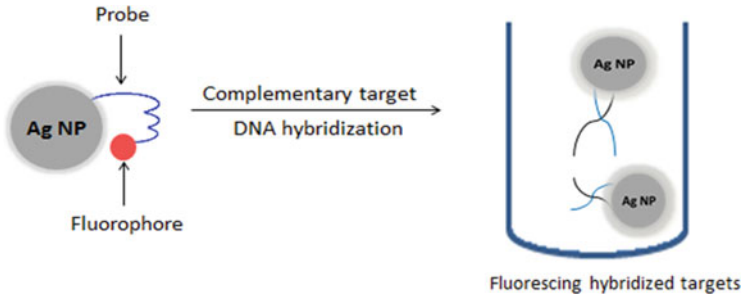

Fig. 23.7 Graphic diagram of silver nano-based sensor

cost-effective were beneficial. In short, this test is a very desirable one in DNA analysis candidate who does not need sophisticated and costly solvents (Mao et al. 2016).

23.3.9 Lanthanide-Doped Polystyrene NPs Enabled Biosensing

The lanthanides have a unique electronic configuration that allows lanthanide doped NPs to have many attractive optical properties, including a long luminescence life, a large and sharp emission band. Due to the long luminescence period, lanthanide doped NPs are often used for highly sensitive biosensor applications (Banerjee and Jaiswal 2018). Using lanthanide doped NPs, a lateral flow immunoassay (LFIA)-based biosensors have been described as a diagnostic point for the treatment of infectious agents (Chen et al. 2020). The LFIA developed is based on the principle of detection of antiCoV-2 IgG in human serum samples. This biosensor platform was made using lanthanide doped polystyrene nanoparticles (LNP) which were made by a mini emulsion polymerization process. In addition, surface modification of the LNP, which acts as a fluorescent probe, was carried out using murine anti-human IgG (MH-IgG) and rabbit IgG (R-IgG) antibodies after the EDC/NHS chemical reaction. The nitrocellulose membrane is used as a template to immobilize the recombinant nucleocapsid-phospho-protein from CoV-2, which is responsible for the specific IgG configuration. The resulting LIFA can detect anti-CoV-2 IgG in human serum in about 10 min. To confirm the clinical application of LIFA, the authors also compared the detection results of anti-CoV-2 IgG using the RT-PCR technique. The LIFA observations were the same as those obtained by the RT-PCR technique, except for one sample which showed the opposite result. Thus, the authors confirm that the developed LFIA does not provide precise quantitative results due to the lack of an anti-CoV-2 IgG standard but can be of great interest for the rapid diagnosis of a suspicious COVID-19 case (Ma et al. 2019).

23.3.10 Nanowire Affinity-Based Biosensors

Nanowire (NW) is one-dimensional nanostructures in the form of wires that can consist of non-metallic and metallic elements with a nanometer and micrometer diameter. NW is strong and has high physical strength, which is directly due to the unique crystal structure and morphology of 1D, as well as its mechanical, electrical, magnetic optical and thermal properties. Silica NW has been extensively researched for biosensor applications due to its photonic, optical, and electronic properties with excellent biocompatibility for sensory applications (Ambhorkar et al. 2018; Patolsky et al. 2006). Due to their wide band, which extends the detection range from purely electrochemical or FET-based detection to simpler optical methods, NW silicon and indium oxides are primarily investigated as new biosensors for the detection of highly sensitive viruses (Kaushik et al. 2014; Arora et al. 2013).

23.4 Fungal Infections as Post-COVID Symptoms

People suffering from COVID-19 threat, such as those in an intensive care unit (ICU), are especially susceptible to fungal infections. Aspergillosis or invasive candidiasis (caused by white fungi) is the most frequent fungal infections in people with COVID-19 (Koehler et al. 2020a, b; Song et al. 2020). These fungal co-infections are becoming more common and have been linked to serious sickness and mortality. It is critical to be aware of the likelihood of fungal co-infection in order to decrease delays in detection and treatment and thereby assist prevent serious disease and death from these infections (Beer et al. 2020; Lansbury et al. 2020). Previously, researchers believed that aspergillosis only occurred in persons with extremely compromised immune systems. However, aspergillosis is increasingly being documented in people who do not have impaired immune systems but have severe viral respiratory infections, such as influenza. Several recent studies have described COVID-19-related pulmonary aspergillosis (Benedetti et al. 2020; Marr et al. 2021; Dellière et al. 2020; Koehler et al. 2020a, b; Verweij et al. 2020). In individuals with severe COVID-19, fungal infections that are resistant to antifungal therapy have also been reported. Early detection and monitoring for Candida infections and antifungal resistance infections (e.g., *Candida auris*, azole-resistant *Aspergillus*) are critical for minimizing COVID-19 mortality in individuals with severe COVID-19 fungal infections (Posteraro et al. 2020; Meijer et al. 2020).

23.4.1 Biosensors for Fungal Diagnosis

Current and future advances in biosensor technology, which employ a slide of approaches that have yet to be utilized in the framework of medical mycology, are anticipated to improve fungal diagnostic research greatly. Biosensor technologies are projected to play an increasingly essential part in the detection and monitoring of all infectious illnesses, with particular relevance in the initial identification of fungal

Table 23.2 Methods documented for fungal diagnosis based on nanomaterials

Fungi	Nanomaterials	Specimen	Limit of detection	References
Paracoccidioides brasiliensis	Gold nanoparticles	Fungal DNA	Greater than 4 mg mL	Martins et al. (2012)
Candida albicans	Carbon nanotubes	Fungal solutions	50 Colony Forming Unit/mL	Villamizar et al. (2009)
Candida spp.	Gold nanoparticles	Wastewater effluent	–	Naja et al. (2008)
Candida albicans	Peptide nuclei acids	Blood culture	100%	Rigby et al. (2002)
Aspergillus fumigatus				
C. glabrata	Gold nanowire	Fungal DNA	100 fM	Yoo et al. (2011)
C. krusei				
Cryptococcus neoformans				
Candida spp.	Nanoparticles	Whole blood	1 Colony Forming Unit/mL	Neely et al. (2013)

infection. Furthermore, biosensors provide continuous monitoring of analytes, which may aid in assessing therapy response. Recently, most fungal diagnostic approaches include invasive sample, are time demanding, and/or have limitations in terms of specificity or sensitivity (Hussain et al. 2020). As a result, creating new analytical methods that overcome these constraints is critical in establishing improved fungal infection control techniques. The development of nanotechnology has enabled the combining of numerous analytical approaches to develop new options in this arena. For example, spectrophotometric methods have been applied to diagnose *Paracoccidioides brasiliensis*, and currently an amalgamation of artificial intelligence and metabolomics was documented for paracoccidioidomycosis (Martins et al. 2012; De Oliveira et al. 2020). Fluorescence in situ hybridization approaches for aspergillosis and candidiasis have been published (Rickerts et al. 2011; Da Silva et al. 2015). MALDI-TOF analysis (matrix-assisted laser desorption/ionization time of flight) currently allows the exact and quick detection procedures for Candida and other yeast species, which are quickly being adapted for filamentous fungus (Lacroix et al. 2014). On primary patient samples, this methodology is also being examined for its value as a diagnostic approach. However, this is a costly approach that needs specialized technical expertise and equipment and cannot be easily deployed in field conditions. Recent studies have also concentrated on the synthesis of new nanomaterials for the building of analytical stages in order to increase sensitivity and specificity. Quantum dots, molecular beacons, DNA dendrimers, and other nanomaterials have been used in numerous techniques (Table 23.2).

Microfluidic-based approaches for fungal detection have lately emerged as an active research topic (Zhou et al. 2019). There have been published literatures based

on various detection methodologies. Using a real-time PCR-based microfluidic device, *Candida albicans* DNA was identified in human blood (Busser et al. 2020). Other reports documented approaches including the use of gold nanoparticles, peptide nucleic acid, gold-nanowire, and colloidal gold and silver (Rigby et al. 2002; Yoo et al. 2011; Naja et al. 2008; Neely et al. 2013). A polymerase free technique for *C. albicans* identification in clinical samples was just published (Chen et al. 2020). The suggested approach was based on single molecule tethering where the movement of the beads tethered by DNA probes provided the signal, and the suggested approach could detect 1 colony forming unit per milliliter in blood sample. A colorimetric approach for the diagnosis of *Aspergillus niger* spores was established based on interfaces between fungal spores and gold nanoparticles altered with a specific binding peptide that was recognized by phage display screening. These binding peptides can be identified 50 spores within 10 min (Lee et al. 2020).

Generally, for the manufacturing and development of fungal biosensors for clinical application, there are two important needs: first, unique biomarkers that would allow particular identification of the target organism, ideally from a variety of clinical specimens, must be identified. Secondly, the chosen biomarkers must be successfully immobilized on the sensing surface. Biomarkers must have features that can be quantified and utilized to determine if the circumstances are ordinary or pathogenic. They are cellular or molecular in origin and may be tested in tissue biopsies, or in cerebrospinal fluid, and blood, etc. A diverse range of fungal biomarkers have been investigated as potential candidates such as galactomannan, mannan, beta-glucan, and cryptococcal antigen (Huppler et al. 2017; Kauffman et al. 2011). These biomarkers, which have been identified and established into diagnostic investigations, might be studied for their possible adaption for detection via biosensor, which could then be reduced and put into a portable instrument. Other biomarkers, as siderophore, mycotoxins,, dendritic cell-associated lectin-2, offer promise but have not yet to be turned into diagnostic tools (Kong et al. 2019; Chauhan et al. 2016; Vendele et al. 2020).

23.5 Conclusion

The traditional virus detection methods have difficulties such as cost, high risk of contamination, and the viral load. However, biosensors offer highly precise sensitive and specific systems able to calculate very low viral concentrations. Nanomaterials have succeeded in integrating in biosensing applications to easily, sensitively, and reliably recognize influenza A, hepatitis B virus, papilloma virus, and SARS-CoV-2. Magnetic nanoparticles have provided significant interest to biosensing methods because they provide unrivaled benefits over other techniques. The development of magnetic nanoparticles, for example, is cost-effective, mechanically and chemically stable and biocompatible, and they are functionalized with antibodies or DNA/RNA probes that can precisely bind to target SARS-CoV-2, herpes simplex virus, adenovirus, and influenza viruses. Also, using of black phosphorus nano-based sensor

provides high sensitivity at ng levels for detection of SARS-CoV-2 antibodies. Other nano-based sensors like carbon, silica, silver, and copper nano-based sensors used to detect the viral DNA according to the particular characteristics. In brief, major challenges still need to be tackled, and tremendous efforts should be made to improve future nano-based sensor studies for virus detections because of their potential for early, precise diagnosis and avoiding further outbreaks of the pandemic. The protein constituents of coronaviruses that have been used for the attacking of these respiratory viruses and their replication were essential in nanobiosensors for human coronavirus detection.

Nowadays, fungal infections are becoming a growing health and economic problems. Especially, black or white fungi are the most frequent fungal infections in people with COVID-19. Accurate and timely identification of fungal infections is critical to supplement efforts in the development of therapeutic medicines, because late identification of fungal infections dramatically reduces the possibility of therapeutic treatments being successful. Traditional diagnostic techniques have already made tremendous progress in identifying and managing invasive fungal diseases. The problem is to determine the causal agent precisely and quickly since this typically directs the right antifungal therapy. Recent diagnosis will be progressively supplemented as nanotechnologies evolve, perhaps leading to the creation of less invasive and miniaturized detection systems. The next age of biosensors is now being created by immobilizing particular protein indicators onto gold nanoparticles. Photonic approaches can be used to identify biosensor interactions with particular targets on the surface of pathogenic fungus. These innovative approaches provide significant potential in addressing the issues connected with the quick identification of fungal infection in human medicine.

Funding No funding

Availability of Data The datasets generated or analyzed during the review are available from the corresponding author on reasonable request.

Author Contribution Abeer E. aly wrote the abstract, Hossam M Mohamed, and Reem M. Mohamed El sapagh share in writing the introduction and biosensors, Heba S Abbas wrote nanobiosensors, fungi parts, conclusion, reviewing, and editing introduction and draw Figs. 23.1 and 23.3. Manal A. Nabil drew the rest of figures, and Doha Abou Baker wrote Lanthanide-doped polystyrene NPs enabled biosensing and nanowire parts and abstract.

Declarations Ethics Approval and Consent No specific approval committee was required as for this review. No informed consent was needed for this review.

Competing Interests The authors declare no competing interests.

References

Ambhorkar P, Wang Z, Ko H, Lee S et al (2018) Nanowire-based biosensors: from growth to applications. Micromachines (Basel) 9(12):679. https://doi.org/10.3390/mi9120679. PMID: 30572645; PMCID: PMC6316191

Aminul Islam M, Ziaul Ahsan M (2020) Plausible approach for rapid detection of SARS-CoV-2 virus by magnetic nanoparticle based Biosensors. Am J Nanosci 2(6):6–13. https://doi.org/10.11648/j.ajn.20200602.11

Antiochia R (2020) Nanobiosensors as new diagnostic tools for SARS, MERS and COVID-19: from past to perspectives. Microchim Acta 187(12):639. https://doi.org/10.1007/s00604-020-04615-x

Arora P, Sindhu A, Dilbaghi N et al (2013) Engineered multifunctional nanowires as novel biosensing tools for highly sensitive detection. Appl Nanosci 3(5):363–372. https://doi.org/10.1007/s13204-012-0142-4

Aytur T, Foley J, Anwar M et al (2006) A novel magnetic bead bioassay platform using a microchip-based sensor for infectious disease diagnosis. J Immunol Methods 314:21–29

Banerjee R, Jaiswal A (2018) Recent advances in nanoparticle-based lateral flow immunoassay as a point-of-care diagnostic tool for infectious agents and diseases. Analyst 143(9):1970–1996. https://doi.org/10.1039/c8an00307f. PMID: 29645058

Beer KD, Jackson BR, Chiller T et al (2020) Does pulmonary aspergillosis complicate COVID-19? Crit Care Exp 2(9):e0211

Bellan LM, Wu D, Langer RS (2011) Current trends in nanobiosensor technology. Wiley Interdiscip Rev Nanomed Nanobiotechnol 3(3):229–246. https://doi.org/10.1002/wnan.136

Benedetti MF, Alava KH, Sagardia J et al (2020) COVID-19 associated pulmonary aspergillosis in ICU patients: report of five cases from Argentina. Med Mycol Case Rep 31:24–28

Bollella P, Schulz C, Favero G et al (2017) Green synthesis and characterization of gold and silver nanoparticles and their application for development of a third generation lactose biosensor. Electroanalysis 29(1):77–86. https://doi.org/10.1002/elan.201600476

Busser FD, Coelho VC, de Abreu Fonseca C et al (2020) A real time PCR strategy for the detection and quantification of Candida albicans in human blood. Rev Inst Med Trop Sao Paulo 62:e9

Cao Q, Teng Y, Yang X et al (2015) A label-free fluorescent molecular beacon based on DNA-Ag nanoclusters for the construction of versatile Biosensors. Biosens Bioelectron 74:318–321. http://europepmc.org/abstract/MED/26159151

Cesewski E, Johnson BN (2020) Electrochemical biosensors for pathogen detection. Biosens Bioelectron 159:112214. https://doi.org/10.1016/j.bios.2020.112214. Epub 2020 Apr 12. PMID: 32364936; PMCID: PMC7152911

Chauhan R, Singh J, Sachdev T et al (2016) Recent advances in mycotoxins detection. Biosens Bioelectron 81:532–545

Chemla YR, Grossman HL, Poon Y et al (2000) Ultrasensitive magnetic biosensor for homogeneous immunoassay. Proc Natl Acad Sci U S A 97:14268–14272

Chen L, Qi Z, Chen R et al (2010) Sensitive detection of Epstein-Barr virus-derived latent membrane protein 1 based on CdTe quantum dots-capped silica nanoparticle labels. Clin Chim Acta 411(23–24):1969–1975. https://doi.org/10.1016/j.cca.2010.08.012. Epub 2010 Aug 13. PMID: 20713034

Chen Z, Zhang Z, Zhai X et al (2020) Rapid and sensitive detection of anti-SARS-CoV-2 IgG, using lanthanide-doped nanoparticles-based lateral flow immunoassay. Anal Chem 92(10):7226–7231. https://doi.org/10.1021/acs.analchem.0c00784

Chou TC, Hsu W, Wang CH et al (2011) Rapid and specific influenza virus detection by functionalized magnetic nanoparticles and mass spectrometry. J Nanobiotechnol 16(9):52. https://doi.org/10.1186/1477-3155-9-52. PMID: 22088100; PMCID: PMC3248366

Chowell G, Abdirizak F, Lee S et al (2015) Transmission characteristics of MERS and SARS in the healthcare setting: a comparative study. BMC Med 13(1):210. https://doi.org/10.1186/s12916-015-0450-0

Chu DKW, Pan Y, Cheng SMS et al (2020) Molecular diagnosis of a novel Coronavirus (2019-nCoV) causing an outbreak of pneumonia. Clin Chem 66(4):549–555. http://europepmc.org/abstract/MED/32031583

Da Silva RM, Da Silva Neto JR, Santos CS et al (2015) Evaluation of fluorescence in situ hybridisation (FISH) for the detection of fungi directly from blood cultures and cerebrospinal fluid from patients with suspected invasive mycoses. Ann Clin Microbiol Antimicrob 14:6

De Oliveira Lima E, Navarro LC, Morishita KN et al (2020) Metabolomics and machine learning approaches combined in pursuit for more accurate paracoccidioidomycosis diagnoses. mSystems 5(3):00258–00220

Dellière S, Dudoignon E, Fodil S et al (2020) Risk factors associated with Covid-19-associated pulmonary aspergillosis in ICU patients: a French multicentric retrospective cohort. Clin Microbiol Infect 27(5):790.e1–790.e5

Draz MS, Shafiee H (2018) Applications of gold nanoparticles in virus detection. Theranostics 8(7): 1985–2017. https://doi.org/10.7150/thno.23856. PMID: 29556369; PMCID: PMC5858513

Duc Chinh V, Speranza G, Migliaresi C et al (2019) Synthesis of gold nanoparticles decorated with multiwalled carbon nanotubes (Au-MWCNTs) via cysteaminium chloride functionalization. Sci Rep 9(1):5667. https://doi.org/10.1038/s41598-019-42055-7. PMID: 30952876; PMCID: PMC6450879

Gerrard JA (2013) Protein nanotechnology: what is it? Methods Mol Biol 996:1–15. https://doi.org/10.1007/978-1-62703-354-1_1. PMID: 23504415

Gopinath PG, Anitha VR, Aruna Mastani S (2018) Design of biosensor array with current boost and signal conditioning circuits for HPV detection. Alexandria Eng J 57(2):671–681. https://doi.org/10.1016/j.aej.2017.01.012

Hasanzadeh M, Sadeghi S, Bageri L et al (2016) Poly-dopamine-beta-cyclodextrin: a novel nanobiopolymer towards sensing of some amino acids at physiological pH. Mater Sci Eng C Mater Biol Appl 1(69):343–357. https://doi.org/10.1016/j.msec.2016.06.081. Epub 2016 Jun 29. PMID: 27612722

Hasanzadeh M, Mokhtari F, Shadjou N et al (2017) Poly arginine-graphene quantum dots as a biocompatible and non-toxic nanocomposite: layer-by-layer electrochemical preparation, characterization and non-invasive malondialdehyde sensory application in exhaled breath condensate. Mater Sci Eng C Mater Biol Appl 1(75):247–258. https://doi.org/10.1016/j.msec.2017.02.025. Epub 2017 Feb 14. PMID: 28415460

Haun JB, Yoon TJ, Lee H et al (2010) Magnetic nanoparticle biosensors. Wiley Interdiscip Rev Nanomed Nanobiotechnol 2(3):291–304. https://doi.org/10.1002/wnan.84. PMID: 20336708

Huang Y, Yang C, Xu XF et al (2020) Structural and functional properties of SARS-CoV-2 spike protein: potential antivirus drug development for COVID-19. Acta Pharmacol Sin 41(9): 1141–1149. https://doi.org/10.1038/s41401-020-0485-4. Epub 2020 Aug 3. PMID: 32747721; PMCID: PMC7396720

Huppler AR, Fishe BT, Lehrnbecher T et al (2017) Role of molecular biomarkers in the diagnosis of invasive fungal diseases in children. J Pediatr Infect Dis Soc 6:32–44

Hussain K, Malavia D, Johnson EM et al (2020) Biosensors and diagnostics for fungal detection. J Fungi (Basel) 6(4):349. https://doi.org/10.3390/jof6040349. PMID: 33302535; PMCID: PMC7770582

Josephson L, Perez JM, Weissleder R et al (2001) Magnetic nanosensors for the detection of oligonucleotide sequences. Angew Chem Int Ed Engl 40:3204–3206

Julian TR, Schwab KJ (2012) Challenges in environmental detection of human viral pathogens. Curr Opin Virol 2(1):78–83. https://doi.org/10.1016/j.coviro.2011.10.027

Kampeera J, Pasakon P, Karuwan C et al (2019) Point-of-care rapid detection of Vibrio parahaemolyticus in seafood using loop-mediated isothermal amplification and

graphene-based screen-printed electrochemical sensor. Biosens Bioelectron 1(132):271–278. https://doi.org/10.1016/j.bios.2019.02.060. Epub 2019 Mar 6. PMID: 30878727

Kang CK, Song KH, Choe PG et al (2017) Clinical and epidemiologic characteristics of spreaders of Middle East respiratory syndrome coronavirus during the 2015 outbreak in Korea. J Korean Med Sci 32(5):744–749. https://doi.org/10.3346/jkms.2017.32.5.744. PMID: 28378546; PMCID: PMC5383605

Kauffman CA, Fisher JF, Sobel JD et al (2011) Candida urinary tract infections—diagnosis. Clin Infect Dis 52:452–456

Kaushik A, Kumar R, Huey E et al (2014) Silica nanowires: growth, integration, and sensing applications. Mikrochim Acta 181(15–16):1759–1780. https://doi.org/10.1007/s00604-014-1255-0. PMID: 25382871; PMCID: PMC4219650

Koehler P, Bassetti M, Chakrabarti A et al (2020a) Defining and managing COVID-19-associated pulmonary aspergillosis: the 2020 ECMM/ISHAM consensus criteria for research and clinical guidance. Lancet 21:e149–e162

Koehler P, Cornely OA, Böttiger BW, Dusse F, Eichenauer DA, Fuchs F, Hallek M, Jung N, Klein F, Persigehl T, Rybniker J, Kochanek M, Böll B, Shimabukuro-Vornhagen A (2020) COVID-19 associated pulmonary aspergillosis. Mycoses 63(6):528–534. https://doi.org/10.1111/myc.13096

Kong H, Cheng W, Wei H et al (2019) An overview of recent progress in siderophore-antibiotic conjugates. Eur J Med Chem 182:271–277

Kotitz R, Matz H, Trahms L et al (1997) SQUID based remanence measurements for immunoassays. IEEE Trans Appl Superconduct 7:3678–3681

Krishna VD, Wu K, Perez AM et al (2016) Giant magnetoresistance-based biosensor for detection of influenza A virus. Front Microbiol 29(7):400. https://doi.org/10.3389/fmicb.2016.00400. PMID: 27065967; PMCID: PMC4809872

Krishna VD, Wu K, Su D, Cheeran MCJ et al (2018) Nanotechnology: review of concepts and potential application of sensing platforms in food safety. Food Microbiol 75:47–54. https://doi.org/10.1016/j.fm.2018.01.025. Epub 2018 Feb 6. PMID: 30056962

Kumar V, Brent JR, Shorie M et al (2016) Nanostructured aptamer-functionalized black phosphorus sensing platform for label-free detection of myoglobin, a cardiovascular disease biomarker. ACS Appl Mater Interfaces 8(35):22860–22868. https://doi.org/10.1021/acsami.6b06488

Lacroix C, Gicquel A, Sendid B et al (2014) Evaluation of two matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) systems for the identification of Candida species. Clin Microbiol Infect 20:153–158

Lansbury L, Lim B, Baskaran V, Lim WS (2020) Co-infections in people with COVID-19: a systematic review and meta-analysis. J Infect 81(2):266–275

Lee JI, Jang SC, Chung J et al (2020) Colorimetric allergenic fungal spore detection using peptide-modified gold nanoparticles. Sensors Actuators B Chem 327:128894

Li G, Sun S, Wilson RJ, White RL et al (2006) Spin valve sensors for ultrasensitive detection of superparamagnetic nanoparticles for biological applications. Sensors Actuators A Phys 126:98–106

Li H, Liu S-M, Yu X-H et al (2020) Coronavirus disease 2019 (COVID-19): current status and future perspectives. Int J Antimicrob Agents 55(5):105951. https://doi.org/10.1016/j.ijantimicag.2020.105951

Ma Q, Wang J, Li Z, Lv X (2019) Recent progress in time-resolved biosensing and bioimaging based on lanthanide-doped nanoparticles. Small 15(32):e1804969. https://doi.org/10.1002/smll.201804969. Epub 2019 Feb 14. PMID: 30761729

Ma H, Zeng W, He H et al (2020) Serum IgA, IgM, and IgG responses in COVID-19. Cell Mol Immunol 17(7):773–775. https://doi.org/10.1038/s41423-020-0474-z

Magdassi S, Grouchko M, Kamyshny A (2010) Copper nanoparticles for printed electronics: routes towards achieving oxidation stability. Materials 3(9):4626–4638. https://doi.org/10.3390/ma3094626. PMID: 28883344; PMCID: PMC5445770

Mao X, Liu S, Yang C et al (2016) Colorimetric detection of hepatitis B virus (HBV) DNA based on DNA-templated copper nanoclusters. Anal Chim Acta 25(909):101–108. https://doi.org/10.1016/j.aca.2016.01.009. Epub 2016 Jan 11. PMID: 26851090

Marr KA, Platt A, Tornheim JA et al (2021) Aspergillosis complicating severe coronavirus disease. Emerg Infect Dis 27(1):18–25

Martins JFS, Castilho ML, Cardoso MAG et al (2012) Identification of paracoccidioides brasiliensis by gold nanoprobes. In: Proceedings of the biomedical vibrational spectroscopy V: advances in research and industry, San Francisco, CA, USA, 31 Jan 2012

Meijer EFJ, Dofferhoff ASM, Hoiting O et al (2020) Azole-resistant COVID-19-associated pulmonary aspergillosis in an immunocompetent host: a case report. J Fungi 6(2):79

Nabaei V, Chandrawati R, Heidari H (2018) Magnetic biosensors: modelling and simulation. Biosens Bioelectron 30(103):69–86. https://doi.org/10.1016/j.bios.2017.12.023. Epub 2017 Dec 20. PMID: 29278815

Naja G, Hrapovic S, Male K et al (2008) Rapid detection of microorganisms with nanoparticles and electron microscopy. Microsc Res Tech 71:742–748

Neely LA, Audeh M, Phung NA, Min M et al (2013) T2 magnetic resonance enables nanoparticle-mediated rapid detection of candidemia in whole blood. Sci Transl Med 5(182):182ra54

Nguyen BT, Koh G, Lim HS et al (2009) Membrane-based electrochemical nanobiosensor for the detection of virus. Anal Chem 81(17):7226–7234. https://doi.org/10.1021/ac900761a. PMID: 19663392

Oh J, Yoo S, Chang YW et al (2009) Carbon nanotube-based biosensor for detection hepatitis B. Curr Appl Phys 9(4):e229–e231. https://www.sciencedirect.com/science/article/pii/S1567173909003381

Osterfeld SJ, Yu H, Gaster RS et al (2008) Multiplex protein assays based on real-time magnetic nanotag sensing. Proc Natl Acad Sci U S A 105:20637–20640

Ozer T, Geiss BJ, Henry CS (2020) Review—chemical and biological sensors for viral detection. J Electrochem Soc 167:037523. https://doi.org/10.1149/2.0232003jes

Pashazadeh P, Mokhtarzadeh A, Hasanzadeh M et al (2017) Nano-materials for use in sensing of salmonella infections: recent advances. Biosens Bioelectron 87:1050–1064

Patolsky F, Zheng G, Lieber CM (2006) Nanowire-based biosensors. Anal Chem 78(13):4260–4269. https://doi.org/10.1021/ac069419j

Payne S (2017) Methods to study viruses. Viruses 37–52. https://doi.org/10.1016/B978-0-12-803109-4.00004-0

Perez JM, Josephson L, O'Loughlin T et al (2002) Magnetic relaxation switches capable of sensing molecular interactions. Nat Biotechnol 20:816–820

Perez JM, Simeone FJ, Saeki Y et al (2003) Viral-induced self-assembly of magnetic nanoparticles allows the detection of viral particles in biological media. J Am Chem Soc 125(34):10192–10193. https://doi.org/10.1021/ja036409g

Pinheiro AV, Han D, Shih WM et al (2011) Challenges and opportunities for structural DNA nanotechnology. Nat Nanotechnol 6(12):763–772. https://doi.org/10.1038/nnano.2011.187. PMID: 22056726; PMCID: PMC3334823

Posteraro B, Torelli R, Vella A et al (2020) Pan-echinocandin-resistant *Candida glabrata* bloodstream infection complicating COVID-19: a fatal case report. J Fungi 6(3):163

Qiu G, Gai Z, Tao Y et al (2020) Dual-functional plasmonic photothermal biosensors for highly accurate severe acute respiratory syndrome coronavirus 2 detection. ACS Nano [Internet] 14(5):5268–5277. https://doi.org/10.1021/acsnano.0c02439

Rickerts V, Khot PD, Myerson D et al (2011) Comparison of quantitative real time PCR with sequencing and ribosomal RNA-FISH for the identification of fungi in formalin fixed, paraffin-embedded tissue specimens. BMC Infect Dis 11:202. [CrossRef] [PubMed]

Rigby S, Procop GW, Haase G et al (2002) Fluorescence in situ hybridization with peptide nucleic acid probes for rapid identification of Candida albicans directly from blood culture bottles. J Clin Microbiol 40:2182–2186

Saylan Y, Erdem Ö, Ünal S, Denizli A (2019) An alternative medical diagnosis method: biosensors for virus detection. Biosensors 9(2):65. https://doi.org/10.3390/bios9020065. Published 2019 May 21

Schotter J, Kamp PB, Becker A et al (2004) Comparison of a prototype magnetoresistive biosensor to standard fluorescent DNA detection. Biosens Bioelectron 19(10):1149–1156. https://doi.org/10.1016/j.bios.2003.11.007. PMID: 15046745

Seo G, Lee G, Kim MJ et al (2020) Rapid detection of COVID-19 causative virus (SARS-CoV-2) in human nasopharyngeal swab specimens using field-effect transistor-based biosensor. ACS Nano 14(4):5135–5142. https://doi.org/10.1021/acsnano.0c02823

Sheridan C (2020) Fast, portable tests come online to curb coronavirus pandemic. Nat Biotechnol 38(5):515–518. https://doi.org/10.1038/d41587-020-00010-2. PMID: 32203294

Song G, Liang G, Liu W (2020) Fungal co-infections associated with global COVID-19 pandemic: a clinical and diagnostic perspective from China. Mycopathologia 185(4):599–606

Storch GA (2000) Diagnostic virology. Clin Infect Dis 31(3):739–751. https://doi.org/10.1086/314015

Suo T, Liu X, Feng J et al (2020) ddPCR: a more accurate tool for SARS-CoV-2 detection in low viral load specimens. Emerg Microbes Infect 9(1):1259–1268. https://doi.org/10.1080/22221751.2020.1772678. PMID: 32438868; PMCID: PMC7448897

Tahamtan A, Ardebili A (2020) Real-time RT-PCR in COVID-19 detection: issues affecting the results. Expert Rev Mol Diagn 20(5):453–454. https://doi.org/10.1080/14737159.2020.1757437. Epub 2020 Apr 22. PMID: 32297805; PMCID: PMC7189409

Vendele I, Willment JA, Silva LM et al (2020) Mannan detecting C-type lectin receptor probes recognise immune epitopes with diverse chemical, spatial and phylogenetic heterogeneity in fungal cell walls. PLoS Pathog 16:e1007927

Verweij PE, Gangneu J, Bassetti M et al (2020) Diagnosing COVID-19-associated pulmonary aspergillosis. Lancet Microbe 1(2):e53–e55

Villamizar RA, Maroto A, Rius FX (2009) Improved detection of Candida albicans with carbon nanotube field-effect transistors. Sensors Actuators B Chem 136:451–457

Wang Q, Lin T, Tang L et al (2002) Icosahedral virus particles as addressable nanoscale building blocks. Angew Chem Int Ed Engl 41(3):459–462. https://doi.org/10.1002/1521-3773(20020201)41:3<459::aid-anie459>3.0.co;2-o. PMID: 12491378

Wang SX, Bae SY, Li G et al (2005) Towards a magnetic microarray for sensitive diagnostics. J Magn Magn Mater 293:731–736

Wang Y, Wang W, Yu L, Tu L, Feng Y, Klein T et al (2015) Giant magnetoresistive-based biosensing probe station system for multiplex protein assays. Biosens Bioelectron 70:61–68. https://doi.org/10.1016/j.bios.2015.03.011

Wu K, Su D, Saha R et al (2020) Magnetic particle spectroscopy: a short review of applications using magnetic nanoparticles. ACS Appl Nano Mater 3(6):4972–4989. https://doi.org/10.1021/acsanm.0c00890

Yang N, Chen X, Ren T et al (2015) Carbon nanotube based biosensors. Sensors Actuators B Chem 207:690–715

Yoo SM, Kang T, Kang H et al (2011) Combining a nanowire SERRS sensor and a target recycling reaction for ultrasensitive and multiplex identification of pathogenic fungi. Small 7:3371–3376

Zhou W, Le J, Chen Y et al (2019) Recent advances in microfluidic devices for bacteria and fungus research. TrAC Trends Anal Chem 112:175–195

Platform Technologies Based on Virus-Like Particles (VLPs) for Infectious Diseases

24

Iram Saba, Kaiser Wani, Suriya Rehman, and Vipin Singh

Abstract

Platform technologies provide an effective tool where peptides and protein antigens are incorporated onto virus-like particles (VLPs) to enhance their immunogenicity. Virus-like particles (VLPs) constitute the virus-derived entities composed of single or several distinct molecules that have the potential to self-assemble, simulate a virus particle however missing the genetic part to infect the host cell. VLPs are considered as tailorable material as we can modify their function using a number of techniques based on genetic as well as chemical engineering. These modular platforms help in overcoming the limitations faced by conventional vaccines and help in their rapid, safe, and cost-effective production. In this chapter we will discuss about this promising platform technology based on VLPs, its principles, procedures, and usage in treating infectious diseases.

I. Saba (✉)
Research and Scientific Center, Sultan Bin Abdulaziz Humanitarian City, Riyadh, Saudi Arabia

Amity Institute of Biotechnology, Amity University Haryana, Manesar, Haryana, India

K. Wani
Department of Biochemistry, College of Science, King Saud University, Riyadh, Saudi Arabia

University Institute of Biotechnology, Chandigarh University, Mohali, India
e-mail: kwani@ksu.edu.sa

S. Rehman
Department of Epidemic Disease Research, Institute for Research and Medical Consultations (IRMC), Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia
e-mail: surrehman@iau.edu.sa

V. Singh
University Institute of Biotechnology, Chandigarh University, Mohali, India
e-mail: Vipin.singh@cumail.in

S. Hameed, S. Rehman (eds.), *Nanotechnology for Infectious Diseases*,
https://doi.org/10.1007/978-981-16-9190-4_24

Keywords

Enveloped virus · Immune response · Platform technologies · Vaccine · Virus-like particles

24.1 Introduction

In order to defend against infectious pathogens, vaccination is considered as a leading prevention strategy (Khan et al. 2020). Vaccines provide a long-lasting protective immunity and elicit a strong immune response by first producing neutralizing antibodies which activate the cellular immunity followed by inducing immune memory (Pulendran and Ahmed 2011; Rehman et al. 2020a, b, 2021a). Traditional vaccines which are either live attenuated or inactivated subunits have been useful to control or eliminate various diseases such as polio, measles, mumps, rubella, malaria, hepatitis B, rabies, anthrax, and cholera (Scorpio et al. 2006). Vaccination drives against specific diseases have been successful and have had strong socioeconomic impact in the past (Cunningham et al. 2016). Notable examples include eradication of smallpox in 1980, and then the eradication of rinderpest in 2011. Despite these successes, these traditional vaccines possess major drawbacks, such as:

- They require the lengthy culturing process in order to produce a large volume of pathogens (viruses and bacteria) thus a huge lag time is generated in between the synthesis and delivery of antigens.
- Owing to the infectious property of their material, they are not considered environmentally safe, thus require the specialized facility in order to keep the harmful material under control.
- They have ability to revert back to virulent strains.
- We also fail to produce vaccines against those pathogens which have high mutation rate as well as high antigenicity, e.g., influenza virus, human immunodeficiency virus, rotavirus, enterovirus and group A streptococcus, and most recently Corona virus where the vaccine efficacy is still being debated.
- Outbreak of infectious disease during the last decade such as Ebola, Zika, H1N1 Influenza, MERS, and the current COVID-19 pandemic gives us reminders that that there is a need of a new vaccine technology which will reduce the time between development and production short (Keller et al. 2010).

A platform technology which is based on production of targeted antigens with a calculated platform base can help us to overcome the above-mentioned drawbacks. A standard approach towards vaccine platform technologies based on virus-like particles (VLPs) is a customized platform that offers safe and easy production of antigens of choice. Virus-like particles (VLPs) are the nanoparticles which lack infectious nucleic acid and possess an assembly of biocompatible capsid proteins (Boisgérault et al. 2002; Rehman et al. 2021a, b). VLPs are composed of proteins

which can self-assemble with each other and do not contain any accessory proteins or nucleic acids, thus forming a hollow interior with outside coat of multiunit protein (Rehman et al. 2020a, b, c, d). VLPs are ideal systems even in absence of adjuvants (Roy and Noad 2008). A technique which is based on self-assembly of capsid protein derived from target virus is an important approach to VLP manufacture (Rehman et al. 2019). VLPs are composed of protein components with highly ordered structures and varying degree of complexity (Manolova et al. 2008). Due to their multivalent structure, they can be combined with an adjuvant in order to elicit robust cellular and humoral immune response (Elsharif et al. 2019). The antigens which activate the B cell crosslinking as well as pathogen associated molecular patterns (PAMPs) are displayed in repeated array thereby inducing stronger and long-lasting antigen specific immune response (Demento et al. 2011). The size of VLPs which ranges from 20 to 500 nm makes them efficient in entering various antigen-presenting cells (APCs) including dendritic cells (DC) and phagocytes, hence an alluring technique for enhanced immunogenicity of antigens (Jennings and Bachmann 2008). VLPs lack viral genome and thus there is no risk associated with viral replication and inactivation (Crisci et al. 2012). Platform based VLPs are produced on the basis of technologies that allows to regulate upstream and downstream process. Multivalent vaccines are generated which act against multiple strains of an antigenically diverse pathogen. They provide a facility to generate flexible multiproduct (Peacey et al. 2007). In order to generate a set up for production facility, previous knowledge and experience are very beneficial. Platform technology helps in production of vaccines of lower cost in developing countries where vaccine availability is still considered a challenge (Plotkin et al. 2017). When platform technology is combined with modular single use technology, modern vaccines may be potentially of low cost (Zhang et al. 2014a). In this chapter we will discuss the platform technologies based on virus-like particles (VLPs), with emphasis on challenges and opportunities offered by them.

24.2 Platform Technologies Based on Genetic Engineering of VLPs

VLPs can be engineered in order to produce recombinant VLPs possessing antigenic epitopes on the surface or adding some terminal regions of coat proteins. Recombinant VLPs containing certain immuno-stimulatory molecules are considered of great value and are produced by bio-conjugation (Tissot et al. 2010) (Fig. 24.1).

Three major approaches are used in genetically engineering the VLPs:

1. Direct fusion, in which foreign protein is directly linked to the N/C terminal regions of outer surface.
2. Linker fusion in which short amino acid sequence acts as a linker between coat proteins and foreign peptides.
3. Protein overcoat strategy, in which a short amino acid sequence from foot and mouth disease virus (FMDV) acts as linker of foreign peptide and coat protein

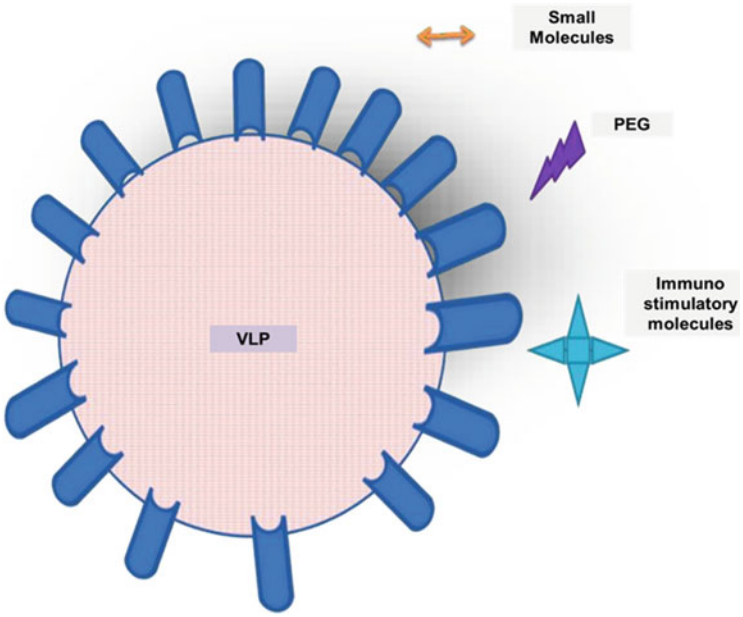

Fig. 24.1 Polyethylene glycol (PEG) conjugation to virus-like particles. Bio-conjugation of VLPs in which viral structural protein is genetically fused with a foreign antigen, and then the chimeric protein is expressed in an appropriate host system with direct coupling of proteins, nucleic acid, and small molecules to surface of VLPs

resulting in ribosomal skip during translation (Turpen et al. 1995). Due to this, a large amalgam of coat proteins and fusion proteins is produced which help in presenting assembly of virus.

The three approaches are diagrammatically shown in Fig. 24.2.

Cloned coat protein genes synthesized from Hepatitis B core antigen (HBcAg), Hepatitis B surface antigen (HBsAg) and from tobacco mosaic virus (TMV) were used to synthesize the first recombinant VLPs (Denis et al. 2007). Thus initial attempts of synthesizing recombinant VLPs highlighted some important features for further synthesis:

- The aligned oligonucleotides are utilized for getting required nucleic acids and peptides coding for carrier coat protein(s)
- Without disturbing the self-assembly of VLPs, foreign peptides are added to carrier molecule
- Using heterologous host for synthesizing VLPs has potential advantages and better functional activities over original viruses.

The first step in constructing recombinant VLPs is cloning of structural genes coding for viral structural proteins of interest (Zeltins 2013). In maximum cases this

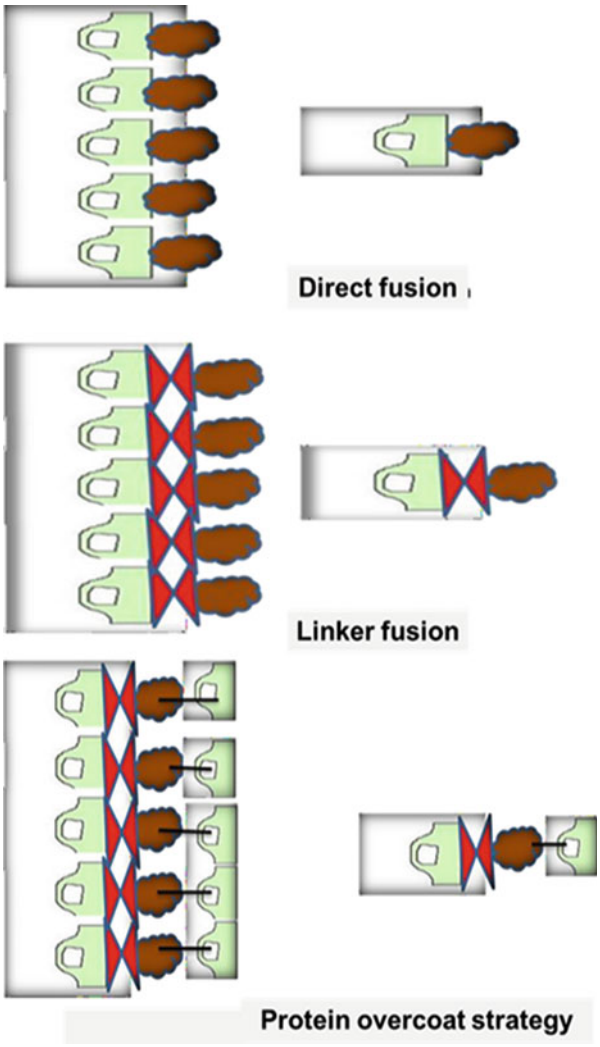

Fig. 24.2 Three major approaches in genetically engineering VLPs. The first approach being where a foreign protein is directly connected to the outer surface's N/C terminal regions, second one where a linker between coat proteins and foreign peptides is formed by a short amino acid sequence, and the last one where amino acids sequence functions as a linker causing ribosomal skipping during translation

nucleotide sequence is enough for the gene synthesis which permits the inclusion of typical AA codon which helps in enhancing the expression level of target proteins (Bachmann and Zinkernagel 1997). Viruses which are characterized well on the basis of full length nucleotide sequences and genome organization are typically used for producing VLPs.

New VLPs require an expression system for their successful construction. Data shows that bacterial and plant based VLPs which constitute about 28% of total VLPs utilize the bacterial expression system. Yeast based and insect based VLPs constitute 20% and 28% of total VLPs, respectively, whereas 9% plant and 15% mammalian host are utilized for the synthesis of VLPs with targeted properties (Zeltins 2013).

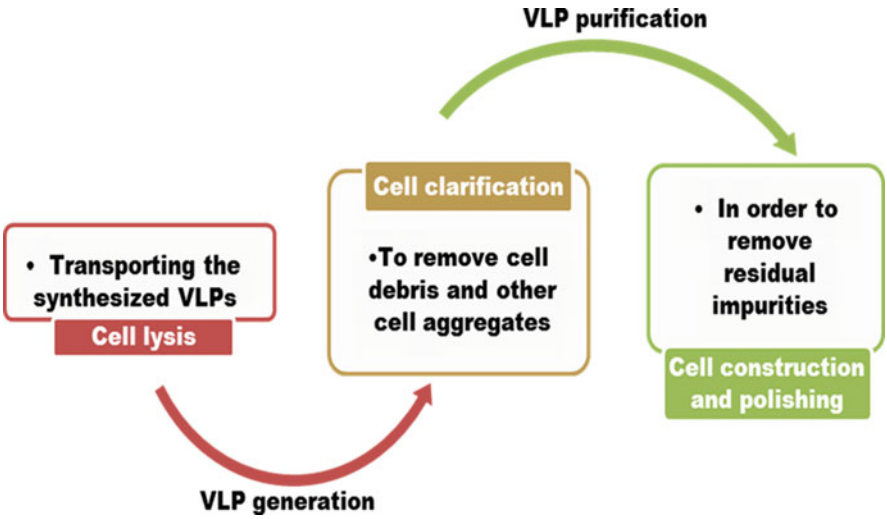

Fig. 24.3 Purification and characterization steps in generation of VLPs

After construction and expression of gene of interest, purification and characterization are considered as of the utmost importance and include the following steps:

- Cell lysis, in order to transport synthesized VLPs into it.
- Cell clarification, in order to remove cell debris and other cell aggregates.
- Cell construction and polishing, in order to remove residual impurities.

These steps has been summarized in Fig. 24.3.

For the purification of VLPs produced in non-secreting systems like eukaryotic cells, detergent solutions are required (King 2011). On the other hand, VLPs produced from bacterial, plant, and yeast cells require other mechanical treatments such as ultra-sonication, repeated freeze/thawing cycles, and enzymatic treatment for purification process (Vicente et al. 2011a). Some uncharacterized VLPs whose stability properties are not known are purified by different cell lysis and extraction buffers which preserves the integrity of particles due to oxidation and host proteases (Schwarz 2016). Such buffers are mostly having chelating agents, reducing agents, and protease inhibitors (Vicente et al. 2011b). After all the purification steps including centrifugation and ultrafiltration in order to purify the VLP containing solutions, precipitation is done using ammonium sulfate and polyethylene glycol which decreases the volume of solution as well as reduces the host derived impurities (Cull and McHenry 1990; Salazar and Asenjo 2007). Based on the characteristics of some VLPs, several purification methods are applied such as size-exclusion, ion exchange, and affinity columns in order to obtain vaccine grade purity level (Hardy et al. 2000).

Currently, purification steps are not just confined to removal of impurities from VLP solution but also disassembling and reassembling steps were added in order to

enhance the activities of target VLPs (Kalnciema et al. 2012). Post purification, several properties of VLPs are characterized by a valuable tool of mass spectroscopy. This tool helps in knowing the VLP composition, proteolytically degraded proteins, post-translational modifications and structural proteins modifications (Freivalds et al. 2011; Branco et al. 2010). The concentration of VLPs is measured by UV spectroscopy and tells us about the protein and nucleic acid content of these particles (Huang et al. 2007). For the measurement of isoelectric point, capillary isoelectric point measurement is considered as a useful tool (Porterfield and Zlotnick 2010). VLPs are further packed with different plasmid DNAs that range from 9 Kbp to 14.5 kbp in size (Lee et al. 2009). The presence of positively charged surface regions-AAs in VLP acts as target for initiation of different functional molecules. Thus these genetic techniques have been extensively applied for introduction of peptides, epitopes to VLP structure which help in broadening of potential VLP applications (Pumpens and Grens 2002). The integration of total protein into VLP structure can also be obtained by this genetic fusion. Protein-A domain which happens to exist with the constant regions of IgG antibody has also been recently incorporated in VLPs. These VLPs were able to bin 2 g of IgG per 1 g of carrier thus helping in purification of different monoclonal antibodies (Werner et al. 2006). These domains are also useful in delivery of drug nucleic acid to target cells or diagnostic agents.

24.3 Platform Technologies Based on Chemical Conjugation of VLPs

Chemically reactive amino acid side chains are used in chemical conjugation with proteinaceous viral capsids in order to modify the viruses (Douglas and Young 2006). Five amino acids out of 20 naturally occurring amino acids are used in chemical modification strategies in which an antigenic peptide sequence is added to virus coat protein. The most common conjugation involves:

- Lysine, which are accessible to *N*-hydroxysuccinimide (NHS)
- Cysteine, involving the Michael addition to maleimides and
- Aspartic/glutamic acid residues, which help in carbodiimide activation (Evans 2008).

These conjugation strategies help in attaching nucleic acids, polymers, and small molecules to the surface of viral capsids (Young et al. 2008; Steinmetz 2010). In addition to above direct conjugation reactions, bifunctional linkers are also utilized to insert additional functional groups usually not found in virus coat proteins. "Click Chemistry" is one such approach in which copper catalyzed azide-alkyne cycloaddition (CuAAC) in presence of Cu and a ligand to form 1,4 substituted triazole proceeds more quickly and has high fidelity (Strable et al. 2008). This reaction was first tested and modified in case of Cow pea mosaic virus (CMV), hepatitis B virus (HBV), and bacteriophage Qβ by a group of scientist from Finland (Steinmetz et al.

2009). In this reaction VLPs are first decorated with an azide and linked with CuAAC reaction and allowing conjugation of proteins, small molecules, and even small RNA fragments (Prasuhn Jr et al. 2007). Azo coupling is a conjugation between aniline and tyrosine side chain. A functional group is introduced onto the VLPs to form diazonium salt. Azo coupling also helps in introduction of aldehydes onto VLPs which helps to alter surface exposed tyrosine residues in case of TMV (Kovacs et al. 2007). Some chemical coupling reactions which take place inside the living systems—biorthogonal are considered as selective and efficient coupling reactions. For these reactions, an aldehyde is added onto VLPs scaffold by forming hydrazine or oxime bond and helps in targeting vascular epithelial growth receptor-1 and in tumor homing (Schlick et al. 2005). A second strategy for modifying VLPs is introduction of synthetic amino acids into VLPs scaffolds. These unnatural amino acids like azidohomoalanine (AHA) and homopropargylglycine (HPG) were introduced and helped in conjugation of small dye molecules, MRI agents and biotin (Strable et al. 2008). These chemically modified structural elements of VLPs help in target selective introduction of various molecules onto its surface and thus help in cell specific drug targeting and cytotoxicity.

24.4 Vaccines Based on Platform Technologies in VLPs Against Infectious Diseases

A number of viral proteins have been assembled in the form of VLPs to produce vaccines and licensed for clinical use in humans such as Recombivax HB for hepatitis B virus, Gardasil for papillomavirus, Hecolin for hepatitis E virus (Nooraei et al. 2021). There is an extensive research going on in developing these types of vaccines against a number of other infectious diseases. Heptavax-B consisting of HBsAg is a genetically engineered VLP based vaccine that has shown greater immunogenicity and safety than the earlier blood-derived hepatitis vaccine (Tornesello et al. 2022). VLP based vaccines against a major cause of cervical cancer causing virus called human papillomavirus (HPV) uses L1 protein as a major constituent in four currently producing vaccines produced by Merck, GlaxoSmithKline, and Innovax (Kim et al. 2014). Another VLP based vaccine generated from a shortened version of a structural protein called PORF2 in hepatitis E virus called Hecolin VLP, developed by Xiamen Innovax Biotech Co., can protect against infection from this deadly virus (Zhang et al. 2014b). Likewise VLPs against influenza consisting of hemagglutinin (HA) and neuraminidase (NA) proteins have shown promising immune response than inactivated influenza virus (Bright et al. 2007; Al-ahdal et al. 2018). The VLPs produced using the envelop glycoproteins of human immunodeficiency virus (HIV) are in clinical trial stage (Zhang et al. 2021). The structural proteins (VP1 and VP2) of human parvovirus (HPV) and norovirus (NV) have been used to produce VLP based vaccines which are in clinical trials (Havlikova et al. 2013; Herbst-Kralovetz et al. 2010). VLP based vaccines are also being developed against the deadly Coronavirus which is the causative agent for the current global pandemic COVID-19 (Shin et al. 2020; Belete 2021).

The repetitive protein structures on virus capsids, which are utilized to produce VLP based vaccines, can initiate the stimulation of innate immunity and activate B cells to produce neutralizing antibodies (Kelly et al. 2019; Rehman et al. 2021a, b). In addition, since dendritic cells can engulf particles of 100–500 nm size via process of phagocytosis, the nanostructures of VLPs are best suited for key epitopes to the immune system (Nooraei et al. 2021). This in turn leads to the stimulation of proinflammatory cytokines like IL-1β leading to cyclic recruitment of antigen-presenting cells and presentation of molecules like MHC class II peptide, CD80, etc. on the surface of dendritic cells (Suresh and Mosser 2013). Ultimately, B and T cell proliferation and differentiation as an outcome of VLP based vaccines interacting with the immune response lead to antiviral antibody titer and act as barrier to different strains of the virus. In addition to their immunogenic properties, VLPs have been considered as drug delivery and gene therapy nanoparticles recently as well (Ferrer-Miralles et al. 2015). Owing to their capacity to bypass the endosomes prior to degradation by lysosomes, VLPs make excellent nanocarriers for drug delivery. As a result of the virus from which they originated, some VLPs tend to target particular tissues in a natural manner. For example, because hepatitis B normally infects the liver, HBV derived VLPs target liver cells.

There are still challenges in manufacturing VLP based vaccines, such as purification and storage. They possess greater stability in general than subunit vaccines, however because the viral genome is not present in them, they are susceptible to instability when conditions change, especially via downstream processing (Vicente et al. 2011b). The temperature and chemical treatment of VLPs can cause them to lose their integrity, resulting in the reduction of their immunogenicity (Tornesello et al. 2022). Also, viral proteins present in VLPs are expressed differently in different platforms which may increase the difficulty levels in purification stages. Additionally, various impurities such as DNA, lipids, host cell proteins, etc. co-purified with VLPs pose an overwhelming challenge. Further research and endeavors to improve VLP based vaccines, keeping antigenicity and immunogenicity of VLPs as prime objective, will lead to the advancement of novel and productive VLP based vaccines in future.

24.5 Conclusion and Future Perspective

Both genetic engineering and chemical conjugation approach represent the platform technologies that are used as a base for development of VLPs in various applications and processes. The future of VLPs lies in the modification of these technologies which allows introduction of multifunctional modalities, for example, targeting ligands, therapeutic moieties, and imaging molecules. Platform technologies based on VLPs are considered significant strategy to generate multivalent vaccines. Single platform as well as multiple platform vaccine modules can be strategized for immunization for different strains of an antigenically diverse organism. VLPs based vaccines offer a promising alternative to other vaccines. A vast number of methods have been used to modify VLPs so they can be tailorable for various

biomedical applications. This is considered as a new era in the field of nanotechnology. Further work in this discipline will help optimize the methodologies to ensure success of modern vaccines. Such studies show a great potential, because of the fact that viral engineering is a well-known area which will lead rapidly to in vivo stage. In spite of the drawbacks that VLP production can be complex and difficult procedure, it still offers an attractive field holding a potential for development of next generation therapeutics.

References

Al-Ahdal MN, Murugaiah V, Varghese PM, Abozaid SM, Saba I, Al-Qahtani AA, Pathan AA, Kouser L, Nal B, Kishore U (2018) Entry inhibition and modulation of pro-inflammatory immune response against influenza A virus by a recombinant truncated surfactant protein D. Front Immunol 9:1586

Bachmann MF, Zinkernagel RM (1997) Neutralizing antiviral B cell responses. Annu Rev Immunol 15(1):235–270

Belete TM (2021) Review on up-to-date status of candidate vaccines for COVID-19 disease. Infect Drug Resist 14:151

Boisgérault F, Moron G, Leclerc C (2002) Virus-like particles: a new family of delivery systems. Expert Rev Vaccines 1(1):101–109

Branco LM, Grove JN, Geske FJ, Boisen ML, Muncy IJ, Magliato SA, Henderson LA, Schoepp RJ, Cashman KA, Hensley LE (2010) Lassa virus-like particles displaying all major immunological determinants as a vaccine candidate for Lassa hemorrhagic fever. Virol J 7(1):1–19

Bright RA, Carter DM, Daniluk S, Toapanta FR, Ahmad A, Gavrilov V, Massare M, Pushko P, Mytle N, Rowe T (2007) Influenza virus-like particles elicit broader immune responses than whole virion inactivated influenza virus or recombinant hemagglutinin. Vaccine 25(19): 3871–3878

Crisci E, Bárcena J, Montoya M (2012) Virus-like particles: the new frontier of vaccines for animal viral infections. Vet Immunol Immunopathol 148(3–4):211–225

Cull M, McHenry CS (1990) [12] Preparation of extracts from prokaryotes. Methods Enzymol 182: 147–153

Cunningham AL, Garçon N, Leo O, Friedland LR, Strugnell R, Laupèze B, Doherty M, Stern P (2016) Vaccine development: from concept to early clinical testing. Vaccine 34(52):6655–6664

Demento SL, Siefert AL, Bandyopadhyay A, Sharp FA, Fahmy TM (2011) Pathogen-associated molecular patterns on biomaterials: a paradigm for engineering new vaccines. Trends Biotechnol 29(6):294–306

Denis J, Majeau N, Acosta-Ramirez E, Savard C, Bedard M-C, Simard S, Lecours K, Bolduc M, Pare C, Willems B (2007) Immunogenicity of papaya mosaic virus-like particles fused to a hepatitis C virus epitope: evidence for the critical function of multimerization. Virology 363(1): 59–68

Douglas T, Young M (2006) Viruses: making friends with old foes. Science 312(5775):873–875

Elsharif AM, Youssef TE, Al-Jameel SS, Mohamed HH, Ansari MA, Rehman S, Akhtar S (2019) Synthesis of an activatable tetra-substituted nickel phthalocyanines-4 (3H)-quinazolinone conjugate and its antibacterial activity. Adv Pharmacol Sci 2019:5964687

Evans DJ (2008) The bionanoscience of plant viruses: templates and synthons for new materials. J Mater Chem 18(32):3746–3754

Ferrer-Miralles N, Rodríguez-Carmona E, Corchero JL, García-Fruitós E, Vázquez E, Villaverde A (2015) Engineering protein self-assembling in protein-based nanomedicines for drug delivery and gene therapy. Crit Rev Biotechnol 35(2):209–221

Freivalds J, Dislers A, Ose V, Pumpens P, Tars K, Kazaks A (2011) Highly efficient production of phosphorylated hepatitis B core particles in yeast Pichia pastoris. Protein Expr Purif 75(2): 218–224

Hardy E, Martınez E, Diago D, Dıaz R, González D, Herrera L (2000) Large-scale production of recombinant hepatitis B surface antigen from Pichia pastoris. J Biotechnol 77(2–3):157–167

Havlikova S, Ličková M, Klempa B (2013) Non-viraemic transmission of tick-borne viruses. Acta Virol 57(2):123–129

Herbst-Kralovetz M, Mason HS, Chen Q (2010) Norwalk virus-like particles as vaccines. Expert Rev Vaccines 9(3):299–307

Huang X, Bronstein LM, Retrum J, Dufort C, Tsvetkova I, Aniagyei S, Stein B, Stucky G, McKenna B, Remmes N (2007) Self-assembled virus-like particles with magnetic cores. Nano Lett 7(8):2407–2416

Jennings GT, Bachmann MF (2008) The coming of age of virus-like particle vaccines. Biol Chem 389(5):521–536

Kalnciema I, Skrastina D, Ose V, Pumpens P, Zeltins A (2012) Potato virus Y-like particles as a new carrier for the presentation of foreign protein stretches. Mol Biotechnol 52(2):129–139

Keller SA, Bauer M, Manolova V, Muntwiler S, Saudan P, Bachmann MF (2010) Cutting edge: limited specialization of dendritic cell subsets for MHC class II-associated presentation of viral particles. J Immunol 184(1):26–29

Kelly HG, Kent SJ, Wheatley AK (2019) Immunological basis for enhanced immunity of nanoparticle vaccines. Expert Rev Vaccines 18(3):269–280

Khan S, Tombuloglu H, Hassanein SE, Rehman S, Bozkurt A, Cevik E, Abdel-Ghany S, Nabi G, Ali A, Sabit H (2020) Coronavirus diseases 2019: current biological situation and potential therapeutic perspective. Eur J Pharmacol 886:173447

Kim KS, Park SA, Ko K-N, Yi S, Cho YJ (2014) Current status of human papillomavirus vaccines. Clin Exp Vaccine Res 3(2):168–175

King BJ (2011) Characterisation of the membrane and protein interactions of the Hepatitis C Virus NS2. University of Leeds

Kovacs EW, Hooker JM, Romanini DW, Holder PG, Berry KE, Francis MB (2007) Dual-surface-modified bacteriophage MS2 as an ideal scaffold for a viral capsid-based drug delivery system. Bioconjug Chem 18(4):1140–1147

Lee LA, Niu Z, Wang Q (2009) Viruses and virus-like protein assemblies—chemically programmable nanoscale building blocks. Nano Res 2(5):349–364

Manolova V, Flace A, Bauer M, Schwarz K, Saudan P, Bachmann MF (2008) Nanoparticles target distinct dendritic cell populations according to their size. Eur J Immunol 38(5):1404–1413

Nooraei S, Bahrulolum H, Hoseini ZS, Katalani C, Hajizade A, Easton AJ, Ahmadian G (2021) Virus-like particles: preparation, immunogenicity and their roles as nanovaccines and drug nanocarriers. J Nanobiotechnol 19(1):1–27

Peacey M, Wilson S, Baird MA, Ward VK (2007) Versatile RHDV virus-like particles: incorporation of antigens by genetic modification and chemical conjugation. Biotechnol Bioeng 98(5):968–977

Plotkin S, Robinson JM, Cunningham G, Iqbal R, Larsen S (2017) The complexity and cost of vaccine manufacturing–an overview. Vaccine 35(33):4064–4071

Porterfield JZ, Zlotnick A (2010) A simple and general method for determining the protein and nucleic acid content of viruses by UV absorbance. Virology 407(2):281–288

Prasuhn DE Jr, Yeh RM, Obenaus A, Manchester M, Finn M (2007) Viral MRI contrast agents: coordination of Gd by native virions and attachment of Gd complexes by azide–alkyne cycloaddition. Chem Commun 12:1269–1271

Pulendran B, Ahmed R (2011) Immunological mechanisms of vaccination. Nat Immunol 12(6): 509–517

Pumpens P, Grens E (2002) Artificial genes for chimeric virus-like particles. In: Artificial DNA: methods and applications, vol 249. p 327

Rehman S, Asiri SM, Khan FA, Jermy BR, Khan H, Akhtar S, Jindan RA, Khan KM, Qurashi A (2019) Biocompatible tin oxide nanoparticles: synthesis, antibacterial, anticandidal and cytotoxic activities. ChemistrySelect 4(14):4013–4017

Rehman S, Asiri SM, Khan FA, Jermy BR, Ravinayagam V, Alsalem Z, Al Jindan R, Qurashi A (2020a) Anticandidal and in vitro anti-proliferative activity of sonochemically synthesized indium tin oxide nanoparticles. Sci Rep 10(1):1–9

Rehman S, Farooq R, Jermy R, Mousa Asiri S, Ravinayagam V, Al Jindan R, Alsalem Z, Shah MA, Reshi Z, Sabit H (2020b) A wild fomes fomentarius for biomediation of one pot synthesis of titanium oxide and silver nanoparticles for antibacterial and anticancer application. Biomolecules 10(4):622

Rehman S, Majeed T, Ansari MA, Ali U, Sabit H, Al-Suhaimi EA (2020c) Current scenario of COVID-19 in pediatric age group and physiology of immune and thymus response. Saudi J Biol Sci 27(10):2567–2573

Rehman S, Majeed T, Ansari MA, Al-Suhaimi EA (2020d) Syndrome resembling Kawasaki disease in COVID-19 asymptomatic children. J Infect Public Health 13(12):1830–1832

Rehman S, Ravinayagam V, Nahvi I, Aldossary H, Al-Shammari M, Amiri MS, Kishore U, Al-Suhaimi EA (2021a) Immunity, sex hormones, and environmental factors as determinants of COVID-19 disparity in women. Front Immunol 3279

Rehman S, Ansari MA, Al-Dossary HA, Fatima Z, Hameed S, Ahmad W, Ali A (2021b) Current perspectives on mycosynthesis of nanoparticles and their biomedical application. In: Modeling and control of drug delivery systems. Academic, pp 301–311

Roy P, Noad R (2008) Virus-like particles as a vaccine delivery system: myths and facts. Hum Vaccin 4(1):5–12

Salazar O, Asenjo JA (2007) Enzymatic lysis of microbial cells. Biotechnol Lett 29(7):985–994

Schlick TL, Ding Z, Kovacs EW, Francis MB (2005) Dual-surface modification of the tobacco mosaic virus. J Am Chem Soc 127(11):3718–3723

Schwarz B (2016) Biomimetic structural engineering of P22 virus-like particles for catalysis and immune modulation. Indiana University

Scorpio A, Blank T, Day W, Chabot D (2006) Anthrax vaccines: Pasteur to the present. Cell Mol Life Sci 63(19-20):2237–2248

Shin MD, Shukla S, Chung YH, Beiss V, Chan SK, Ortega-Rivera OA, Wirth DM, Chen A, Sack M, Pokorski JK (2020) COVID-19 vaccine development and a potential nanomaterial path forward. Nat Nanotechnol 15(8):646–655

Steinmetz NF (2010) Viral nanoparticles as platforms for next-generation therapeutics and imaging devices. Nanomedicine 6(5):634–641

Steinmetz NF, Hong V, Spoerke ED, Lu P, Breitenkamp K, Finn M, Manchester M (2009) Buckyballs meet viral nanoparticles: candidates for biomedicine. J Am Chem Soc 131(47): 17093–17095

Strable E, Prasuhn DE Jr, Udit AK, Brown S, Link AJ, Ngo JT, Lander G, Quispe J, Potter CS, Carragher B (2008) Unnatural amino acid incorporation into virus-like particles. Bioconjug Chem 19(4):866–875

Suresh R, Mosser DM (2013) Pattern recognition receptors in innate immunity, host defense, and immunopathology. Adv Physiol Educ 37(4):284–291

Tornesello AL, Tagliamonte M, Buonaguro FM, Tornesello ML, Buonaguro L (2022) Virus-like particles as preventive and therapeutic cancer vaccines. Vaccines 10(2):227

Tissot AC, Renhofa R, Schmitz N, Cielens I, Meijerink E, Ose V, Jennings GT, Saudan P, Pumpens P, Bachmann MF (2010) Versatile virus-like particle carrier for epitope based vaccines. PLoS One 5(3):e9809

Turpen TH, Reinl SJ, Charoenvit Y, Hoffman SL, Fallarme V, Grill LK (1995) Malaria epitopes expressed on the surface of recombinant tobacco mosaic virus. Biotechnology 13(1):53–57

Vicente T, Mota JP, Peixoto C, Alves PM, Carrondo MJ (2011a) Rational design and optimization of downstream processes of virus particles for biopharmaceutical applications: current advances. Biotechnol Adv 29(6):869–878

Vicente T, Roldão A, Peixoto C, Carrondo MJ, Alves PM (2011b) Large-scale production and purification of VLP-based vaccines. J Invertebr Pathol 107:S42–S48

Werner S, Marillonnet S, Hause G, Klimyuk V, Gleba Y (2006) Immunoabsorbent nanoparticles based on a tobamovirus displaying protein A. Proc Natl Acad Sci 103(47):17678–17683

Young M, Debbie W, Uchida M, Douglas T (2008) Plant viruses as biotemplates for materials and their use in nanotechnology. Annu Rev Phytopathol 46:361–384

Zeltins A (2013) Construction and characterization of virus-like particles: a review. Mol Biotechnol 53(1):92–107

Zhang W, Wang L, Liu Y, Chen X, Liu Q, Jia J, Yang T, Qiu S, Ma G (2014a) Immune responses to vaccines involving a combined antigen–nanoparticle mixture and nanoparticle-encapsulated antigen formulation. Biomaterials 35(23):6086–6097

Zhang X, Wei M, Pan H, Lin Z, Wang K, Weng Z, Zhu Y, Xin L, Zhang J, Li S (2014b) Robust manufacturing and comprehensive characterization of recombinant hepatitis E virus-like particles in Hecolin®. Vaccine 32(32):4039–4050

Zhang S, Nguyen HT, Ding H, Wang J, Zou S, Liu L, Guha D, Gabuzda D, Ho DD, Kappes JC (2021) Dual pathways of human immunodeficiency virus type 1 envelope glycoprotein trafficking modulate the selective exclusion of uncleaved oligomers from virions. J Virol 95(3): e01369–e01320

Part VI

Nanotechnology in Gene Therapy

Therapeutic Applications of CRISPR/Cas9 Technology for Infectious Diseases

25

Garima Sharma, Suriya Rehman, and Ashish Ranjan Sharma

Abstract

The concept of genome alteration in both plant and animal kingdom has come a long way and has transformed medicinal research in the modern era. The predetermined gene expression may be modulated (i.e., upregulated or downregulated) as a result of the specific genetic change. CRISPR/Cas9 is a hopeful gene editing technique that can modify DNA sequences, correcting the genes for gain-of-function or loss-of-functions mutation to treat genetic illnesses. Although other gene editing techniques are also known, CRISPR/Cas9 is the most efficient and precise technique to gene correction. In the last decade, CRISPR/Cas9 has entered under clinical trials and very soon it might enter into clinics. In this chapter we discuss the origins of CRISPR-Cas9 systems and their biomedical applications.

Keywords

CRISPR/Cas9 · Genetic disorders · Therapy · Genome editing

G. Sharma (✉)
Department of Biomedical Science and Institute of Bioscience and Biotechnology, Kangwon National University, Chuncheon, Republic of Korea

S. Rehman
Department of Epidemic Diseases Research, Institute for Research and Medical Consultations (IRMC), Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia

A. R. Sharma
Institute for Skeletal Aging and Orthopedic Surgery, Hallym University-Chuncheon Sacred Heart Hospital, Chuncheon, Gangwon-Do, Republic of Korea

S. Hameed, S. Rehman (eds.), *Nanotechnology for Infectious Diseases*,
https://doi.org/10.1007/978-981-16-9190-4_25

Abbreviations

AAVs	Adeno-associated virus
AIDS	Acquired immunodeficiency syndrome
APOBEC3G	Apolipoprotein B mRNA editing enzyme
APP	Amyloid precursor protein
BCL-2	B-cell lymphoma 2
BLs	Burkitt lymphoma
Cas9	CRISPR-associated protein 9
cccDNAs	covalently closed circular DNAs
CDKs	Cyclin-dependent kinases
CFTR	Cystic fibrosis transmembrane conductance regulator
CRISPR	Clustered regulatory interspaced short palindromic repeats
DSB	Double stranded break
EGFR	Epidermal growth factor receptor
EphA2	Ephrin receptor tyrosine kinase A2
FA	Fanconi anemia
HAART	Highly active antiretroviral therapy
HbS	Hemoglobin-S
HDR	Homology-directed repair
HITI	Homology-independent targeted insertion
HTT	Huntingtin
JAK3	Janus family kinase3
KLHL	Kelch-like
LCLs	Lymphoblastoid cell lines
LTRs	Long terminal repeats
LV	Lentivirus
MCL-1	Myeloid cell leukemia-1
MDR	Multidrug resistance
NHEJ	Non-homologous end joining
NK	Natural killer
PCSK9	Proprotein convertase subtilisin/Kexin type 9
PD-1	Programmed death-1
RNAi	RNA interference
SC	Sickle cell
sgRNA	Single guide RNA
SHCBP1	SHC SH2-binding protein 1
STDs	Sexually transmitted disease
TNBC	Triple-negative breast cancer
XHIGM	X-linked hyper immunoglobulin M syndrome

25.1 Introduction

In the last few decades, CRISPR/Cas9 has emerged as a cost-effective and easily scalable genome editing technique (Hsu et al. 2014; Peng et al. 2016; Chakraborty et al. 2017). Recently, USA (Baylis and McLeod 2017) and China (Cyranoski 2016) have allowed the CRISPR technology for cancer treatment and are under clinical trials. CRISPR/Cas9 technology is nowadays applied for genome editing on plants, microorganisms, Drosophila, zebrafish, and animals (Platt et al. 2014; Bortesi and Fischer 2015; Gratz et al. 2015; Ryan et al. 2016; Liu et al. 2017; Zhu et al. 2017). In CRISPR/Cas9 technology, sgRNA sequence helps to aim the gene of interest and create DSB in the DNA that is further repaired by either deletion or insertion of nucleotides via HDR or NHEJ (Brouns et al. 2008) (Fig. 25.1). These changes can provide both the loss-of-function and gain-of-functions.

In 1987, Inshino's research group introduced CRISPR-biology as a collection of 14 repeats of 29 bp nucleotide, in *E. coli's iap gene.* These repeats were again separated by spacers made up of non-repetitive tiny sequences (Ishino et al. 1987). Just after the discovery of these repetitive and non-repetitive sequences, the role of these sequences was not evident. That was only after discovering more repetitive elements (repeats) in other bacteria that scientists around the world started exploring the biological importance/functions of these sequences (Hermans et al. 1991; Mojica et al. 1995; Bult et al. 1996; Nelson et al. 1999). This family of repeats was given the term of CRISPR in 2002 by Jansen's group. Moreover, Jansen's group also recognized the Cas genes located near the CRISPR locus and suggested a possible association between the CRISPR loci and the Cas genes. They also suggested CRISPR sequences as bacteria adaptive defense mechanism to resist phage infections (Jansen et al. 2002).

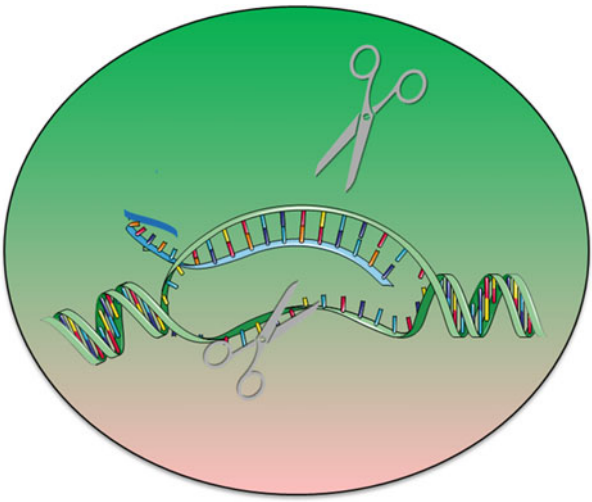

Fig. 25.1 Schematic diagram of CRISPR/Cas9 technology

Later in 2007, Barrangou's group reported the role of CRISPR/Cas9 sequences in providing acquired immunity to prokaryotes against viruses (Barrangou et al. 2007). This antiviral defense mechanism is either because the mature CRISPR RNAs function as sgRNA or prevent bacteria's plasmid transformation and conjugation (Marraffini and Sontheimer 2008). These features enabled CRISPR gene editing technology to enter into the biomedical applications. This chapter will detail the role of CRISPR/Cas9 technology in biomedical applications.

25.2 CRISPR/Cas9 Technology for Cancer Treatment

25.2.1 Modulation of Tumor Suppressor Genes

The role of the tumor suppressor genes as smart therapeutic targets in cancer cells suggested using CRISPR/Cas9 technology in cancer treatment (Hanahan and Weinberg 2011; Sanchez-Rivera and Jacks 2015). CRISPR/Cas9 technology can modulate tumor suppressor genes in cancer cells and induce cell death via apoptosis. Various tumor suppressor genes are known to be regulated by CRISPR/Cas9 technology. CRISPR/Cas9 was used in bladder cancer cells to regulate hBax, E-cadherin, and p21 tumor suppressor genes to induce apoptosis and inhibit cell proliferation (Liu et al. 2014). Similarly, in myeloid malignancies (i.e., leukemia), CRISPR/Cas9 technology edited the ASXL1 tumor suppressor gene in mouse xenografts, which is often mutated during myeloid malignancies, thus re-establishing ASXL1 protein expression and suppressing leukemia cell growth (Murati et al. 2012; Valletta et al. 2015). For this purpose, the LV vectors are used in CRISPR/Cas9 technology to delete essential genes in cancer cells. These important genes are responsible for cancer cells' growth and proliferation. For example, an LV vector was used to induce apoptosis in lymphoma cells by deleting MCL-1 genes, a member of the BCL-2 genes family (Aubrey et al. 2015).

25.2.2 Modulation of Cyclin-Dependent Kinases (CDKs) in Cancer Cells

Besides tumor suppressor genes, CRISPR/Cas9 technology can also manipulate genes responsible for cell cycle regulation, such as CDKs. Since CDKs are important factors to regulate the normal process of the cell cycle, it is well-known that dysregulation in CDKs functioning can cause tumor progression (Zhou et al. 2015; Niu et al. 2019). In osteosarcoma cells, CRISPR/Cas9 technology suppressed CDK11 genes (Feng et al. 2015) and in TNBC cells to correct CDK, CDK7 (Wang et al. 2015).

25.2.3 Modulation of Multidrug Resistance Gene (MDR) in Cancer Cells

Another approach for CRISPR/Cas9 technology-mediated cancer treatment is deleting the MDR gene, such as *MDR1* encodes for membrane efflux pump P-glycoprotein that is associated by removal of the drugs from the cancer cells rendering resistance towards anticancer drugs to the cancer cells. In a study, suppressing or deleting MDR genes restored cancer cells' sensitivity towards anticancer drugs in osteosarcoma cell lines (Liu et al. 2016b). Similarly, CRISPR/Cas9 technology can manage drug-resistant mutations in EGFR genes in lung cancer (Tang and Shrager 2016).

25.2.4 Modulation of Cell Proliferation-Related Genes in Cancer Cells

Since overexpression of cells' proliferation genes causes tumor progression and thus they are also well-known biomarkers, suppression of these genes can be a potential cancer treatment option. One of the cell proliferation-related genes is SHCBP1 (Feng et al. 2016). In breast cancer cell, CRISPR/Cas9 technology suppressed SHCBP1 to inhibit proliferation and induce apoptosis (Feng et al. 2016).

25.2.5 Modulation of Genes Responsible for Ubiquitination, Actin Dynamics, and Cell Cycle Pathways in Cancer Cells

Since various proteins responsible for cancer progression are encoded by KLHL gene family (Dhanoa et al. 2013), deleting or suppressing KLHL genes using CRISPR/Cas9 technology can be a potential cancer treatment option. The KLHDC4 gene was edited by CRISPR/Cas9 technology in a nasopharyngeal carcinoma cell line to induce apoptosis and inhibit cancer cell growth progression and migration (Lian et al. 2016).

25.3 CRISPR/Cas9 Technology to Treat Viral Infection

25.3.1 Modulation of Genes Responsible for Human Immunodeficiency Virus (HIV)

HIV is the causative agent of AIDS. Although HAART therapy can manage, it cannot provide the life-long treatment. This generates the need to develop a permanent cure for HIV. The best possible method for permanent cure for HIV is to knockout the chromosomally integrated viral DNAs. As studied, LTRs (repeated similar sequences of DNA) induce the expression of HIV-1 genes. LTRs assist in inserting retroviral DNA into the host chromosome. Therefore, alterations in the

binding sites of LTRs may affect the expression on HIV-1 genes expression (Shah et al. 2014).

Human CD4+ T cell is used to analyze the role of HIV infection and pathogenesis. In CD4+ T cells, CRISPR/Cas9 technology was applied for mechanistic examination of HIV host factors through the validation, gene knockout, and finally spreading HIV infection in 2–3 weeks (Hultquist et al. 2019). Using CRISPR/Cas9-technology, B cells were edited to reduce HIV-1 infection effect (Hartweger et al. 2019). Expression of some factors such as catalytic polypeptide-like APOBEC3G and TRIM5α genes creates resistant in host against HIV infection. Thus, enhancing the expression of these genes via CRISPR/Cas9 technology can increase host restriction against HIV infection (Bogerd et al. 2015).

25.3.2 Modulation of Genes Responsible for Hepatitis C Virus (HCV)

HCV is a single-stranded RNA virus that is the causative agent of hepatitis C, an inflammatory condition of liver. This virus causes infection of hepatocytes in liver. It has been estimated that about 170 million population are to be infected with HCV throughout the world. It has been found that the Cas9 enzyme/endonuclease, isolated from *Francisella novicida* (FnCas9), can aim endogenous RNA (Sampson et al. 2013). Using the same Cas9 endonuclease from this bacterium, CRISPR/FnCas9 was used for the inhibition of HCV within eukaryotic cells (Price et al. 2015). CRISPR/Cas9 shows also promises to the RNA virus like HCV.

25.3.3 Modulation of Genes Responsible for Hepatitis B Virus (HBV)

Around 350 million people are chronic carrier of HBV throughout the world making HBV a chronic hepatitis which is a frequent infectious disease worldwide. The cccDNAs of HBVs reside inside the infected cell that makes it very difficult to be excised by current therapeutics. Thus, the cccDNA of HBV-targeted CRISPR/Cas9 approach cleaves the genome and cause its cellular clearance. For example, in reporter cell lines CRISPR/Cas9 can interfere in the episomal cccDNA causing disruption in HBV sequences that are integrated with chromosomes. CRISPR/Cas9-mediated interference in HBV cccDNA can also inhibit viral replication. CRISPR/Cas9-mediated cleavage also suppresses HBV through the reduction of cccDNA. Wang et al. (2017) used RNAi technique by means of sgRNA-miRNA-gRNA cassette joining CRISPR/Cas9 which eliminate the chronic HBV infection through cccDNA clearance. The disruption of the HBV genome through CRISPR/Cas9-thecnology shows promises as an anti HBV therapy.

25.3.4 Modulation of Genes Responsible for Human Papilloma Virus (HPV)

HPV infection is ds DNA virus which infects mucosal cells or skin, causing STDs and accounts for an estimated 11% of the global cancer incidences in women. The RNA-guided endonuclease offers a therapeutic approach for HPV. It was reported that the CRISPR/Cas9-mediated cervical cancer treatment can be done by targeting HPV E6 (Yoshiba et al. 2019). HPV6/HPV11 stains are the main cause of genital warts. Researcher uses CRISPR/Cas9 technology which targets the conserved regions of HPV6/11 E7 genes and this work have the potentiality for the development therapy for genital warts (Liu et al. 2016a).

25.3.5 Modulation of Genes Responsible for Epstein−Barr Virus (EBV)

EBV is a ds DNA virus which spreads primarily through saliva and causes mononucleosis. Yuen et al. (2015) used CRISPR/Cas9 technology for genome editing of EBV in human cells. They modified BART promoter gene which encodes viral miRNAs. Ma et al. (2017) have performed analysis in lymphoma cell lines LCLs and BL using CRISPR/Cas9 technology. The result showed that 57 BL and 87 LCL genes are significant for survival and growth. This work indicated possible therapeutic application of the CRISPR/Cas9 technology EBV. The EphA2 is an entry receptor for EBV in human cells. Using knockout by CRISPR/Cas9 technology, Chen et al. (2018) show that EphA2 extracellular domain can bind with the EBV-glycoprotein gHgL and provide the entry to the cell. It is a new potential target for therapeutic development.

25.4 CRISPR/Cas9 Technology for the Treatment of Allergy and Immunological Disorders

25.4.1 Modulation of Cell Surface Glycoprotein Genes

In general, the expression of cell surface glycoproteins increases in the alveolar macrophages during respiratory disorders. For example, the expression of MUC18 (113 kDa) protein is increased in microbial infections-caused chronic obstructive pulmonary disease (Kuske and Johnson 1999). CRISPR/Cas9 technology-mediated MUC18 knockout reduced pro-inflammatory marker, i.e., interleukine-8, in toll-like receptors-2, 3, and 4 agonists-treated human primary nasal airway epithelial cells to mimic microbial infection (Chu et al. 2015).

25.4.2 Modulation of Genes Responsible for Immune Cells Functioning

The checkpoints proteins, such as CTLA-4/B7-1/B7-2 and PD-1/PD-L1, interact with other proteins and prevent T cells from strong immune responses. The suppression of these checkpoints allows T cells to stimulate immune responses and kill cells. CRISPR/Cas9-mediated editing in T cells' PD-1 receptor gene increased the production of interferon-γ, resulting in toxicity towards cells (Su et al. 2016). Similarly, gene editing via CRISPR/Cas9 technology can also correct CD40 ligand mutations in B cells during XHIGM, an immune system disorder (Kuo et al. 2015). In addition, immunoglobulin (Ig) genes can also be edited for increased production of antibodies (Cheong et al. 2016).

The deficiency in the production of a protein tyrosine kinase, JAK3 protein, is associated with a reduction in the circulating numbers of immune cells, such as NK cells and T cells, and circulating of a sufficient number of abnormally functioning B cells (Chang et al. 2015). Since mutations in JAK3 (Janus family kinase gene) can cause immunodeficiencies, correction in JAK3 via CRISPR/Cas9 technology can re-establish the number and functioning of immune cells (Chang et al. 2015).

25.5 CRISPR/Cas9 Technology to Treat Liver-Related Disorders

25.5.1 Modulation of Genes Responsible for Metabolic Liver Disease (MLD)

Defects in genes responsible for the synthesis of transporter proteins can lead to metabolic liver disease (MLD) due to the irregular metabolism of biomolecules. Intravenous injection of CRISPR/Cas9-based gene editing system in mice was found to correct phenylalanine hydroxylase Pah^{enu2} gene responsible for causing an autosomal recessive liver disease called phenylketonuria (Villiger et al. 2018). In another study, the murine factor IX (F9) gene was targeted using a CRISPR/Cas9-mediated gene editing system in hepatocytes to treat MLD-related conditions (Singh et al. 2018). CRISPR/Cas9 technology also corrected X-linked deficiency linked with ornithine transcarbamylase in an infant mice model to treat MLD (Yang et al. 2016).

25.5.2 Modulation of Genes Responsible for Hereditary Tyrosinemia (HT)

Tyrosinemia, an inherited and recessive metabolic disease, results in toxin accumulation leading to liver failure, cirrhosis, and cancer. It is caused by fumaryl acetoacetate hydrolase deficiency due to mutations in the *Fah* gene (Nasrallah et al. 2015; Morrow and Tanguay 2017). Cas9 nuclease-mediated repair of the

mutation in *Fah* gene can treat HT (Yin et al. 2014; VanLith et al. 2018; Shao et al. 2019), thus treating various metabolic liver disorders.

25.5.3 Modulation of Genes Responsible for Cystic Fibrosis (CF)

Mutations in the *CFTR* gene cause CF, an autosomal recessive monogenic disease leading to lung and digestive system damage (Skov et al. 2019). Using CRISPR/Cas9 technology, the mutation of *CFTR* was corrected in the stem cells via homologous recombination (Schwank et al. 2013; Crane et al. 2015). The *CFTR*−/− and *CFTR*+/− knockout sheep models were produced using CRISPR/Cas9 to study CF pathogenesis (Fan et al. 2018).

25.6 CRISPR/Cas9 Technology for the Treatment of Neurological Disorders

25.6.1 Modulation of Genes Responsible for Huntington Disease (HD)

HD is a rare, progressive, and inheritable brain disorder characterized by depression, mood swings, memory lapses, and clumsiness. HD is initiated by the gain-of-function mutation in the exon 1 of *HTT* gene's CAG repeats (Rub et al. 2016; Saudou and Humbert 2016). Therefore, suppression of HD genes via CRISPR/Cas9 technology can be used to treat HD (Shin et al. 2016; Monteys et al. 2017; Yang et al. 2017).

25.6.2 Modulation of Genes Responsible for Alzheimer's Disease (AD)

Progressive memory loss is the main characteristic of AD disease that is classified as a neurodegenerative disorder. The AD is caused by the mutations in presenilin 1 & 2 genes, *PSEN1 & 2* (Levy-Lahad et al. 1995). *PSEN1 & 2* genes are γ-secretase's catalytic subunit that produces amyloid-β (Aβ) by cleaving APP. It has been known that the mutations in the *PSEN1* gene decrease Aβ40 and increase the ratio of Aβ42/Aβ40. Therefore, correcting the mutation in the *PSEN1* gene via CRISPR/Cas9 technology can potentially treat AD (Pires et al. 2016; Poon et al. 2016). Moreover, CRISPR/Cas9-mediated interference in the *APP* gene can also decrease Aβ pathogenesis (Gyorgy et al. 2018).

25.7 CRISPR/Cas9 Technology to Treat Anemia and β-Thalassemia

Mutations in the genes for DNA repair pathway cause an autosomal recessive disorder called Fanconi anemia (FA). Examples of such genes are *Fancf* gene and *FANCI* genes. The CRISPR/Cas9 technology can correct mutations in these genes to treat FA and can also work against bone marrow failure (Skvarova Kramarzova et al. 2017). The SC anemia is another blood disorders due to the production of abnormal HbS protein due to mutations in the β-globin gene (Williams and Thein 2018). CRISPR/Cas9 can correct *Hbs* gene in SC anemia patient's blood-derived hematopoietic stem cells to reinstate normal functioning of hemoglobin (Wen et al. 2017). CRISPR/Cas9-mediated correction in SC anemia patient's bone marrow-derived CD34+ hematopoietic stem and progenitor cells was also reported (Hoban et al. 2016). Reduced production of hemoglobin tetramer's β-globin chains cause β-thalassemia due to the reduction in hemoglobin synthesis (Cao and Galanello 2010; Xie et al. 2014). Cas9-mediated correction of β-thalassemia splice mutation iPSCs is a modernized therapeutic approach for treating β-thalassemia (Alateeq et al. 2018).

25.8 CRISPR/Cas9 Technology to Treat Eye-Related Disorders

The RP is a genetic diseases or disorder. This disorder affects the eyes and causes loss of vision. It is an inherited pigmentary retinal dystrophy (Shintani et al. 2009; Vezinaw et al. 2019). Presently, there is no cure for this diseases. The *RP1*, *RHO*, and *RPGR* gene mutations are noted causative of RP (Wang et al. 2005). A guide RNA/Cas9 plasmid, when injected subretinally, can edit the Rho (S334) mutation, protecting against retinal degeneration and improve visual function (Bakondi et al. 2016). The HITI method was applied to improve the visual condition via inserting gene in non-dividing cells (Suzuki et al. 2016; Suzuki and Izpisua Belmonte 2018).

Cataract can cause the cloudiness of the crystalline lens due to genetic mutations, such as αA-crystallin gene (Shiels and Hejtmancik 2007, 2017). For study purposes, congenital cataracts was developed by generating mutations in αA-crystallin gene in animals (Yuan et al. 2017), and in GJA8 knockout rabbit model via CRISPR/Cas9 system (Yuan et al. 2016).

25.9 CRISPR/Cas9 Technology for the Treatment of Cardiovascular Disorders (CVDs)

Dysregulation in various types of genes contributed to genetic CVDs (Lara-Pezzi et al. 2012; Roberts et al. 2013). For example, the over-functioning of PCSK9, which regulates homeostasis in cholesterol level, causes hypercholesterolemia and atherosclerosis (Seidah 2013; Bergeron et al. 2015). Therefore, suppression of the PCSK9 gene via the CRISPR/Cas9 delivery system can treat atherosclerosis (Jiang et al.

2017; Jarrett et al. 2018). CRISPR/Cas9 technology was also applied in the zebrafish model to edit genes responsible for genetic CVDs (Tessadori et al. 2018).

25.10 Challenges

CRISPR/Cas9 system is now paying attention immensely for its potentiality in therapy of human diseases. Recently, exciting advances have been noted to treat human diseases. However, several challenges need to be discussed before CRISPR/Cas9 enters in the therapeutic domain. The major difficulty to the therapeutic application of CRISPRCas9 is the off-target effects. Off-target effects of CRISPRCas9 comprise off-target binding, off-target editing, and several other functional complications. On the other hand, very low off-target editing is also harmful. Another challenge is the suitable delivery system of the CRISPR/Cas9. The treatment of human diseases needs specific delivery of CRISPR/Cas9 into target tissues. For this purpose, AAVs and LVs are used (Xu et al. 2019). Although AAVs possess various advantages, such as less immunogenicity, better safety index, serotype specificity, and ability to transduce both dividing and non-dividing cells, some toxicities at high dose are reported in animal models. Furthermore, LVs are also non-immunogenicity and have high transducing efficiency. Another problem to use viral delivery vectors is their packing limitation. However, after modifications, the carrying capacity of AAVs can be increased up to 35 kb. Another challenge for the therapeutic applications of CRISPR/Cas9 is the host immune response activate by the Cas9 proteins. CRISPR/Cas9 editing may alter the functionality of edited cells which is the another challenge. These challenges need to be addressed immediately to develop the CRISPR/Cas9 therapy of human diseases.

25.11 Conclusions

Therefore, it can be concluded that CRISPR/Cas9 will have promising applications in future clinics. Since the last few decades the CRISPR/Cas9-based gene editing in animal models has made unexpected progress and it will be very soon when CRISPR/Cas9 technology will be able to treat incurable genetic disorders (Fig. 25.2).

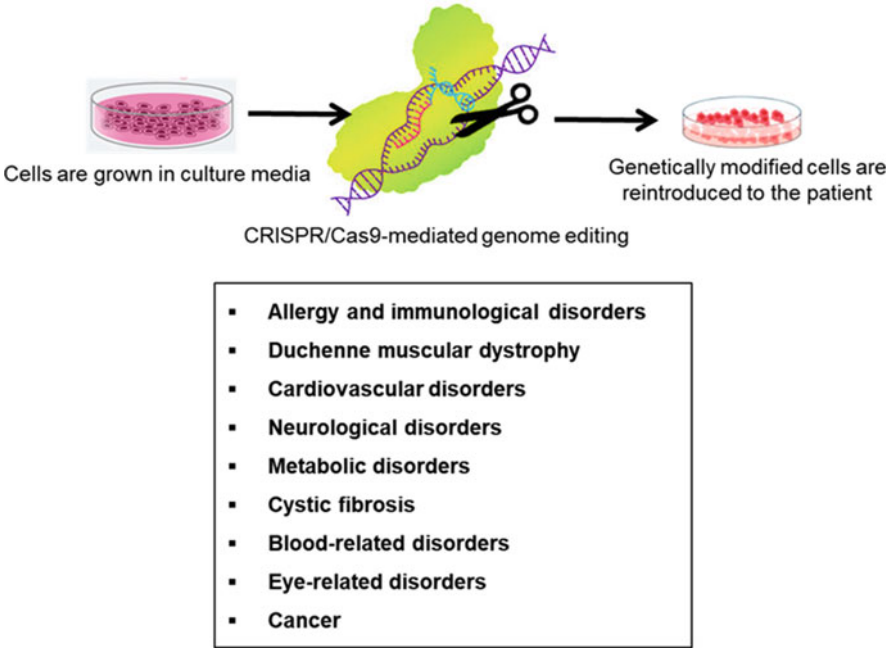

Fig. 25.2 CRISPR/Cas9-mediated treatment of disorders

References

Alateeq S, Ovchinnikov D, Tracey T, Whitworth D, Al-Rubaish A, Al-Ali A, Wolvetang E (2018) Identification of on-target mutagenesis during correction of a beta-thalassemia splice mutation in iPS cells with optimised CRISPR/Cas9-double nickase reveals potential safety concerns. APL Bioeng 2(4):046103

Aubrey BJ, Kelly GL, Kueh AJ, Brennan MS, O'Connor L, Milla L, Wilcox S, Tai L, Strasser A, Herold MJ (2015) An inducible lentiviral guide RNA platform enables the identification of tumor-essential genes and tumor-promoting mutations in vivo. Cell Rep 10(8):1422–1432

Bakondi B, Lv W, Lu B, Jones MK, Tsai Y, Kim KJ, Levy R, Akhtar AA, Breunig JJ, Svendsen CN, Wang S (2016) In vivo CRISPR/Cas9 gene editing corrects retinal dystrophy in the S334ter-3 rat model of autosomal dominant retinitis pigmentosa. Mol Ther 24(3):556–563

Barrangou R, Fremaux C, Deveau H, Richards M, Boyaval P, Moineau S, Romero DA, Horvath P (2007) CRISPR provides acquired resistance against viruses in prokaryotes. Science 315(5819): 1709–1712

Baylis F, McLeod M (2017) First-in-human phase 1 CRISPR gene editing cancer trials: are we ready? Curr Gene Ther 17(4):309–319

Bergeron N, Phan BA, Ding Y, Fong A, Krauss RM (2015) Proprotein convertase subtilisin/kexin type 9 inhibition: a new therapeutic mechanism for reducing cardiovascular disease risk. Circulation 132(17):1648–1666

Bogerd HP, Kornepati AV, Marshall JB, Kennedy EM, Cullen BR (2015) Specific induction of endogenous viral restriction factors using CRISPR/Cas-derived transcriptional activators. Proc Natl Acad Sci U S A 112:E7249–E7256

Bortesi L, Fischer R (2015) The CRISPR/Cas9 system for plant genome editing and beyond. Biotechnol Adv 33(1):41–52

Brouns SJ, Jore MM, Lundgren M, Westra ER, Slijkhuis RJ, Snijders AP, Dickman MJ, Makarova KS, Koonin EV, van der Oost J (2008) Small CRISPR RNAs guide antiviral defense in prokaryotes. Science 321(5891):960–964

Bult CJ, White O, Olsen GJ, Zhou L, Fleischmann RD, Sutton GG, Blake JA, FitzGerald LM, Clayton RA, Gocayne JD, Kerlavage AR, Dougherty BA, Tomb JF, Adams MD, Reich CI, Overbeek R, Kirkness EF, Weinstock KG, Merrick JM, Glodek A, Scott JL, Geoghagen NS, Venter JC (1996) Complete genome sequence of the methanogenic archaeon, Methanococcus jannaschii. Science 273(5278):1058–1073

Cao A, Galanello R (2010) Beta-thalassemia. Genet Med 12(2):61–76

Chakraborty C, Teoh SL, Das S (2017) The smart programmable CRISPR technology: a next generation genome editing tool for investigators. Curr Drug Targets 18(14):1653–1663

Chang CW, Lai YS, Westin E, Khodadadi-Jamayran A, Pawlik KM, Lamb LS Jr, Goldman FD, Townes TM (2015) Modeling human severe combined immunodeficiency and correction by CRISPR/Cas9-enhanced gene targeting. Cell Rep 12(10):1668–1677

Chen J, Sathiyamoorthy K, Zhang X, Schaller S, Perez White BE, Jardetzky TS et al (2018) Ephrin receptor A2 is a functional entry receptor for Epstein-Barr virus. Nat Microbiol 3:172–180

Cheong TC, Compagno M, Chiarle R (2016) Editing of mouse and human immunoglobulin genes by CRISPR-Cas9 system. Nat Commun 7:10934

Chu HW, Rios C, Huang C, Wesolowska-Andersen A, Burchard EG, O'Connor BP, Fingerlin TE, Nichols D, Reynolds SD, Seibold MA (2015) CRISPR-Cas9-mediated gene knockout in primary human airway epithelial cells reveals a proinflammatory role for MUC18. Gene Ther 22(10):822–829

Crane AM, Kramer P, Bui JH, Chung WJ, Li XS, Gonzalez-Garay ML, Hawkins F, Liao W, Mora D, Choi S, Wang J, Sun HC, Paschon DE, Guschin DY, Gregory PD, Kotton DN, Holmes MC, Sorscher EJ, Davis BR (2015) Targeted correction and restored function of the CFTR gene in cystic fibrosis induced pluripotent stem cells. Stem Cell Reports 4(4):569–577

Cyranoski D (2016) Chinese scientists to pioneer first human CRISPR trial. Nature 535(7613): 476–477

Dhanoa BS, Cogliati T, Satish AG, Bruford EA, Friedman JS (2013) Update on the Kelch-like (KLHL) gene family. Hum Genomics 7:13

Fan Z, Perisse IV, Cotton CU, Regouski M, Meng Q, Domb C, Van Wettere AJ, Wang Z, Harris A, White KL, Polejaeva IA (2018) A sheep model of cystic fibrosis generated by CRISPR/Cas9 disruption of the CFTR gene. JCI Insight 3(19):e123529

Feng Y, Sassi S, Shen JK, Yang X, Gao Y, Osaka E, Zhang J, Yang S, Yang C, Mankin HJ, Hornicek FJ, Duan Z (2015) Targeting CDK11 in osteosarcoma cells using the CRISPR-Cas9 system. J Orthop Res 33(2):199–207

Feng W, Li HC, Xu K, Chen YF, Pan LY, Mei Y, Cai H, Jiang YM, Chen T, Feng DX (2016) SHCBP1 is over-expressed in breast cancer and is important in the proliferation and apoptosis of the human malignant breast cancer cell line. Gene 587(1):91–97

Gratz SJ, Rubinstein CD, Harrison MM, Wildonger J, O'Connor-Giles KM (2015) CRISPR-Cas9 genome editing in Drosophila. Curr Protoc Mol Biol 111:31.32.31–31.32.20

Gyorgy B, Loov C, Zaborowski MP, Takeda S, Kleinstiver BP, Commins C, Kastanenka K, Mu D, Volak A, Giedraitis V, Lannfelt L, Maguire CA, Joung JK, Hyman BT, Breakefield XO, Ingelsson M (2018) CRISPR/Cas9 mediated disruption of the Swedish APP allele as a therapeutic approach for early-onset Alzheimer's disease. Mol Ther Nucleic Acids 11:429–440

Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144(5):646–674

Hartweger H, McGuire AT, Horning M, Taylor JJ, Dosenovic P, Yost D et al (2019) HIV-specific humoral immune responses by CRISPR/Cas9-edited B cells. J Exp Med 216:1301–1310

Hermans PW, van Soolingen D, Bik EM, de Haas PE, Dale JW, van Embden JD (1991) Insertion element IS987 from Mycobacterium bovis BCG is located in a hot-spot integration region for

insertion elements in Mycobacterium tuberculosis complex strains. Infect Immun 59(8): 2695–2705

Hoban MD, Lumaquin D, Kuo CY, Romero Z, Long J, Ho M, Young CS, Mojadidi M, Fitz-Gibbon S, Cooper AR, Lill GR, Urbinati F, Campo-Fernandez B, Bjurstrom CF, Pellegrini M, Hollis RP, Kohn DB (2016) CRISPR/Cas9-mediated correction of the sickle mutation in human CD34+ cells. Mol Ther 24(9):1561–1569

Hsu PD, Lander ES, Zhang F (2014) Development and applications of CRISPR-Cas9 for genome engineering. Cell 157(6):1262–1278

Hultquist JF, Hiatt J, Schumann K, McGregor MJ, Roth TL, Haas P et al (2019) CRISPR-Cas9 genome engineering of primary CD4(+) T cells for the interrogation of HIV-host factor interactions. Nat Protoc 14:1–27

Ishino Y, Shinagawa H, Makino K, Amemura M, Nakata A (1987) Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in Escherichia coli, and identification of the gene product. J Bacteriol 169(12):5429–5433

Jansen R, Embden JD, Gaastra W, Schouls LM (2002) Identification of genes that are associated with DNA repeats in prokaryotes. Mol Microbiol 43(6):1565–1575

Jarrett KE, Lee C, De Giorgi M, Hurley A, Gillard BK, Doerfler AM, Li A, Pownall HJ, Bao G, Lagor WR (2018) Somatic editing of Ldlr with adeno-associated viral-CRISPR is an efficient tool for atherosclerosis research. Arterioscler Thromb Vasc Biol 38(9):1997–2006

Jiang C, Mei M, Li B, Zhu X, Zu W, Tian Y, Wang Q, Guo Y, Dong Y, Tan X (2017) A non-viral CRISPR/Cas9 delivery system for therapeutically targeting HBV DNA and pcsk9 in vivo. Cell Res 27(3):440–443

Kuo CY, Hoban MD, Joglekar AV, Kohn DB (2015) Site specific gene correction of defects in CD40 ligand using the Crispr/Cas9 genome editing platform. J Allergy Clin Immunol 135(2): AB17

Kuske MD, Johnson JP (1999) Assignment of the human melanoma cell adhesion molecule gene (MCAM) to chromosome 11 band q23.3 by radiation hybrid mapping. Cytogenet Cell Genet 87(3–4):258

Lara-Pezzi E, Dopazo A, Manzanares M (2012) Understanding cardiovascular disease: a journey through the genome (and what we found there). Dis Model Mech 5(4):434–443

Levy-Lahad E, Wijsman EM, Nemens E, Anderson L, Goddard KA, Weber JL, Bird TD, Schellenberg GD (1995) A familial Alzheimer's disease locus on chromosome 1. Science 269(5226):970–973

Lian YF, Yuan J, Cui Q, Feng QS, Xu M, Bei JX, Zeng YX, Feng L (2016) Upregulation of KLHDC4 predicts a poor prognosis in human nasopharyngeal carcinoma. PLoS One 11(3): e0152820

Liu Y, Zeng Y, Liu L, Zhuang C, Fu X, Huang W, Cai Z (2014) Synthesizing AND gate genetic circuits based on CRISPR-Cas9 for identification of bladder cancer cells. Nat Commun 5:5393

Liu YC, Cai ZM, Zhang XJ (2016a) Reprogrammed CRISPR-Cas9 targeting the conserved regions of HPV6/11 E7 genes inhibits proliferation and induces apoptosis in E7-transformed keratinocytes. Asian J Androl 18:475–479

Liu T, Shen JK, Li Z, Choy E, Hornicek FJ, Duan Z (2016b) Development and potential applications of CRISPR-Cas9 genome editing technology in sarcoma. Cancer Lett 373(1): 109–118

Liu J, Zhou Y, Qi X, Chen J, Chen W, Qiu G, Wu Z, Wu N (2017) CRISPR/Cas9 in zebrafish: an efficient combination for human genetic diseases modeling. Hum Genet 136(1):1–12

Ma Y, Walsh MJ, Bernhardt K, Ashbaugh CW, Trudeau SJ, Ashbaugh IY et al (2017) CRISPR/Cas9 screens reveal Epstein-Barr virus-transformed B cell host dependency factors. Cell Host Microbe 21:580–591.e7

Marraffini LA, Sontheimer EJ (2008) CRISPR interference limits horizontal gene transfer in staphylococci by targeting DNA. Science 322(5909):1843–1845

Mojica FJ, Ferrer C, Juez G, Rodriguez-Valera F (1995) Long stretches of short tandem repeats are present in the largest replicons of the Archaea Haloferax mediterranei and Haloferax volcanii and could be involved in replicon partitioning. Mol Microbiol 17(1):85–93

Monteys AM, Ebanks SA, Keiser MS, Davidson BL (2017) CRISPR/Cas9 editing of the mutant huntingtin allele in vitro and in vivo. Mol Ther 25(1):12–23

Morrow G, Tanguay RM (2017) Biochemical and clinical aspects of hereditary tyrosinemia type 1. Adv Exp Med Biol 959:9–21

Murati A, Brecqueville M, Devillier R, Mozziconacci MJ, Gelsi-Boyer V, Birnbaum D (2012) Myeloid malignancies: mutations, models and management. BMC Cancer 12:304

Nasrallah F, Hammami MB, Ben Rhouma H, Fradj SH, Azzouz H, Omar S, Feki M, Ben Youssef IT, Messaoud T, Tebib N, Kaabachi N (2015) Clinical and biochemical profile of tyrosinemia type 1 in Tunisia. Clin Lab 61(5–6):487–492

Nelson KE, Clayton RA, Gill SR, Gwinn ML, Dodson RJ, Haft DH, Hickey EK, Peterson JD, Nelson WC, Ketchum KA, McDonald L, Utterback TR, Malek JA, Linher KD, Garrett MM, Stewart AM, Cotton MD, Pratt MS, Phillips CA, Richardson D, Heidelberg J, Sutton GG, Fleischmann RD, Eisen JA, White O, Salzberg SL, Smith HO, Venter JC, Fraser CM (1999) Evidence for lateral gene transfer between Archaea and bacteria from genome sequence of Thermotoga maritima. Nature 399(6734):323–329

Niu Y, Xu J, Sun T (2019) Cyclin-dependent kinases 4/6 inhibitors in breast cancer: current status, resistance, and combination strategies. J Cancer 10(22):5504–5517

Peng R, Lin G, Li J (2016) Potential pitfalls of CRISPR/Cas9-mediated genome editing. FEBS J 283(7):1218–1231

Pires C, Schmid B, Petraeus C, Poon A, Nimsanor N, Nielsen TT, Waldemar G, Hjermind LE, Nielsen JE, Hyttel P, Freude KK (2016) Generation of a gene-corrected isogenic control cell line from an Alzheimer's disease patient iPSC line carrying a A79V mutation in PSEN1. Stem Cell Res 17(2):285–288

Platt RJ, Chen S, Zhou Y, Yim MJ, Swiech L, Kempton HR, Dahlman JE, Parnas O, Eisenhaure TM, Jovanovic M, Graham DB, Jhunjhunwala S, Heidenreich M, Xavier RJ, Langer R, Anderson DG, Hacohen N, Regev A, Feng G, Sharp PA, Zhang F (2014) CRISPR-Cas9 knock in mice for genome editing and cancer modeling. Cell 159(2):440–455

Poon A, Schmid B, Pires C, Nielsen TT, Hjermind LE, Nielsen JE, Holst B, Hyttel P, Freude KK (2016) Generation of a gene-corrected isogenic control hiPSC line derived from a familial Alzheimer's disease patient carrying a L150P mutation in presenilin 1. Stem Cell Res 17(3): 466–469

Price AA, Sampson TR, Ratner HK, Grakoui A, Weiss DS (2015) Cas9-mediated targeting of viral RNA in eukaryotic cells. Proc Natl Acad Sci U S A 112:6164–6169

Roberts R, Marian AJ, Dandona S, Stewart AFR (2013) Genomics in cardiovascular disease. J Am Coll Cardiol 61(20):2029–2037

Rub U, Seidel K, Heinsen H, Vonsattel JP, den Dunnen WF, Korf HW (2016) Huntington's disease (HD): the neuropathology of a multisystem neurodegenerative disorder of the human brain. Brain Pathol 26(6):726–740

Ryan OW, Poddar S, Cate JH (2016) CRISPR-Cas9 genome engineering in Saccharomyces cerevisiae cells. Cold Spring Harb Protoc 2016(6)

Sampson TR, Saroj SD, Llewellyn AC, Tzeng YL, Weiss DS (2013) A CRISPR/Cas system mediates bacterial innate immune evasion and virulence. Nature 497:254–257

Sanchez-Rivera FJ, Jacks T (2015) Applications of the CRISPR-Cas9 system in cancer biology. Nat Rev Cancer 15(7):387–395

Saudou F, Humbert S (2016) The biology of huntingtin. Neuron 89(5):910–926

Schwank G, Koo BK, Sasselli V, Dekkers JF, Heo I, Demircan T, Sasaki N, Boymans S, Cuppen E, van der Ent CK, Nieuwenhuis EE, Beekman JM, Clevers H (2013) Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients. Cell Stem Cell 13(6):653–658

Seidah NG (2013) Proprotein convertase subtilisin kexin 9 (PCSK9) inhibitors in the treatment of hypercholesterolemia and other pathologies. Curr Pharm Des 19(17):3161–3172

Shah S, Alexaki A, Pirrone V, Dahiya S, Nonnemacher MR, Wigdahl B (2014) Functional properties of the HIV-1 long terminal repeat containing single nucleotide polymorphisms in Sp site III and CCAAT/enhancer binding protein site I. Virol J 11:92

Shao Y, Wang L, Guo N, Wang S, Yang L, Li Y, Wang M, Yin S, Han H, Zeng L, Zhang L, Hui L, Ding Q, Zhang J, Geng H, Liu M, Li D (2019) Correction: Cas9-nickase-mediated genome editing corrects hereditary tyrosinemia in rats. J Biol Chem 294(21):8348

Shiels A, Hejtmancik JF (2007) Genetic origins of cataract. Arch Ophthalmol 125(2):165–173

Shiels A, Hejtmancik JF (2017) Mutations and mechanisms in congenital and age-related cataracts. Exp Eye Res 156:95–102

Shin JW, Kim KH, Chao MJ, Atwal RS, Gillis T, MacDonald ME, Gusella JF, Lee JM (2016) Permanent inactivation of Huntington's disease mutation by personalized allele-specific CRISPR/Cas9. Hum Mol Genet 25(20):4566–4576

Shintani K, Shechtman DL, Gurwood AS (2009) Review and update: current treatment trends for patients with retinitis pigmentosa. Optometry 80(7):384–401

Singh K, Evens H, Nair N, Rincon MY, Sarcar S, Samara-Kuko E, Chuah MK, VandenDriessche T (2018) Efficient in vivo liver-directed gene editing using CRISPR/Cas9. Mol Ther 26(5): 1241–1254

Skov M, Hansen CR, Pressler T (2019) Cystic fibrosis—an example of personalized and precision medicine. APMIS 127(5):352–360

Skvarova Kramarzova K, Osborn MJ, Webber BR, DeFeo AP, McElroy AN, Kim CJ, Tolar J (2017) CRISPR/Cas9-mediated correction of the FANCD1 gene in primary patient cells. Int J Mol Sci 18(6):1269

Su S, Hu B, Shao J, Shen B, Du J, Du Y, Zhou J, Yu L, Zhang L, Chen F, Sha H, Cheng L, Meng F, Zou Z, Huang X, Liu B (2016) CRISPR-Cas9 mediated efficient PD-1 disruption on human primary T cells from cancer patients. Sci Rep 6(1):20070

Suzuki K, Izpisua Belmonte JC (2018) In vivo genome editing via the HITI method as a tool for gene therapy. J Hum Genet 63(2):157–164

Suzuki K, Tsunekawa Y, Hernandez-Benitez R, Wu J, Zhu J, Kim EJ, Hatanaka F, Yamamoto M, Araoka T, Li Z, Kurita M, Hishida T, Li M, Aizawa E, Guo S, Chen S, Goebl A, Soligalla RD, Qu J, Jiang T, Fu X, Jafari M, Esteban CR, Berggren WT, Lajara J, Nunez-Delicado E, Guillen P, Campistol JM, Matsuzaki F, Liu GH, Magistretti P, Zhang K, Callaway EM, Belmonte JC (2016) In vivo genome editing via CRISPR/Cas9 mediated homology-independent targeted integration. Nature 540(7631):144–149

Tang H, Shrager JB (2016) CRISPR/Cas-mediated genome editing to treat EGFR-mutant lung cancer: a personalized molecular surgical therapy. EMBO Mol Med 8(2):83–85

Tessadori F, Roessler HI, Savelberg SMC, Chocron S, Kamel SM, Duran KJ, van Haelst MM, van Haaften G, Bakkers J (2018) Effective CRISPR/Cas9-based nucleotide editing in zebrafish to model human genetic cardiovascular disorders. Dis Model Mech 11(10):dmm035469

Valletta S, Dolatshad H, Bartenstein M, Yip BH, Bello E, Gordon S, Yu Y, Shaw J, Roy S, Scifo L, Schuh A, Pellagatti A, Fulga TA, Verma A, Boultwood J (2015) ASXL1 mutation correction by CRISPR/Cas9 restores gene function in leukemia cells and increases survival in mouse xenografts. Oncotarget 6(42):44061–44071

VanLith C, Guthman R, Nicolas CT, Allen K, Du Z, Joo DJ, Nyberg SL, Lillegard JB, Hickey RD (2018) Curative ex vivo hepatocyte-directed gene editing in a mouse model of hereditary tyrosinemia type 1. Hum Gene Ther 29(11):1315–1326

Vezinaw CM, Fishman GA, McAnany JJ (2019) Visual impairment in retinitis pigmentosa. Retina 40(8):1630–1633

Villiger L, Grisch-Chan HM, Lindsay H, Ringnalda F, Pogliano CB, Allegri G, Fingerhut R, Haberle J, Matos J, Robinson MD, Thony B, Schwank G (2018) Treatment of a metabolic liver disease by in vivo genome base editing in adult mice. Nat Med 24(10):1519–1525

Wang DY, Chan WM, Tam PO, Baum L, Lam DS, Chong KK, Fan BJ, Pang CP (2005) Gene mutations in retinitis pigmentosa and their clinical implications. Clin Chim Acta 351(1–2):5–16

Wang Y, Zhang T, Kwiatkowski N, Abraham BJ, Lee TI, Xie S, Yuzugullu H, Von T, Li H, Lin Z, Stover DG, Lim E, Wang ZC, Iglehart JD, Young RA, Gray NS, Zhao JJ (2015) CDK7-dependent transcriptional addiction in triple-negative breast cancer. Cell 163(1):174–186

Wang J, Chen R, Zhang R, Ding S, Zhang T, Yuan Q et al (2017) The gRNA miRNA-gRNA ternary cassette combining CRISPR/Cas9 with RNAi approach strongly inhibits hepatitis B virus replication. Theranostics 7:3090–3105

Wen J, Tao W, Hao S, Zu Y (2017) Cellular function reinstitution of offspring red blood cells cloned from the sickle cell disease patient blood post CRISPR genome editing. J Hematol Oncol 10(1):119

Williams TN, Thein SL (2018) Sickle cell anemia and its phenotypes. Annu Rev Genomics Hum Genet 19:113–147

Xie F, Ye L, Chang JC, Beyer AI, Wang J, Muench MO, Kan YW (2014) Seamless gene correction of beta-thalassemia mutations in patient-specific iPSCs using CRISPR/Cas9 and piggyBac. Genome Res 24(9):1526–1533

Xu CL, Ruan MZC, Mahajan VB, Tsang SH (2019) Viral delivery systems for CRISPR. Viruses 11(1):28

Yang Y, Wang L, Bell P, McMenamin D, He Z, White J, Yu H, Xu C, Morizono H, Musunuru K, Batshaw ML, Wilson JM (2016) A dual AAV system enables the Cas9-mediated correction of a metabolic liver disease in newborn mice. Nat Biotechnol 34(3):334–338

Yang S, Chang R, Yang H, Zhao T, Hong Y, Kong HE, Sun X, Qin Z, Jin P, Li S, Li XJ (2017) CRISPR/Cas9-mediated gene editing ameliorates neurotoxicity in mouse model of Huntington's disease. J Clin Invest 127(7):2719–2724

Yin H, Xue W, Chen S, Bogorad RL, Benedetti E, Grompe M, Koteliansky V, Sharp PA, Jacks T, Anderson DG (2014) Genome editing with Cas9 in adult mice corrects a disease mutation and phenotype. Nat Biotechnol 32(6):551–553

Yoshiba T, Saga Y, Urabe M, Uchibori R, Matsubara S, Fujiwara H et al (2019) CRISPR/Cas9-mediated cervical cancer treatment targeting human papillomavirus E6. Oncol Lett 17:2197–2206

Yuan L, Sui T, Chen M, Deng J, Huang Y, Zeng J, Lv Q, Song Y, Li Z, Lai L (2016) CRISPR/Cas9-mediated GJA8 knockout in rabbits recapitulates human congenital cataracts. Sci Rep 6:22024

Yuan L, Yao H, Xu Y, Chen M, Deng J, Song Y, Sui T, Wang Y, Huang Y, Li Z, Lai L (2017) CRISPR/Cas9-mediated mutation of alphaA-crystallin gene induces congenital cataracts in rabbits. Invest Ophthalmol Vis Sci 58(6):BIO34–BIO41

Yuen KS, Chan CP, Wong NM, Ho CH, Ho TH, Lei T et al (2015) CRISPR/Cas9-mediayoungted genome editing of Epstein-Barr virus in human cells. J Gen Virol 96:626–636

Zhou Y, Han C, Li D, Yu Z, Li F, An Q, Bai H, Zhang X, Duan Z, Kan Q (2015) Cyclin-dependent kinase 11(p110) (CDK11(p110)) is crucial for human breast cancer cell proliferation and growth. Sci Rep 5:10433

Zhu S, Zhou Y, Wei W (2017) Genome-wide CRISPR/Cas9 screening for high-throughput functional genomics in human cells. Methods Mol Biol 1656:175–181

Brain Infectious Diseases and Nanotherapy 26

Maharudra Pratap Singh, Santosh Kumar Yadav, Mohammad Meraj Khan, Sharique Ahmad, Rehan Khan, Abdul Quaiyoom Khan, Rizwanul Haque, and Syed Shadab Raza

Maharudra Pratap Singh and Santosh Kumar Yadav contributed equally and share equal first co-authorship.

M. P. Singh
Laboratory for Stem Cell and Restorative Neurology, Department of Biotechnology, Era's Lucknow Medical College Hospital, Era University, Lucknow, India

Department of Biotechnology, Central University of South Bihar, Gaya, India

S. K. Yadav
Laboratory for Stem Cell and Restorative Neurology, Department of Biotechnology, Era's Lucknow Medical College Hospital, Era University, Lucknow, India

M. M. Khan
Department of Pharmaceutics, Centre for Pharmaceutical Nanotechnology, National Institute of Pharmaceutical Education and Research, S.A.S Nagar, Sector 67, Mohali, Punjab, India

S. Ahmad
Department of Pathology, Era's Lucknow Medical College Hospital, Era University, Lucknow, India

R. Khan
Institute of Nanoscience and Technology, Mohali, India
e-mail: rehankhan@inst.ac.in

A. Q. Khan
Hammad Medical Co-operation, Doha, Qatar

R. Haque
Department of Biotechnology, Central University of South Bihar, Gaya, India
e-mail: rhaque@cub.ac.in

S. S. Raza (✉)
Laboratory for Stem Cell and Restorative Neurology, Department of Biotechnology, Era's Lucknow Medical College Hospital, Era University, Lucknow, India

Department of Stem Cell Biology and Regenerative Medicine, Era University, Lucknow, India
e-mail: drshadab@erauniversity.in

S. Hameed, S. Rehman (eds.), *Nanotechnology for Infectious Diseases*,
https://doi.org/10.1007/978-981-16-9190-4_26

Abstract

Indubitably brain is a highly complex organ and has the responsibility to regulate all the cognitive, behavioral, and emotional activities. The meninges and blood–brain barrier act as a safeguard to protect it from pathogenic attacks and blood-borne insults, however, the absence of an indigenous defense mechanism makes it an easy target for many diseases and disorders. Infectious diseases are the main reason for casualties, long-term disability, and economic hardship and negatively impacting healthcare and socioeconomic development on a global scale. For decades, the scientific community struggled to produce medications that can trespass the barriers of the brain and target the intended locations with minimum side effects. However, most of the promising drugs, which proved to enhance brain repair and function at the laboratory stage in animal models, confront numerous hurdles, including specificity, delivery, development of resistance, and toxicity. Thus the development of new techniques is a prerequisite. Nanotechnology is a technique that can modify substances at the molecular level to achieve the desired structure. The nanostructures, interacting with microorganisms, are rapidly transforming the biomedical area, providing benefits in both diagnostic and therapeutic applications. This chapter gives a basic overview of how nanosized materials can be used to diagnose and treat common brain infections.

Keywords

Nanotechnology · Nanoparticle · Central nervous system · Blood–brain barrier · Pathogenic microorganisms · Brain infections

26.1 Introduction

The pathogenic microorganisms occasionally enter the body, infect various parts of the body resulting in the development of a variety of symptoms ranging from minor irritation to major illness. Brain infection is a pathophysiological condition when viruses, bacteria, fungi, or sporadically protozoa and parasites infect the brain, spinal cord, or surrounding area. Although infections of the brain are rare, they can be hazardous with a poor prognosis. Due to the lack of typical host defensive systems, such as antibody and complement activity, the Central Nervous System (CNS) is especially susceptible to pathogens (Ekizoğlu 2017). Pathogenic microbes such as neuro-invasive bacteria, viruses, parasites, and fungus can penetrate the CNS and induce a variety of health issues, which can be life-threatening if left untreated (Ekizoğlu 2017). Despite significant progress achieved with antibiotics ranging from nucleic acids, antibodies, tiny molecules, and proteins/peptides, efficient treatment of infectious diseases remains a major issue (De Rycker et al. 2018; Kaufmann

et al. 2018; Metcalf and Lessler 2017). The employment of antimicrobial drugs for the cure of brain diseases has efficiently decreased the fatality rate, however, the systemic exposure of currently available antiviral and antibacterial drugs caused severe side effects along with the development of swift drug resistance (Baker et al. 2007; Meylan et al. 2018). Furthermore, fresh viral and bacterial pathogens are continually developing as a result of evolution or other biological processes, making management and prevention of infectious illnesses an ongoing problem. As a result, in addition to developing novel antiviral and antibiotic medicines for brain infection management, innovative methodologies to maximize the efficacy of already available treatments must be created (Willing et al. 2011; Dickey et al. 2017).

26.2 Types of Infectious Diseases of the Brain

Brain infections are classified as per their anatomical components, for example, infection of brain parenchyma (encephalitis), meninges (meningitis), spinal cord (myelitis), brain parenchyma and extradural (abscess), subdural (empyema), or simultaneous infections of multiple regions (meningoencephalitis and encephalomyelitis). They can cause a variety of symptoms, such as fever, headache, seizures, and behavioral or cognitive abnormalities. Their effects might range from mild brain damage to strokes and even death in extreme situations.

26.3 Potential Causative Agents of Brain Infections

Almost all the known microbial types are known to infect the brain and cause various brain diseases; however, the frequency for the type of causative agent varies greatly.

1. **Viruses:** Broadly, viral diseases are classified into two groups—acute ailments and chronic infections. Acute viral illnesses have a short incubation time, but chronic viral diseases have a longer incubation period. Their symptoms appear gradually and lead to a deadly conclusion. The common examples of acute viral diseases are meningitis (aseptic), flaccid paralysis, encephalitis, post-infectious, and encephalomyelitis, etc. While most frequent chronic viral diseases are subacute sclerosing panencephalitis, spongiform encephalopathies, progressive multifocal leukoencephalopathy (JC virus), retrovirus disease. Examples of widespread viral pathogens are herpes simplex virus (HSV), Varicella-zoster virus (VZV), Epstein–Barr virus (EBV), Human Herpesvirus 6 (HHV6), Human Herpesvirus 7 (HHV7), *Adenovirus, Measles, mumps, rubella, HIV, West Nile, chikungunya, Saint Louis, Powassan, equine encephalitis, Hendra, Toscana, rabies, la Crosse Simian herpes, Nipah.*
2. **Bacteria:** Bacteria can invade the body by several routes and may persist in the bloodstream. The CNS is separated and protected from the blood-borne threats with the help of meninges, the three membranes that enclose the CNS. In addition, the barriers (BBB, BCSFB) not only maintain the neuronal

environment's homeostasis but also protect the CNS from infections. Due to the fact, despite many microbes can enter the bloodstream, only a small percentage of them make it to the meninges, especially those who have devised unique mechanisms to get over these barriers. One of the widely spread infectious brain diseases is bacterial meningitis, which is the most commonly reported in infants and adults worldwide, is usually caused by an extracellular infectious agent such as meningococcus (*Neisseria meningitidis*), *Streptococcus pneumoniae* (also known as pneumococcus), and *Haemophilus influenzae* (Coureuil et al. 2017). Moreover, *S. suis*, a swine pathogen, is recently identified as zoonotic pathogen causing human meningitis, particularly in Asia (Segura et al. 2014). Other known bacterial pathogens are as follows: *Listeria monocytogenes, Mycobacterium, Mycoplasma pneumoniae, Coxiella burnetii, Chlamydia pneumoniae, Borrelia burgdorferi, Treponema, Brucella* sp., *Rickettsia, Ehrlichia, Bartonella, Tropheryma whipplei, Francisella tularensis, Legionella.*

3. **Parasites:** Parasites are a diverse set of life forms that are categorized into two types: single-celled organisms (protozoa) and multicellular helminths. CNS diseases caused by parasitic pathogens are continued to be the leading cause of morbidness and deaths globally (Carpio et al. 2016). Each year, these parasites afflict millions of people in undeveloped or developing nations; nevertheless, occasional instances are reported in non-endemic regions due to increased cross-country journey, and suppression of immune system either due to the medications after transplantation remedies (5–10% of transplant recipients) or infection of HIV (~19% in AIDS patients) (Walker et al. 2006). Cysticercosis of the brain (by *Taenia solium*) is increasingly common parasite infection of the central nervous system; toxoplasmosis (by *Toxoplasma gondii*), echinococcosis (*Echinococcus multilocularis*), and schistosomiasis (*Schistosoma* sp.) are less common illnesses. Paragonimiasis (*Paragonimus westermani*), American and African trypanosomiasis (*Trypanosoma cruzi* and *T. brucei*, respectively), Malaria (*Plasmodium* sp.), and angiostrongyliasis (by *Angiostrongylus cantonensis*) are all rare parasite disorders that affect the CNS (Abdel Razek et al. 2011).
4. **Fungi:** Fungal infections of CNS are uncommon clinical conditions as out of thousands of identified fungal species only 300 may be virulent to humans, of them only 10–15% could infect the CNS (Sharma 2010; Köhler et al. 2014; Bryan et al. 2015). From single cellular yeasts to filamentous and dimorphic fungi are the clinically relevant etiological pathogens of CNS. In comparison to viral, bacterial, or parasitic CNS diseases, symptomatic fungal CNS infections have a higher risk of morbidity and mortality with a broad array of clinical symptoms, diagnostic problems, and unique treatment obstacles (Sharma 2010). Some widespread disease-causing fungi of CNS are *Histoplasma capsulatum, Cryptococcus neoformans, Sporotrichum, Candida albicans, Coccidioides immitis, Paracoccidioides brasiliensis, Blastomyces, Aspergillus spp., and zygomycetes* (Bariola et al. 2010; Gottfredsson and Perfect 2000; Kleinschmidt-DeMasters 2002; Liu et al. 2012; Murthy 2007; Pedroso et al. 2013; Scully et al. 2008).

26.4 Routes of Contamination of the Central Nervous System (CNS)

The bony structure around the brain and spinal cord, the meninges, and the BBB collectively make the brain resistant to bacterial pathogens. Viruses may enter the human body through bodily fluids such as saliva, blood, urine, feces, sperm, and mucosal secretions and infect the CNS (Koeller and Shih 2017). If the pathogens somehow initiate the infection in the body, the CNS becomes more vulnerable compared to other parts of the body due to the lack of a host defense mechanism. There are three primary avenues by which the CNS can be contaminated: hematogenic, adjacency, and neural.

26.4.1 Hematogenic Route

26.4.1.1 Arterial Way

Irrespective of whether bacteria, parasites, or viruses are responsible for the infection and the region of infection, this pathway is the primary method of infection for CNS. Most bacterial or parasite pathogens follow this route for infection. In the case of viral pathogens, most of the viruses enter the CNS by this route only, except the neurotrophic viruses which breach the BBB (if damaged) or through transcytotic passage in the epithelial cells of BBB, as well as by infesting leucocytes by following "Trojan horse" mechanism. Due to this variation in route of infection, the viral infections are less prevalent at the junction of white matter/gray matter and in the area of the lenticulo-striated arteries, while in the case of bacteria they are predominantly found in the abovementioned areas.

26.4.1.2 Venous Way

Venous dissemination is quite rare. In schistosomiasis, it is likely to be the source of CNS damage (especially in the bone marrow). Thrombophlebitis, on the other hand, is an increased risk in a certain number of illnesses, and the veins are a channel for the infection's spread.

26.4.1.3 Damage via Contiguity

Damage from proximity is the next most common type of CNS infection. Sinusitis or petrous bone infection leads to a trans-osseous infection, resulting in either an intracerebral abscess or a peri-cerebral collection, causing the brain infection (extra- or subdural empyema). In this way, the frontal or temporal region is primarily affected.

26.4.2 Neural Way

In contrast to the hematogenic route, the neural route is much less prevalent and accountable for a definite category of viral encephalitis, like HSV, as well as the

harm caused by rabies and varicella zona viruses. Also, the neural pathway undoubtedly explains listeriosis-induced injury to cranial nerve nuclei.

26.4.3 Direct Infection

Direct entry of pathogens to CNS can occur due to injury of the cranium and/or vertebra or post-intracranial surgical treatment.

26.4.4 Unknown

Though, to date, for most brain infections the route of infection is determined. However, according to the CDC in ~20% of reports, the source of pathogenicity is unidentified.

26.4.4.1 Meningitis

Meningitis develops when the protection measures of the brain and spinal cord, viz. meninges and/or CSF become inflamed. Bacteria and viruses are responsible for the distinctive alterations in the CSF (Ekizoğlu 2017). Meningitis can also be caused by a fungus or a parasite in rare cases. Bacterial meningitis is a life-threatening illness responsible for more than 1.2 million hospitalizations per year, thus it must be treated right away (Borchorst and Møller 2012). Bacterial meningitis causes hypoxic brain injury and could lead to fatality by causing brain edema, increasing intracranial pressure, and altering blood circulation to the brain. Several species of bacteria can infect the upper respiratory tract and then migrate to the brain via circulation. Bacterial meningitis can also arise when certain bacteria directly penetrate the meninges. The common symptoms of meningitis include a deep fever, rigorous headache, rigid neck, photophobia, nausea, and vomiting. Meningitis is characterized by the inability to lower your chin to your chest. About 50% of recovered patients are likely to experience neurological adverse consequences like the loss of hearing ability, developmental abnormalities, and neuropsychological injury.

S. pneumoniae, *S. agalactiae*, *N. meningitidis*, and *L. monocytogenes* are the most prevalent organisms that cause acute bacterial meningitis. Approximately 10–20% of patients recovered from bacterial meningitis face degeneration of neurons and long-term vision and hearing impairment. Neuronal degeneration, predominantly in the hippocampal region, has been recognized as a probable source of long-term cognitive effects in survivors (Nau et al. 1999; McGill et al. 2017). The subarachnoid space is deficient in complement features, polymorphonuclear leukocytes, and some plasma cells, which renders the host defense ability too weak to control the infection in this region. The increase of bacterial population in the CSF induces fever, inflammation, and in turn secretion of specific cytokines like interleukin-1beta, interleukin-6, tumor necrosis factor-α, matrix metalloproteinases, and free radicals, leading to BBB disruption and neuronal injury. Neuronal damage is also caused by

bacterial toxins and virulence factors (Van Furth et al. 1996). There is a noticeable increase in the number of white blood cells that cross the BBB and enter CNS tissue. Moreover, the inflammation of meninges also enhances the BBB permeability and inhibits the elimination pump, in addition to increasing the permeability of the BBB. Antibiotic treatment reduces inflammation, allowing the BBB to slowly return to normal (Spector 1990). The prolonged use of antibiotics like beta-lactams causes lysis of bacterial cells and release of cell components, viz. lipopolysaccharide (LPS), lipoteichoic acid (LTA), and murein (peptidoglycans), which could overstimulate the immune cells and intensify the inflammatory response (McGill et al. 2017; Liechti et al. 2015; Ribes et al. 2014).

In the medical dictionary, viral meningitis is described as a nonbacterial inflammation of the brain's tissues. However, several others have also been recognized with the capability to induce meningitis, like mumps virus, arboviruses, lymphocytic choriomeningitis virus (LCMV), bunyaviruses, HSV-1 and -2, and HIV-1 (Swanson and McGavern 2015). Human enteroviruses (HEVs) are accountable for most of the viral meningitis cases, with children being the most common victims. HSV encephalitis most frequently affects humans under the age of 20 and over the age of 50 years. Meningitis caused by HSV-2 accounts for 17% of all instances of aseptic meningitis. In adults, 8% of viral meningitis cases were caused by the varicella-zoster virus. Generally, the symptoms of viral meningitis are modest and resolve following antiviral treatment with no long-term consequences (Ekizoğlu 2017). As a result, as compared to meningitis caused by bacteria and fungi, viral meningitis is generally regarded as the least lethal kind of meningitis.

26.4.4.2 Encephalitis

Encephalitis, inflammation of the brain parenchyma due to viruses, is the result of viral incursion into the brain. Acute disseminated encephalomyelitis arises when a virus causes a hypersensitivity reaction in the brain and spinal cord. Viruses can induce inflammations in this way. Symptoms may begin as minor flu-like symptoms and headaches but are swiftly followed by behavioral abnormalities, hallucinations, and confusion.

Viruses are the primary reason for encephalitis in 68.5% of patients with recognized etiology, accounting for 15.8% of all reports. HSV is still the leading pathogen of encephalitis in the developed world. Enteroviruses, VZV, EBV, measles virus, and arboviruses, like JEV, WNV, and MVEV, are other causes of encephalitis, according to the National Institutes of Health. Another probable cause of meningoencephalitis is *L. monocytogenes*, which has symptoms of both meningitis and encephalitis.

Epidemic and sporadic viral infections are the two main forms of viral infections. Arbovirus, echovirus, coxsackievirus, poliovirus (in certain less developed regions), HSV, rabies, varicella-zoster virus, or the mumps virus can produce epidemic diseases. When necessary, antiviral medications are used in conjunction with complementary therapies.

26.4.4.3 Brain Abscesses

Abscesses form when a collection of pus becomes encased in brain tissue, causing inflammation. According to the American Academy of Neurology, brain abscesses caused by bacteria, fungus, or parasites are regarded to be localized infections of the brain parenchyma. About 1–2% of brain abscesses occur in developed countries, while 8% of brain abscesses occur in non-developed countries (Muzumdar et al. 2011). Immunocompromised individuals are more prone to suffer from brain abscesses than healthy individuals. The male population within the age group of 30–50 years is more likely to develop brain abscesses (Patel and Clifford 2014). Over time, an abscess can lead to speech abnormalities, muscular weakness, stiffness, and seizures. Once discovered, an abscess must be located and surgically removed, followed by 4–8 weeks of antibiotic treatment. Patient fatality rates have declined due to improvements in the microbiology-based diagnostic methods (improved methods of anaerobic microbes and advanced methods of neuroradiological imaging); identification (new neurosurgical methodologies); and therapies (wide-range antibiotics).

Cranial infections (e.g., osteomyelitis, sinusitis, and otitis) and cranial trauma, recent neurosurgical operations, and hematogenous dissemination are all possible causes of brain abscesses (e.g., in bacterial endocarditis).

There are three possible causes of brain abscesses; (1) cranial infections (for example, osteomyelitis, sinusitis, and otitis); (2) cranial injury and recent neurosurgery, and hematogenous dissemination (e.g., bacteria-mediated endocarditis). *S. aureus* and Streptococci (e.g., *S. milleri* group and viridian group streptococci) are the highly prevalent bacteria causing brain abscesses due to their ability to extend from the nasopharynx to oropharynx. *Pseudomonas* spp. has been found in brain abscesses caused by otitis media or otitis externa. Brain abscesses caused by head trauma or neurosurgical procedures are often infected with Staphylococcus spp. and aerobic Gram-negative bacilli (Kaya et al. 2008). Anaerobic bacterial brain abscesses are frequently caused by otorhinolaryngitis. The most commonly isolated anaerobic organisms include anaerobic streptococcus spp. and peptostreptococcus; prevotella spp., bacteroides, and fusobacterium spp. (Le Moal et al. 2003; Vishwanath et al. 2016). Brain abscesses may also be caused by *M. hominis* and rare *Mycoplasma* spp. (Raoultm et al. 2009; Ørsted et al. 2011). Parasitic brain abscesses can also be caused by protozoa and helminths. For example, CNS toxoplasmosis is induced by *T. gondii*, and neurocysticercosis is caused by *T. solium* larvae (Honda and Warren 2009). Among fungal pathogens, Candida, Cryptococcus, Histoplasma, Coccidioides, and Blastomyces are a few of the more frequent fungal pathogens that can cause brain abscesses in immunocompromised patients and severe diabetic patients (Honda and Warren 2009; Garcia et al. 2015).

26.4.4.4 Myelitis

As the name implies, myelitis is a spinal cord inflammation that can interfere with regular brain-to-body communication. The general symptoms produced by myelitis are loss of sensory function and paralysis due to myelin and axon damage caused by spinal cord inflammation. Microbial infections are one of the various factors causing

myelitis. The direct infection by various pathogens like HIV, HTLV-I and II, *Treponema pallidum*, *Borrelia* spp., and *Mycobacterium* can lead to the development of myelitis, however, non-infectious methods such as inflammatory pathways can also be responsible for myelitis. Myelitis lesions normally affect a small area; however, they might expand and affect other locations. Depending on the location or source of the lesion, myelitis is categorized into different categories:

- Acute flaccid myelitis is characterized by weak muscles and paralysis.
- Poliomyelitis, caused by a viral infection in the gray matter, the disease is characterized by momentary or lasting paralysis.
- Transverse myelitis is characterized by damage of myelin of axons on both sides of the spinal cord.
- Leukomyelitis is characterized by white matter lesions.
- Meningococcal myelitis (or meningomyelitis) is characterized by injuries affecting the meninges and spinal cord.

A full recovery from myelitis is common, but it can take months to years. Myelitis does not cure, however, the symptoms can be controlled.

26.4.4.5 Empyemas

Empyemas are peri-cerebral collections that arise in the majority of instances as a result of a skull base infection (Karampekios and Hesselink 2005). In rare conditions, it can be developed either due to drainage of subdural hematoma or direction infection via an injury. A specific pathological condition disengages the dura mater from the sections situated in the depth of the bone vault creating a gap where this collection becomes extradural. The common position of extradural hematomas and empyemas is frontal, they migrate forward from the sagittal sinus and are bound by sutures. Alternatively, the collection might be subdural, bridging the dura mater by microthrombophlebitis. After the falx cerebri, this collection travels behind the sagittal sinus in a frontal position and is not restricted by sutures. Due to the abundance of veins in this subdural area, thrombophlebitis, a major complication of brain infection, is possible. From the microbiological point of view, causative bacteria for empyemas is the same as for abscesses, mostly Streptococci are the predominant microbial flora.

26.4.4.6 Meningoencephalitis

Meningoencephalitis, commonly called herpes meningoencephalitis, is a diseased condition that shares the symptoms of meningitis and encephalitis, both. Meningoencephalitis symptoms include strange behavior, personality changes, and thinking difficulties. Headache, fever, pain with neck movement, light sensitivity, and seizures with clear liquid in the lumbar puncture are all possible symptoms (Granerod et al. 2010; Mailles and Stahl 2009). The presence of recurrent or recent herpes infection, fever, headache, changed mental status, convulsions, alteration of consciousness, and localized symptoms should be included in the clinical diagnosis.

Cerebrospinal fluid testing is routinely done. According to the type of pathogen, the categories of meningoencephalitis are as follows:

- **Herpes meningoencephalitis (HME):** The herpes virus causes one of the most frequent types of meningoencephalitis.
- **Fungal meningoencephalitis:** Caused by the spread of the fungus through the bloodstream, it primarily affects persons with a reduced immune system as a result of medicine, cancer, or HIV.
- **Bacterial meningoencephalitis:** This infection, also known as pyogenic meningoencephalitis, is a life-threatening disease with a high risk of fatality and complications like disability.
- **Parasitic meningoencephalitis:** The pathogen is a parasite, which is frequently transferred by contaminated food.
- **Secondary meningoencephalitis:** In this case, the infection spread to the brain from other parts of the body.
- **HIV meningoencephalitis:** Within weeks or months of HIV diagnosis, the virus can approach the brain and meninges also.
- **Aseptic meningoencephalitis:** This term refers to viral and non-infectious causes of meningoencephalitis, and it is typically identified when the CSF tests negative for germs.
- **Primary amebic meningoencephalitis:** This rare and frequently fatal form of meningoencephalitis is caused by *Naegleria fowleri* (an amoeba). It is normally spread by swimming in the water contaminated with the pathogen and also due to the use of neti pots with contaminated water.
- **Japanese encephalitis (JE):** The Japanese encephalitis virus causes this form of meningoencephalitis in Asia. It is vaccine-preventable.

Meningoencephalitis occurs when the meninges and the brain are both inflamed. Also known as encephalomeningitis. Herpes virus infection is the most prevalent cause of meningoencephalitis, which can be caused by viruses, bacteria, and protozoa. For the bacteria that can cause meningoencephalitis, common means of transmission are coughing/sneezing (HIB, *N. meningitides*, and *S. pneumoniae*) and kissing or exchange of saliva during close contact. In the case of herpes simplex type 1 and type 2 meningoencephalitis, the transmission can occur by spreading of droplets, contaminated water or food, mating, oral contacts, and birthing.

The most prevalent form, i.e., Herpes meningoencephalitis (HME), is treated with an intravenous antiviral medicine such as acyclovir for up to 14 days. Vidarabine and famciclovir are two other antiviral medicines that may be used. These antiviral drugs are less effective as the virus progresses. Antibiotics are administered for bacterial causes of meningoencephalitis. The antibiotic used is determined by the causing bacterium.

26.4.4.7 Neurocysticercosis

Larvae of porcine tapeworms are responsible for neurocysticercosis. In the Western Hemisphere, pork tapeworm is the most widespread causative agent of brain

infections. If a human consumes food contaminated with the tapeworm's eggs, the peptic secretions induce the hatching of eggs which develop into larvae. Upon entering the bloodstream, the larvae travel throughout the body, including the brain and spinal cord, before dying. The larvae develop into cysts as they mature (bunches of larvae enveloped in a protective covering). If the cysts form in the brain, the infection is known as neurocysticercosis.

Once the cyst degenerates and the larvae die, symptoms such as inflammation and swelling as well as seizures, personality changes, headaches, and mental problems begin to appear. Cysts can restrict the passage of CSF to the ventricle parts of the brain. This creates pressure on the brain and the condition is known as hydrocephalus. The increase in pressure might result in headaches, nausea, vomiting, and tiredness. This can lead to meningitis when cysts break and leak their contents into the cerebrospinal fluid. Neurocysticercosis can be fatal if left untreated. Antiparasitic drugs, generally called antihelminthic drugs, such as albendazole or praziquantel are used to treat the infection. As the larvae die, corticosteroids are administered to minimize inflammation. In order to treat seizures, antiseizure medications are used. Hydrocephalus may require surgery to remove the extra CSF by inserting a shunt.

26.4.4.8 Piron Disease

Prion disease is an infectious disease caused by the presence of prions, which are generally misfolded versions of normal host proteins called "Prion" proteins. Animals and humans both are affected by this disease, commonly termed transmissible spongiform encephalopathies (TSEs). Prions, an infectious agent, cause them. Few examples of prion diseases in humans and animals are Creutzfeldt–Jakob disease (CJD) in humans, Bovine spongiform encephalopathy (BSE or "mad cow" disease) in cattle, chronic wasting disease (CWD) in deer, and scrapie in sheep. They cause prion disease by self-replicating, causing neuronal cell death, and transmitting themselves. A considerable portion of Prion protein is water-insoluble and resistant to protease breakdown, resulting in the slow but inevitable cellular buildup and neuronal cell death. No specific therapy has been shown to arrest the progression of prion disease.

26.4.4.9 Challenges in the Therapy of Brain Infections

Worldwide, CNS diseases are continuously a prime reason for death and disability for years. One of the major roadblocks in the cure of brain infections is its complex and sensitive nature (Ngowi et al. 2021). Other significant factors include infection management delays and shortages, vaccination distribution failures, the development of antibiotic resistance in microorganisms, and a lack of knowledge about pathophysiology.

26.5 Lack of Knowledge on Microbial Migration into the CNS

Only a restricted number of microbes can infect the CNS in humans. As a result, the underlying mechanisms which pathogens follow to invade BBB and BCSFB to infect the CNS and consequent disease manifestations are largely unresolved (Kim 2008; Zhang and Tuomanen 1999; Schwerk et al. 2015; Nassif et al. 2002). There are two main ways by which pathogens breach the BBB and BCSFB: transcellular and paracellular traverse. In the transcellular passage, the pathogen passes through the barrier cells or tight junctions by disrupting it, however, no sign of pathogen remains. On the other hand, in the case of paracellular traversal (also known as Trojan horse process), it passes through the barrier cells whether or not damaging the tight junction cells. In paracellular traversal, the infected phagocytes facilitate the entry of pathogen through BBB. The common pathogens of CNS like *L. monocytogenes, M. tuberculosis*, and HIV could also follow the Trojan horse mechanism (Kim 2008; Pulzova et al. 2009).

26.6 The Involvement of BBB and BCSFB in the Treatment of CNS Infections Using Antibiotics

Brain microvascular endothelial cells provide physical and functional barriers in the form of BBB and BCSFB. The diffusion of drugs through the BBB varies depending on whether or not inflammation is present. To achieve this, the antibiotics' pharmacokinetics and pharmacodynamics must be optimized. In a condition when there is no meningeal inflammation, the entry of antibiotic compounds into the cerebrospinal fluid and extracellular spaces of the brain depends on molecular size, ability to bind with plasma proteins, lipophilicity, and active transport. Compared to other medications, the antimicrobial compounds with lower molecular weight, for example, rifampin, fluoroquinolones, and sulfonamides, can better permeate the CSF. Hydrophobic medicines (fluoroquinolones, rifamycin, and chloramphenicol) can penetrate the BBB, while antimicrobials with hydrophilic nature (vancomycin and lactams) cannot. Blood–brain barrier (BBB) crossability is also affected by plasma protein binding. When the BBB/BCSFB remains intact, only a tiny amount of medication can traverse the BBB by binding with plasma proteins (primarily albumin and globulins). If there is an infection, the inflammatory responses are activated in response to it, increasing the protein concentration of the CSF. At the site of infection, this could result in decreased activity of highly protein-bound antibiotics. On the other hand, due to inflammation, the permeability of BBB/BCSFB increases, and proinflammatory cytokines can interfere with the active transport system, resulting in an increase in antibiotics traversal rate, regardless of their physicochemical features.

26.7 Antibiotics Resistance

Antibiotic resistance is on the rise among bacteria that cause CNS infections, posing a severe treatment challenge. Drug resistance is becoming more common among CNS diseases, and continuing surveillance of resistant bacteria should be encouraged, along with research into the development of novel medicines. As conventional antibiotic resistance increases, new targets for the management of brain infections must be identified especially in the case of bacterial infections.

26.8 Nanotechnological Strategy for the Management of Brain Infectious Diseases

Apart from the development of drugs, which directly target the disease-causing agents (antibiotics, antivirals, anti-parasites, etc.), the development of vaccines is another effective strategy to cope up with infectious diseases. Vaccination is one of the most straightforward and economic strategies that significantly decreased the morbidity and death associated with infectious illnesses. Lamentably, vaccines against numerous life-threatening pathogens, such as *Chlamydia* and *M. tuberculosis*, are still unavailable (Haque et al. 2018). For some diseases where inactivated or attenuated live pathogens are used for vaccination, safety concerns have been reported and recently produced subunit vaccines often have poor immunogenicity (Pardi et al. 2018). Thus novel, more effective, and safer strategies are required to deal with infectious disease.

Nanotechnology is the study and use of particles having dimensions in the nanoscale range (10^{-9} m) (Nalwa 1999; Parboosing et al. 2012). These particles are created by manipulating the surface of naturally occurring compounds, artificial chemicals, or metals (Ngowi et al. 2021). The study of the interplay of nanoscience with biological systems is referred to as "nanobiotechnology" (Scheller et al. 1995; Niemeyer and Mirkin 2004), while the related field of "nanomedicine" addresses the use of nanosized materials to detect, cure, and impede illnesses (Fig. 26.1) (Medepalli 2008). During the last two decades, nanobiotechnology has emerged as a potential strategy to overcome various shortcomings of antiviral medicines and antibiotics while also increasing their therapeutic effects (Milovanovic et al. 2017; Singh et al. 2017). In this regard, a wide range of nanoparticles has been investigated to advance the efficiency and diminish the adverse effects of different remedies to treat infectious diseases (Fig. 26.2) (Xiang et al. 2014). These milestones were achieved by increasing drug solubility/stability, extending the self-life in the bloodstream, conquering biological barriers, increasing bioavailability, targeting infection sites, and modifying drug discharge profiles in vivo (Zaidi et al. 2017; Gao et al. 2018; Zhou et al. 2018).

Hitherto, various types of nanoparticles possessing different physical and chemical characteristics have been produced, viz. metals and their oxides, liposomal, nanoemulsions, polymeric, chitosan, poly(butyl cyanoacrylate) (PBCA), fullerenes, polylactide-*co*-glycoside (PLGA), solid–lipid (SL), and others. Specially designed

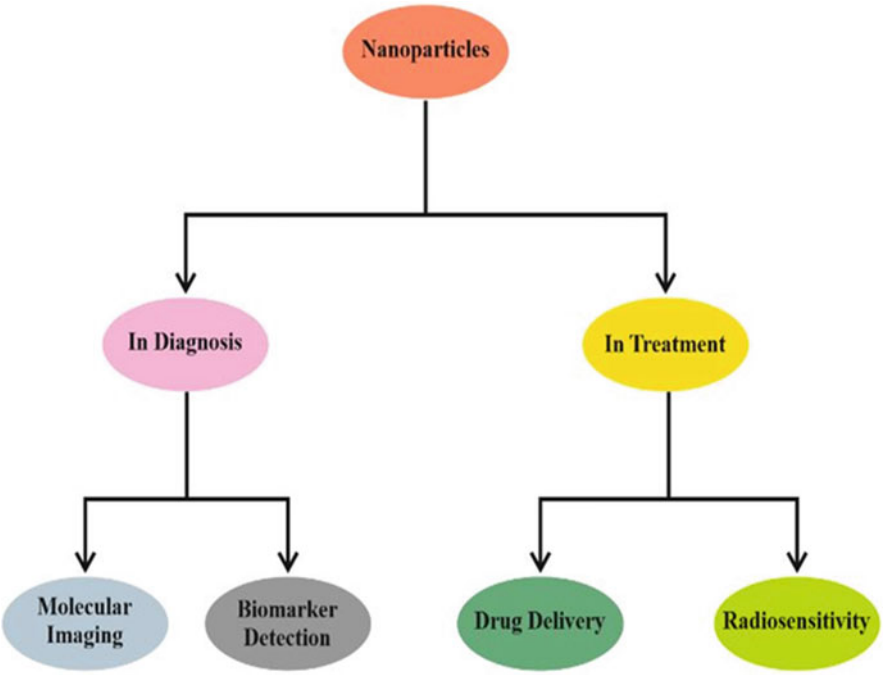

Fig. 26.1 The above diagram depicts the possible use of nanoparticles in brain therapy. Because of their great sensitivity, specificity, and capacity to pass the blood–brain barrier, nanoparticles can be utilized to diagnose and treat brain illnesses and disorders

nanoparticles loaded with probes, ligands, or drugs to serve different purposes were examined in several trials. Here we will discuss different applications of nanomaterials in the detection and treatment of brain infectious diseases.

26.9 Nanotechnology in the Diagnosis of Brain Infectious Diseases

Though the symptoms developed in the patients reveal a lot about the possible causative agents of the disease, however, prompt detection of the pathogen is always advantageous in the treatment of the disease as it allows appropriate administration of antibiotics (Zhang et al. 2017). The sample collection for the diagnosis of some diseases is painful and unsafe for example, currently, to detect meningitis, lumbar puncture or spinal tap is the gold standard method in which a small amount of CSF is collected with the help of injection to check the presence of virus or bacteria. Sample collection by this method can be followed by few complications like bleeding, infection, back pain, headache, and cerebral herniation, etc. (Engelborghs et al. 2017). Thus this method is considered unsafe. Other diagnostic methods like blood tests for infection indications, for example, C-reactive protein, blood count

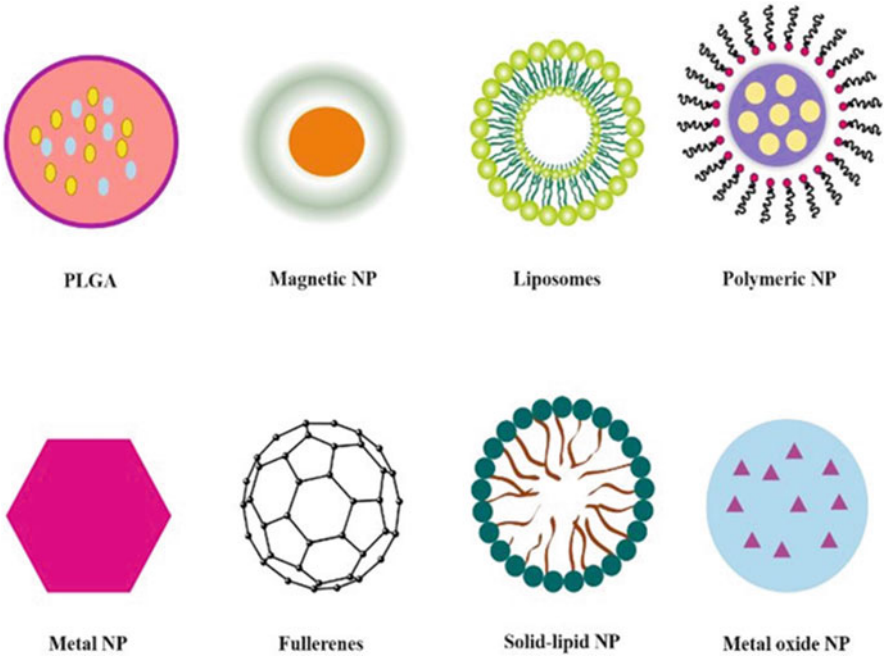

Fig. 26.2 This figures represent some of the common nanoparticles used in diagnostic and therapy, such as the polylactide-*co*-glycoside, polymeric, fullerenes, magnetic, solid-lipid, liposomes, metal, and metal oxide nanoparticles

(full), and culture of blood samples are also done. However, despite amazing effectiveness in preclinical testing and orthodox diagnosis, the vast majority of these diagnostic approaches have suffered during clinical use that could be due to the difficult characters of brain infections and diseases (Dahm et al. 2016). As a result, it is critical to reconsider the utility of traditional diagnostic and to develop new strategies. Because of innately advantageous properties like high surface-to-volume ratio, ease of surface amendments with desired probes, and ability to overcome biological barriers (like BBB), NPs are often regarded as potential materials for diagnosing brain infections (Fig. 26.3) (Hong et al. 2018; Selvan et al. 2019). In this part of the chapter, we will discuss the specifications of various nanomaterials for the diagnosis of brain infections (Fig. 26.4).

26.10 Carbon-Based Nanomaterials

Nanomaterials made up of carbon demonstrate great variation in their structure, morphology, physical characteristics, and chemical properties. Several carbon-derived nanomaterials such as graphene and its derivatives, carbon nano-onions, nanodiamonds, carbon nanotubes, carbon dots, nanohorns, fullerenes, etc. have

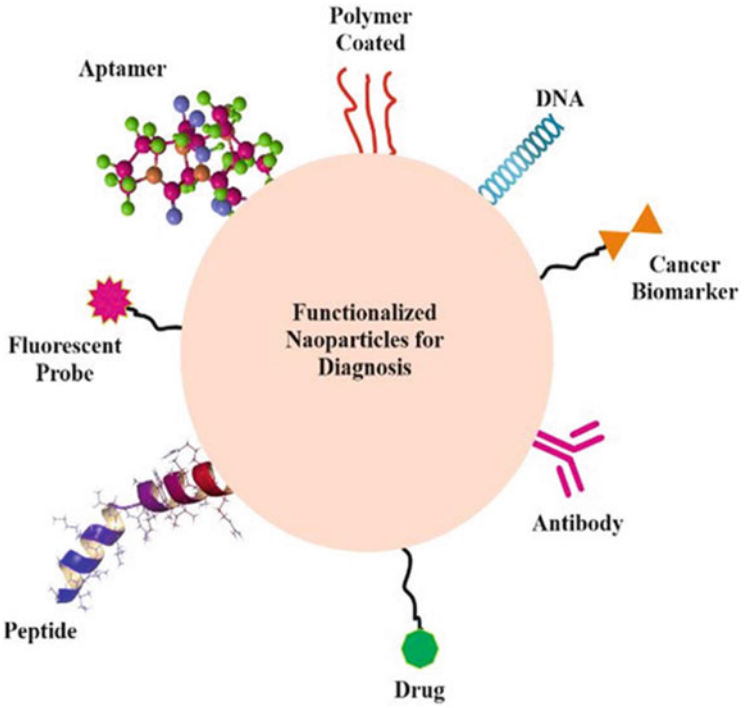

Fig. 26.3 A schematic illustration of multifunctional NPs used in the diagnosis of CNS infections

appeared as potential nanostructures for imaging, diagnosis, and treatment purposes. Since these are constituted of carbon, they exhibit significantly lower toxic potential than other nanomaterials which are synthesized from transition metal or silica (Sohaebuddin et al. 2010; Sharifi et al. 2012). Furthermore, their atypical physical characteristics and shapes exhibit diverse interplay characters inside the cell and tissue. Desired changes can also be made by both covalent and non-covalent modifications to alter their surface charges to tag fluorescent tags of interest (Bartelmess et al. 2015), molecules against cell- and disease-specific targets (Fabbro et al. 2012), contrast agents for magnetic resonance imaging (Hahn et al. 2011), drugs and nucleic acids as well (Bianco et al. 2005; Cheung et al. 2010). Ultimately, these nanomaterials are synthesized from cheap raw materials and involve simple steps making them cost-effective to produce at a large scale (De Volder et al. 2013). One derivative of carbon nanomaterial is graphene; a two-dimensional honeycomb nanomaterial made up of a single layer of sp2 bonded carbon atoms. In recent years it gained immense consideration in biosensing uses owing to its huge surface-to-volume ratio, and outstanding electrical qualities. Roberts et al. (2020) developed a novel, concise, and easy to use graphene field-effect transistor (GraFET) based highly sensitive biosensor consisting of carboxy functionalized graphene on Si/SiO_2 substrate covalently immobilized with monoclonal antibodies of Japanese

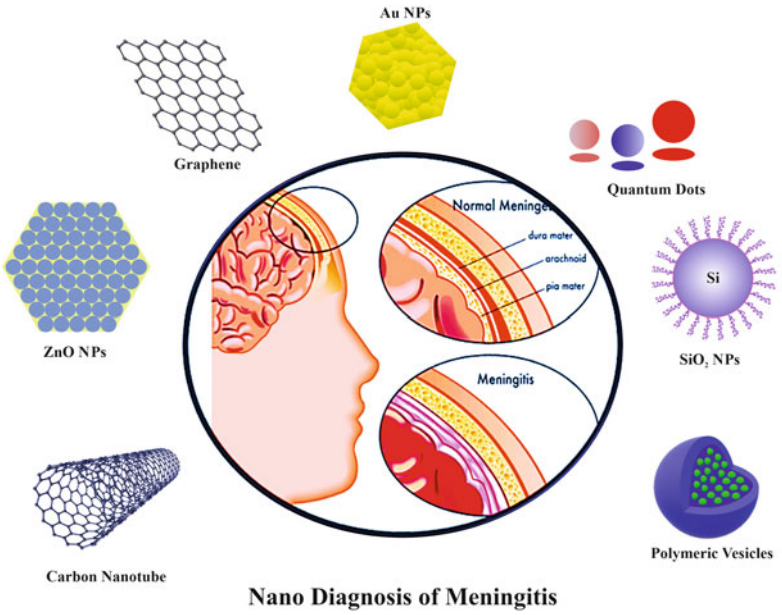

Fig. 26.4 Different nanoparticles for brain infection and meningitis detection

Encephalitis Virus (JEV) and Avian Influenza Virus (AIV) to diagnose the respective pathogens. Perumal et al. (2018) deposited gold nanorods on a 3D graphene nano platform through chemical vapor and employed them for detection of *M. tuberculosis*, a causative agent of tuberculous meningitis. This Au nanorod/3D graphene nanocomposite surprisingly detected TB DNA in a large range of 10 fM to 0.1 μM. Similarly, graphene oxide-based nanoparticles offer exceptional physicochemical properties and are currently widely used in medical diagnostic applications. Furthermore, the usage of graphene oxide has improved the sensitivity and accuracy of novel nanosensors dramatically. Dou et al. (2017) synthesized a microfluidic system by combining ssDNA probe-modified graphene oxide nanosensors and LAMP amplification in a single integrated system. Two bacterial pathogens, viz. *S. pneumoniae* and *N. meningitidis* causing bacterial meningitis were effectively identified using m-mqLAMP, with great accuracy.

26.11 Metals or Metal Oxides

Nanosized metal oxide-derived matrices have caught the attention as a platform for immobilizing a variety of biological molecules, like enzymes, antibodies, bacteria, DNA, and a variety of other proteins, in order to create novel and superior sensing devices, particularly electrochemical biosensors (Tyagi et al. 2013; Yin et al. 2013; Mielecki et al. 2013; Li et al. 2013). For the manufacture of electrochemical

biosensors, a variety of nanostructured metal oxides have been investigated, which includes the oxides of iron (Fe), zinc (Zn), nickel (Ni), tin (Sn), zirconium (Zr), cerium (Ce), and others for different diseases (Zhang et al. 2009; Bai et al. 2010; Das et al. 2011; Mohan et al. 2011). To this purpose, Tak et al. (2014) synthesized ZnO nanostructures by simple hydrothermal technique and immobilize single-strands of thiolated probe DNA for the proficient diagnosis of meningitis caused by *N. meningitides*. Au NPs are widely used for diagnostic and drug delivery purposes because they are inactive, their structure and size can efficiently be regulated by a variety of processes, its easy to modify and activate their surfaces, preparation of stable colloidal forms is simple, and they exhibit minimal cytotoxicity (Draz and Shafiee 2018; Zong et al. 2017). Cheng and colleagues demonstrated that gold nanoparticles containing a transactivator of transcription (TAT) peptide on the surface may be applied to deliver doxorubicin and a contrast agent (gadolinium: Gd3+) to the CNS (Cheng et al. 2014). It has also been reported that Au NPs could freely cross the BBB. Furthermore, AuNPs may be identified non-invasively by Computed Tomography imaging, making them a viable diagnostic tool for brain imaging (Rizvi et al. 2018). Patel and colleagues previously developed an effective electrochemical biosensor to diagnose meningitis that used thin Au films as platforms to immobilize probes (Patel et al. 2009, 2010).

26.12 Polymeric Nanostructures

Polymeric nanostructures can be used to design biosensors against infectious agents of various kinds. The easiness to tailor the properties of these nanostructures according to diverse conditions could significantly increase the responsiveness and efficacy of sensing systems (El Idrissi et al. 2018). Tiwari et al. (2007) coupled antibodies against glycolipid (produced in rabbit and purified by affinity chromatography) to liposome NPs (200–400 nm) along with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride and *N*-hydroxysuccinimide to detect tuberculosis pathogen. To detect the same microbe, Tiwari et al. (2017) developed a rapid and efficient liposome nanosensor by incorporating polyclonal antibodies against TB antigens which exhibited 97.5 and 95.8% sensitivity and specificity, respectively.

26.13 Quantum Dots

Quantum dots (QDs) are a type of nanoparticle that is normally inorganic semiconductor in nature (except carbon QDs) and has a size of fewer than 50 nm. These minute crystals can transport electrons and emit light of different colors when exposed to UV light. Their interesting optical and electronic characteristics, along with a greater degree of brightness and photostability, cause them an obvious alternative for molecular and cellular imaging analyses in biomedical science (Gao et al. 2005). QDs possess a vast surface area which facilitates the binding of a variety

of agents for detection and treatment purposes. Surface-activated QD with antibodies, proteins, and peptides can be directed to selective tissue sites. Though the QD mediated cytotoxicity is controversial, polymer encapsulated QDs have shown to be effective in reducing the toxicity potential of QDs in animal cells and tissues (Mukherjee et al. 2016). QDs prepared from various substances have been synthesized to diagnose infectious agents. Safardoust-Hojaghan et al. (2017) employed the hydrothermal technique to generate graphene QDs with the help of citric acid ($C_6H_8O_7$) and ethylenediamine ($C_2H_8N_2$) for the identification of *S. aureus* and *E. coli*, which was sensitive for a lower number of bacteria. Electrochemical detection of DNA has sparked a lot of attention because of its unique characteristics, such as greater sensitivity, specificity, and stability, together with its cost-effectiveness and quick analysis time (Mateo-Martí et al. 2007; Chen et al. 2008; Gupta et al. 2021). Recently, Gupta et al. (2021) developed a lab on genochip based analytical device embedded with tungsten disulfide QDs for specific identification of DNA of meningitis causing agent.

26.14 Nanoparticles in the Treatment of Brain Infections

26.14.1 Polymeric Nanoparticles

Poly(lactic-*co*-glycolic acid) nanoparticles (PLGA) are among the highly promising nanoparticle formulations to treat CNS infections. PLGA have been cleared for human use by the FDA since 1989. Because PLGA NPs cannot traverse the BBB, their utility in CNS diseases is limited despite their advantages. To transfer PLGA NPs to the brain, various methods have been developed in recent years. Among the highly potential candidate biomolecules for delivery of therapeutics into the brain is cell-penetrating peptide (CPP) (Zou et al. 2013; Kreuter 2014; Sharma et al. 2016). CPPs are positively charged biomolecules at physiological pH with diverse amino acid sequences and sizes. CPPs can carry a vast array of therapeutic compounds like proteins, nucleic acids, small molecules, and nanoparticles, which can be utilized to treat diseases of CNS. First CPPs utilized for this purpose include TAT, a glycoprotein of Rabies virus, Penetratin, Angiopep, and the synthetic peptide family (Qin et al. 2011).

A significant study by Qin et al. (2011) focusing on the transport of TAT-mediated clinically useful liposomes and nanoparticles in vivo, when administered through the tail vein, TAT-modified liposome (TAT-LIP) augmented in the brain after 24 h, despite the fact that not all formulations were brain-targeted. In other investigations, coupled nanocarrier–TAT peptide systems were used to generate a stable medication with increased CNS penetration and decreased adverse effects. Liu et al. (2008) reported that CPPs could be employed to improve the transportation of small compounds across the BBB. TAT-modified micelles were utilized to increase the administration of ciprofloxacin, a popular antibiotic used to treat brain infections. Similar results were obtained by conjugating ritonavir nanoformulation with TAT peptide to increase its CSF concentration (Borgmann

et al. 2011). Additionally, conjugating pharmaceutics to SynB family peptides, which are cationic CPPs, boosted their absorption in the brain and in vivo activity by the adsorptive-mediated transcytosis method (Adenot et al. 2007). It was found by Rousselle et al. (2003) that intravenously injected medicines conjugated to SynB1 or SynB3 dramatically boosted the brain penetration of medications, for instance, benzylpenicillin, doxorubicin, and dalargin. An additional investigation by Adenot et al. (2007) demonstrated that the coupling of SynB3 with benzylpenicillin enhanced the drug's brain penetration without interfering with the tight junction integrity. Besides TAT and SynB peptides, CPPs have been effectively used to transport drugs to the brain. Dendrimers loaded with Amphotericin B (AmB) and Angiopep-2 can be used as a delivery mechanism for treating CNS yeast infections.

26.14.2 Polymeric Micelles

Polymeric micelles (PMs) made up of cholesterol-coupled PEG, attached with transcript or activator TAT peptide, have also been found to be effective at transporting antibiotics through BBB. Micelles were spherical, with a diameter of fewer than 180 nm. In their study, Liu et al. (2008) found that micelles of TAT-PEG-b-Col showed prolonged antibacterial action against *Bacillus subtilis* and *E. coli*, additionally, these NPs existed in the nucleus of neurons after traversing the blood–brain barrier. Owing to this study, nano-delivery methods for treating brain infections can be developed using these micelles. When individual polymer chains are directly mixed in an aqueous solution above critical micelle concentration and critical micelle temperature, nanosized aggregates are formed by spontaneous self-assembly producing polymer micelles. Amphiphilic polymers having limited solubility in aqueous solutions can be solubilized in a volatile organic solvent followed by dialysis against a suitable buffer. For medication delivery, amphiphilic di- or tri-block copolymers with hydrophilic and hydrophobic blocks are most typically utilized. Unimers must be biodegradable and/or should have a low molecular mass (40 kDa) so that they can be cleared from the body by the renal system to avoid the accumulation of polymer in the body and minimize toxicity. The extremely advanced amphiphilic block copolymers are spherical core-shell micelles with a diameter of 10–80 nm. These consist of a hydrophobic core to load drugs and a hydrophilic shell, which serve as a physical barrier to prevent aggregation of micelles, binding with proteins, and opsonization after systemic delivery. FDA-approved excipient poly(ethylene glycol), or poly(ethylene oxide), is the most frequent hydrophilic block utilized to build the hydrophilic shell (PEO). They are both made up of repeating monomer component CH_2CH_2O and can have a variety of end groups depending on how they were synthesized.

Unlike self-assembled micelles, unimolecular micelles are composed of single polymer molecules that are covalently bonded to amphiphile chains. It is common to employ dendrimers as building blocks for unimolecular micelles because of their high branching density and ability to form well-defined globules with well-controlled surface functionality Drug molecules can be trapped in dendritic cores.

By adding a hydrophobic block to the dendrimer core, the carrying capability of the dendrimer can be increased. Researchers Wang et al. (2010) created an amphiphilic 16-arm star polymer with an inner poly(caprolactone) block and an outside poly (ethylene oxide) block, with a polyamidoamine dendrimer core. These unimolecular micelles exhibited a high loading capacity for encapsulating a hydrophobic medication named etoposide.

26.14.3 Cationic Antimicrobial Peptides

When compared to conventional antimicrobial drugs, which target either bacteria or fungi, antimicrobial peptides (AMPs) have a wider range of antimicrobial activity, including bacteria, fungi, and parasites as well as enveloped viruses and cancer cells. "Innate" host resistance relies heavily on these AMPs as their first line of defense against pathogens. Because of this, the advancement of AMPs for the cure of drug-resistant illnesses has attracted considerable attention. AMPs have also been linked to hemolysis and allergic reactions in high doses. Cationic AMPs have been enticed as novel therapies with the potential to cure multidrug-resistant diseases. Cholesterol-conjugated G3R6TAT (CG3R6TAT) self-assembled into cationic nanoparticles, which displayed excellent antibacterial activity in vitro against various microorganisms. Over the past few years, attention has been drawn to antimicrobial peptides that are cationic attributed to their wide range of activity and capacity to battle multidrug-resistant bacteria (Hancock and Sahl 2006). In an investigation, cationic peptide was added to the nanoparticles to make them more effective. It exhibited greater effect in comparison to traditional antibiotics, for instance, wider antimicrobial action, and greater lethality against bacteria and fungi. The authors also discovered that nanoparticles crossed the BBB successfully and targeted the pathogens as effectively as vancomycin. Similar cationic nanoparticles with TAT–cholesterol–G_3 R_6 formulations were found to penetrate the BBB and be effective against *C. neoformans* and *C. albicans* meningitis infections in rabbits.

26.14.4 Liposomes

Because of their unique qualities, including biocompatibility and biodegradability, LPs emerge as a potential system for drug administration because of their ability to shield their cargo from enzyme breakdown phenomena as well as their low toxicity and flexibility. As a result of their limited shelf life and drug loading efficiency, LPs are not ideal for specific therapeutic applications. Several efforts have been undertaken in recent decades toward the ingegnerization of optimum LPs, and new kinds of liposomal carriers have been developed. Liposomal formulations have been utilized to advance the pharmacokinetics of hydrophobic and hydrophilic drugs. Using liposomal formulations that target endothelial cells' transferrin and insulin receptors, medications could be delivered to the brain more efficiently and

effectively. Rat's carotid arteries with Amphotericin B (AmB)-loaded magnetic liposomes showed that this formulation reduced AmB's harmful effects and increased its concentration in the brain.

Fusogenic liposomes are very sensitive to pH and possess a high capability to fuse with the membranes of bacterial cells and induce the release of drugs intracellularly. Furthermore, cell-penetrating peptides can enhance this potential, for example, Tat47–57 (HIV Tat protein-derived peptide). Bartomeu Garcia et al. (2017) designed spherical fusogenic liposomes of 100 nm diameter and loaded with antibiotics such as vancomycin, methicillin, and ampicillin to target *S. pneumoniae*, methicillin-resistant *S. aureus*, and *E. coli* and tagged the nanoparticles with cell-penetrating peptides (Tat47–57). When all these bacteria were treated with these novel liposomes under in vitro conditions, the antibiotic minimum inhibitory concentration decreased dramatically, especially for the liposome loaded with methicillin which completely eradicated the bacterial population at a concentration of 1.7–3 μg/mL (Bartomeu Garcia et al. 2017).

26.14.5 Solid Lipid Nanoparticles

Solid lipid nanoparticles (SLN) have emerged as a viable alternative to conventional lipid particles (LPs) due to their numerous advantages, including regulated and targeted drug release, high stability, and efficient encapsulation efficiency of medicines. To internalize lipophilic and hydrophilic molecules into the SLNs structure there is a lipid matrix (tri-di- and mono-glycerides, fat acids, steroids, or waxes) that defines an aqueous volume. Certain medications could leak out of the SLN shell, which is composed of a single fat, during transit. As a result, the SLN architecture compromises delivery efficacy results. Scientists are using SLNs as a medicine delivery mechanism in the fight against infectious brain disorders. When loaded with antibiotics, SLNs can bypass the P-gp efflux pump and diffuse across the BBB, according to a recent study (Kalhapure et al. 2015). According to tissue distribution experiments, tobramycin (an aminoglycoside antibiotic) successfully cross the BBB by employing SLNs for drug administration (Bargoni et al. 2001). It has been shown that SLNs can carry antiretroviral drugs to the CNS. For antiretroviral drug delivery, dendrimers (a kind of SLN) have been studied. In a recent study, lamivudine-loaded polyamidoamine dendrimers were evaluated in vitro for antiviral activity. The cellular absorption of lamivudine was increased compared to the control group, and viral p24 levels were lowered (Dutta and Jain 2007; Salouti and Ahangari 2014). In spite of these promising outcomes, dendrimers are still in the early stages of development for their applications in brain diseases (Ekizoğlu 2017).

26.14.6 Cell-Mediated Drug Delivery

Targeted cell-based delivery is another option for transporting drug-loaded nanoparticles over biologic barriers (BBB). The chemotactic and phagocytic

properties of mononuclear phagocytes can be used to transport medications to CNS infection sites and other difficult-to-access regions, like lymphoid tissue (MPs) (Wagner et al. 2006; Batrakova et al. 2011). Cell-based drug release systems must deliver an adequate amount of therapeutically active medication to the infection site. To accomplish site-specific drug delivery, the drug must be confined in stable and non-degrading subcellular compartments, which facilitate the swift release of drugs after reaching inside the cell. Because they reduce acidity in lysosomal compartments, cationic NPs are less prone to be degraded in comparison to anionic NPs (Batrakova et al. 2011). Immunocytes, for instance, dendritic cells, monocytes, and macrophages, neutrophils, and lymphocytes are dynamic cells; they can easily traverse the BBB and discharge their cargo at the site of infection and tissue damage. These immune cells can thus be utilized as drug delivery Trojan horses. Thus the application of immune cells as a drug delivery system could be beneficial in several ways, for example, site-specific drug transport, enhance self-life in blood circulation, and minimal or no toxicities to cells and tissues. Attempts have been made to treat the HIV infections of CNS by developing formulations with cell-mediated drug delivery. Researchers have proposed the idea of employing MPs to deliver nanoantiretroviral drugs to raise levels of circulating drugs at specific regions of HIV replication, such as the CNS. As a result of the administration of chloroquine-loaded liposomes, fungal load in the brain was significantly reduced, and macrophage antifungal activity was increased, even when low dosages of this formulation were compared to the free drug at large doses. Amphotericin B (AmB), a water-insoluble chemical, can also be transported by this approach, highlighting the need for further study. Supplemental therapy is widely applied in conjunction with antimicrobials to minimize the inflammatory processes that participate in CNS injury and long-term deficits linked to CNS infectious diseases.

References

Abdel Razek AA, Watcharakorn A, Castillo M (2011) Parasitic diseases of the central nervous system. Neuroimaging Clin N Am 21(4):815–841

Adenot M, Merida P, Lahana R (2007) Applications of a blood-brain barrier technology platform to predict CNS penetration of various chemotherapeutic agents. 2. Cationic peptide vectors for brain delivery. Chemotherapy 53(1):73–76

Bai YH, Li JY, Xu JJ, Chen HY (2010) Ultrasensitive electrochemical detection of DNA hybridization using Au/Fe3O4 magnetic composites combined with silver enhancement. Analyst 135(7):1672–1679

Baker LG, Specht CA, Donlin MJ, Lodge JK (2007) Chitosan, the deacetylated form of chitin, is necessary for cell wall integrity in *Cryptococcus neoformans*. Eukaryot Cell 6(5):855–867

Bargoni A, Cavalli R, Zara GP, Fundarò A, Caputo O, Gasco MR (2001) Transmucosal transport of tobramycin incorporated in solid lipid nanoparticles (SLN) after duodenal administration to rats. Part II—tissue distribution. Pharm Res 43(5):497–502

Bariola JR, Perry P, Pappas PG, Proia L, Shealey W, Wright PW et al (2010) Blastomycosis of the central nervous system: a multicenter review of diagnosis and treatment in the modern era. Clin Infect Dis 50(6):797–804

Bartelmess J, Quinn SJ, Giordani S (2015) Carbon nanomaterials: multi-functional agents for biomedical fluorescence and Raman imaging. Chem Soc Rev 44:4672–4698

Bartomeu Garcia C, Shi D, Webster TJ (2017) Tat-functionalized liposomes for the treatment of meningitis: an in vitro study. Int J Nanomedicine 12:3009–3021
Batrakova EV, Gendelman HE, Kabanov AV (2011) Cell-mediated drug delivery. Expert Opin Drug Deliv 8(4):415–433
Bianco A, Kostarelos K, Prato M (2005) Applications of carbon nanotubes in drug delivery. Curr Opin Chem Biol 9:674–679
Borchorst S, Møller K (2012) The role of dexamethasone in the treatment of bacterial meningitis—a systematic review. Acta Anaesthesiol Scand 56(10):1210–1221
Borgmann K, Rao KS, Labhasetwar V, Ghorpade A (2011) Efficacy of Tat-conjugated ritonavir-loaded nanoparticles in reducing HIV-1 replication in monocyte-derived macrophages and cytocompatibility with macrophages and human neurons. AIDS Res Hum Retrovir 27 (8):853–862
Bryan AM, Del Poeta M, Luberto C (2015) Sphingolipids as regulators of the phagocytic response to fungal infections. Mediat Inflamm 2015:640540
Carpio A, Romo ML, Parkhouse RM, Short B, Dua T (2016) Parasitic diseases of the central nervous system: lessons for clinicians and policy makers. Expert Rev Neurother 16(4):401–414
Chen J, Zhang J, Huang L, Lin X, Chen G (2008) Hybridization biosensor using 2-nitroacridone as electrochemical indicator for detection of short DNA species of chronic myelogenous leukemia. Biosens Bioelectron 24(3):349–355
Cheng Y, Dai Q, Morshed RA, Fan X, Wegscheid ML, Wainwright DA, Han Y, Zhang L, Auffinger B, Tobias AL, Rincón E, Thaci B, Ahmed AU, Warnke PC, He C, Lesniak MS (2014) Blood-brain barrier permeable gold nanoparticles: an efficient delivery platform for enhanced malignant glioma therapy and imaging. Small 10(24):5137–5150
Cheung W, Pontoriero F, Taratula O, Chen AM, He H (2010) DNA and carbon nanotubes as medicine. Adv Drug Deliv Rev 62:633–649
Coureuil M, Lécuyer H, Bourdoulous S, Nassif X (2017) A journey into the brain: insight into how bacterial pathogens cross blood-brain barriers. Nat Rev Microbiol 15(3):149–159
Dahm T, Rudolph H, Schwerk C, Schroten H, Tenenbaum T (2016) Neuroinvasion and inflammation in viral central nervous system infections. Mediat Inflamm 2016:8562805
Das M, Dhand C, Sumana G, Srivastava AK, Nagarajan R, Nain L, Iwamoto M, Manaka T, Malhotra BD (2011) Electrophoretic fabrication of chitosan-zirconium-oxide nanobiocomposite platform for nucleic acid detection. Biomacromolecules 12(3):540–547
DeVolder I, Richman L, Conrad AL, Magnotta V, Nopoulos P (2013) Abnormal cerebellar structure is dependent on phenotype of isolated cleft of the lip and/or palate. Cerebellum 12 (2):236–244
De Rycker M, Baragana B, Duce SL, Gilbert IH (2018) Challenges and recent progress in drug discovery for tropical diseases. Nature 559(7715):498–506
Dickey SW, Cheung GYC, Otto M (2017) Different drugs for bad bugs: antivirulence strategies in the age of antibiotic resistance. Nat Rev Drug Discov 16(7):457–471
Dou M, Sanjay ST, Dominguez DC, Zhan S, Li X (2017) A paper/polymer hybrid CD-like microfluidic SpinChip integrated with DNA-functionalized graphene oxide nanosensors for multiplex qLAMP detection. Chem Commun 53:10886–10889
Draz MS, Shafiee H (2018) Applications of gold nanoparticles in virus detection. Theranostics 8: 1985–2017
Dutta T, Jain NK (2007) Targeting potential and anti-HIV activity of lamivudine loaded mannosylated poly (propyleneimine) dendrimer. Biochim Biophys Acta 1770(4):681–686
Ekizoğlu M (2017) Infectious diseases of the brain. In: Nanotechnology methods for neurological diseases and brain tumors. Elsevier Science, San Diego, pp 291–315
El Idrissi M, Meyer CE, Zartner L, Meier WP (2018) Nanosensors based on polymer vesicles and planar membranes: a short review. J Nanobiotechnol 16:1–14
Engelborghs S, Niemantsverdriet E, Struyfs H, Blennow K, Brouns R, Comabella M, Dujmovic I, Van Der Flier W, Frölich L, Galimberti D et al (2017) Consensus guidelines for lumbar puncture in patients with neurological diseases. Alzheimers Dement (Amst) 8:111–126

Fabbro A, Villari A, Laishram J, Scaini D, Toma FM, Turco A, Prato M, Ballerini L (2012) Spinal cord explants use carbon nanotube interfaces to enhance neurite outgrowth and to fortify synaptic inputs. ACS Nano 6(3):2041–2055

Gao X, Yang L, Petros JA, Marshall FF et al (2005) In vivo molecular and cellular imaging with quantum dots. Curr Opin Biotechnol 16:63–72

Gao F, Jiang X, Li Q, Fan K (2018) Association of miR-182 expression in amniotic fluid with intrauterine infection and brain injury in premature infants. Exp Ther Med 16(3):1905–1909

Garcia RR, Min Z, Narasimhan S, Bhanot N (2015) Fusarium brain abscess: case report and literature review. Mycoses 58(1):22–26

Gottfredsson M, Perfect JR (2000) Fungal meningitis. Semin Neurol 20(3):307–322

Granerod J, Ambrose HE, Davies NW, Clewley JP, Walsh AL, Morgan D et al (2010) Causes of encephalitis and differences in their clinical presentations in England: a multicentre, population-based prospective study. Lancet Infect Dis 10(12):835–844

Gupta J, Chakrabarty B, Singh G, Singh S, Kumar A, Xess I, Jauhari P, Gulati S (2021) A rare infective cause of stroke in an immunocompetent child. Brain and Development 43(1):152–156

Hahn MA, Singh AK, Sharma P, Brown SC, Moudgil BM (2011) Nanoparticles as contrast agents for in-vivo bioimaging: current status and future perspectives. Anal Bioanal Chem 399:3–27

Hancock RE, Sahl H-G (2006) Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. Nat Biotechnol 24(12):1551–1557

Haque A, Akcesme FB, Pant AB (2018) A review of zika virus: hurdles toward vaccine development and the way forward. Antivir Ther 23(4):285–293

Honda H, Warren DK (2009) Central nervous system infections: meningitis and brain abscess. Infect Dis Clin N Am 23(3):609–623

Hong W, Zhang Z, Liu L, Zhao Y, Zhang D, Liu M (2018) Brain-targeted delivery of PEGylated nano-bacitracin A against Penicillin-sensitive and -resistant Pneumococcal meningitis: formulated with RVG29 and Pluronic® P85 unimers. Drug Deliv 25:1886–1897

Kalhapure RS, Suleman N, Mocktar C, Seedat N, Govender T (2015) Nanoengineered drug delivery systems for enhancing antibiotic therapy. J Pharm Sci 104(3):872–905

Karampekios S, Hesselink J (2005) Cerebral infections. Eur Radiol 15:485–493

Kaufmann SHE, Dorhoi A, Hotchkiss RS, Bartenschlager R (2018) Host-directed therapies for bacterial and viral infections. Nat Rev Drug Discov 17(1):35–56

Kaya RA, Türkmenoglu ON, Çolak I, Aydin Y (2008) Brain abscess: analysis of results in a series of 51 patients with a combined surgical and medical approach during an 11-year period. Neurosurg Focus 24(6):E9

Kim KS (2008) Mechanisms of microbial traversal of the blood–brain barrier. Nat Rev Microbiol 6(8):625–634

Kleinschmidt-DeMasters BK (2002) Central nervous system aspergillosis: a 20-year retrospective series. Hum Pathol 33(1):116–124

Koeller KK, Shih RY (2017) Viral and prion infections of the central nervous system: radiologic-pathologic correlation: from the radiologic pathology archives. RadioGraphics 37(1):199–233

Köhler JR, Casadevall A, Perfect J (2014) The spectrum of fungi that infects humans. Cold Spring Harb Perspect Med 5(1):a019273

Kreuter J (2014) Drug delivery to the central nervous system by polymeric nanoparticles: what do we know? Adv Drug Deliv Rev 71:2–14

Le Moal G, Landron C, Grollier G, Bataille B, Roblot F, Nassans P et al (2003) Characteristics of brain abscess with isolation of anaerobic bacteria. Scand J Infect Dis 35(5):318–321

Li J, Li L, Wu F, Zhang L, Liu X (2013) Dispersion–precipitation synthesis of nanorod Mn3O4 with high reducibility and the catalytic complete oxidation of air pollutants. Catal Commun 31: 52–56

Liechti FD, Grandgirard D, Leib SL (2015) Bacterial meningitis: insights into pathogenesis and evaluation of new treatment options: a perspective from experimental studies. Future Microbiol 10(7):1195–1213

Liu L, Guo K, Lu J, Venkatraman SS, Luo D, Ng KC et al (2008) Biologically active core/shell nanoparticles self-assembled from cholesterol-terminated PEG–TAT for drug delivery across the blood–brain barrier. Biomaterials 29(10):1509–1517
Liu TB, Perlin DS, Xue C (2012) Molecular mechanisms of cryptococcal meningitis. Virulence 3 (2):173–181
Mailles A, Stahl JP (2009) Infectious encephalitis in France in 2007: a national prospective study. Clin Infect Dis 49:1838–1847
Mateo-Martí E, Briones C, Pradier CM, Martín-Gago JA (2007) A DNA biosensor based on peptide nucleic acids on gold surfaces. Biosens Bioelectron 22(9–10):1926–1932
McGill F, Heyderman RS, Panagiotou S, Tunkel AR, Solomon T (2017) Acute bacterial meningitis in adults. Lancet 388(10063):3036–3047
Medepalli KK (2008) Advanced nanomaterials for biomedical applications. ProQuest, Cambridge
Metcalf CJE, Lessler J (2017) Opportunities and challenges in modeling emerging infectious diseases. Science 357(6347):149–152
Meylan S, Andrews IW, Collins JJ (2018) Targeting antibiotic tolerance, pathogen by pathogen. Cell 172(6):1228–1238
Mielecki M, Wojtasik J, Zborowska M, Kurzątkowska K, Grzelak K, Dehaen W et al (2013) Oriented immobilization of His-tagged kinase RIO1 protein on redox active N-(IDA-like)-Cu (II) monolayer deposited on gold electrode—the base of electrochemical biosensor. Electrochim Acta 96:147–154
Milovanovic M, Arsenijevic A, Milovanovic J, Kanjevac T, Arsenijevic N (2017) Chapter 14—Nanoparticles in antiviral therapy. In: Grumezescu AM (ed) Antimicrobial nanoarchitectonics. Elsevier, pp 383–410
Mohan S, Srivastava P, Maheshwari SN, Sundar S, Prakash R (2011) Nano-structured nickel oxide based DNA biosensor for detection of visceral leishmaniasis (Kala-azar). Analyst 136 (13):2845–2851
Mukherjee A, Shim Y, Myong Song J (2016) Quantum dot as probe for disease diagnosis and monitoring. Biotechnol J 11(1):31–42
Murthy JM (2007) Fungal infections of the central nervous system: the clinical syndromes. Neurol India 55(3):221–225
Muzumdar D, Jhawar S, Goel A (2011) Brain abscess: an overview. Int J Surg 9(2):136–144
Nalwa HS (1999) Handbook of nanostructured materials and nanotechnology, vol 1. Academic, San Diego, pp 1–3
Nassif X, Bourdoulous S, Eugène E, Couraud P-O (2002) How do extracellular pathogens cross the blood–brain barrier? Trends Microbiol 10(5):227–232
Nau R, Soto A, Brück W (1999) Apoptosis of neurons in the dentate gyrus in humans suffering from bacterial meningitis. J Neuropathol Exp Neurol 58(3):265–274
Ngowi EE, Wang YZ, Qian L, Helmy Y, Anyomi B, Li T, Zheng M, Jiang ES, Duan SF, Wei JS, Wu DD, Ji XY (2021) The application of nanotechnology for the diagnosis and treatment of brain diseases and disorders. Front Bioeng Biotechnol 2(9):629832
Niemeyer CM, Mirkin CA (2004) Nanobiotechnology: concepts, applications and perspectives, vol 1. Wiley, Weinheim
Ørsted I, Gertsen J, Schønheyder H, Jensen J, Nielsen H (2011) Mycoplasma salivarium isolated from brain abscesses. Clin Microbiol Infect 17(7):1047–1049
Parboosing R, Maguire GE, Govender P et al (2012) Nanotechnology and the treatment of HIV infection. Viruses 4:488–520
Pardi N, Hogan MJ, Porter FW, Weissman D (2018) mRNA vaccines—a new era in vaccinology. Nat Rev Drug Discov 17(4):261–279
Patel K, Clifford DB (2014) Bacterial brain abscess. Neurohospitalist 4(4):196–204
Patel MK, Solanki PR, Seth S, Gupta S, Khare S, Kumar A, Malhotra BD (2009) CtrA gene based electrochemical DNA sensor for detection of meningitis. Electrochem Commun 11(5):969–973
Patel MK, Solanki PR, Kumar A, Khare S, Gupta S, Malhotra BD (2010) Electrochemical DNA sensor for Neisseria meningitidis detection. Biosens Bioelectron 25(12):2586–2591

Pedroso VS, Vilela MC, Santos PC, Cisalpino PS, Rachid MA, Teixeira AL (2013) Traffic of leukocytes and cytokine upregulation in the central nervous system in a murine model of neuroparacoccidioidomycosis. Mycopathologia 176(3–4):191–199

Perumal V, Saheed MSM, Mohamed NM, Murthe SS, Gopinath SC, Chiu J-M (2018) Gold nanorod embedded novel 3D graphene nanocomposite for selective bio-capture in rapid detection of Mycobacterium tuberculosis. Biosens Bioelectron 116:116–122

Pulzova L, Bhide MR, Andrej K (2009) Pathogen translocation across the blood-brain barrier. FEMS Immunol Med Microbiol 57(3):203–213

Qin Y, Chen H, Yuan W, Kuai R, Zhang Q, Xie F et al (2011) Liposome formulated with TAT-modified cholesterol for enhancing the brain delivery. Int J Pharm 419(1):85–95

Raoultm D, Al Masalma M, Armougom F, Scheld WM, Dufour H, Roche P-H et al (2009) The expansion of the microbiological spectrum of brain abscesses with use of multiple 16S ribosomal DNA sequencing. Clin Infect Dis 48(9):1169–1178

Ribes S, Djukic M, Eiffert H (2014) Strategies to increase the activity of microglia as efficient protectors of the brain against infections. Front Cell Neurosci 8:138

Rizvi SMD, Hussain T, Ahmed ABF, Alshammari TM, Moin A, Ahmed MQ, Barreto GE, Kamal MA, Ashraf GM (2018) Gold nanoparticles: a plausible tool to combat neurological bacterial infections in humans. Biomed Pharmacother 107:7–18

Roberts F, Hobbs H, Jessop H, Bozzolini C, Burman J, Greco R, Ismail A, Kazmi M, Kirgizov K, Mancardi G, Mawson S, Muraro PA, Puyade M, Saccardi R, Withers B, Verhoeven B, Sharrack B, Snowden JA (2020) Rehabilitation before and after autologous haematopoietic stem cell transplantation (AHSCT) for patients with multiple sclerosis (MS): consensus guidelines and recommendations for best clinical practice on behalf of the autoimmune diseases working party, nurses group, and patient advocacy committee of the European Society for Blood and Marrow Transplantation (EBMT). Front Neurol 11:556141

Rousselle C, Clair P, Smirnova M, Kolesnikov Y, Pasternak GW, Gac-Breton S et al (2003) Improved brain uptake and pharmacological activity of dalargin using a peptide-vector-mediated strategy. J Pharmacol Exp Ther 306(1):371–376

Safardoust-Hojaghan H, Salavati-Niasari M, Amiri O, Hassanpour M (2017) Preparation of highly luminescent nitrogen doped graphene quantum dots and their application as a probe for detection of Staphylococcus aureus and E. coli. J Mol Liq 241:1114–1119

Salouti M, Ahangari A (2014) Nanoparticle based drug delivery systems for treatment of infectious diseases. In: Application of nanotechnology in drug delivery. InTech, pp 155–192

Scheller FW, Bier FF, Pfeiffer D (1995) Biosensoren: grundlagen und anwendungen. TM-Tech Mess 62:213–219

Schwerk C, Tenenbaum T, Kim KS, Schroten H (2015) The choroid plexus—a multi-role player during infectious diseases of the CNS. Front Cell Neurosci 9:80

Scully EP, Baden LR, Katz JT (2008) Fungal brain infections. Curr Opin Neurol 21(3):347–352

Segura M, Zheng H, de Greeff A, Gao GF, Grenier D, Jiang Y, Lu C, Maskell D, Oishi K, Okura M, Osawa R, Schultsz C, Schwerk C, Sekizaki T, Smith H, Srimanote P, Takamatsu D, Tang J, Tenenbaum T, Tharavichitkul P, Hoa NT, Valentin- Weigand P, Wells JM, Wertheim H, Zhu B, Gottschalk M, Xu J (2014) Latest developments on *Streptococcus suis*: an emerging zoonotic pathogen: part 1. Future Microbiol 9(4):441–444

Selvan ST, Padmanabhan P, Gulyás BZ (2019) Nanotechnology-based diagnostics and therapy for pathogen-related infections in the CNS. ACS Chem Neurosci 11:2371–2377

Sharifi S, Behzadi S, Laurent S, Laird Forrest M, Stroeve P, Mahmoudi M (2012) Toxicity of nanomaterials. Chem Soc Rev 41:2323–2343

Sharma RR (2010) Fungal infections of the nervous system: current perspective and controversies in management. Int J Surg 8(8):591–601

Sharma G, Lakkadwala S, Modgil A, Singh J (2016) The role of cell-penetrating peptide and transferring on enhanced delivery of drug to brain. Int J Mol Sci 17(6):806

Singh L, Kruger HG, Maguire GEM, Govender T, Parboosing R (2017) The role of nanotechnology in the treatment of viral infections. Ther Adv Infect Dis 4(4):105–131

Sohaebuddin SK, Thevenot PT, Baker D, Eaton JW, Tang L (2010) Nanomaterial cytotoxicity is composition, size, and cell type dependent. Part Fibre Toxicol 7:22

Spector R (1990) Advances in understanding the pharmacology of agents used to treat bacterial meningitis. Pharmacology 41(3):113–118

Swanson PA, McGavern DB (2015) Viral diseases of the central nervous system. Curr Opin Virol 11:44–54

Tak M, Gupta V, Tomar M (2014) Flower-like ZnO nanostructure based electrochemical DNA biosensor for bacterial meningitis detection. Biosens Bioelectron 15(59):200–207

Tiwari RP, Garg SK, Bharmal RN, Kartikeyan S, Bisen PS (2007) Rapid liposomal agglutination card test for the detection of antigens in patients with active tuberculosis. Int J Tuberc Lung Dis 11:1143–1151

Tiwari D, Haque S, Tiwari RP, Jawed A, Govender T, Kruger HG (2017) Fast and efficient detection of tuberculosis antigens using liposome encapsulated secretory proteins of Mycobacterium tuberculosis. J Microbiol Immunol Infect 50:189–198

Tyagi M, Tomar M, Gupta V (2013) NiO nanoparticle-based urea biosensor. Biosens Bioelectron 41:110–115

Van Furth A, Roord JJ, Van Furth R (1996) Roles of proinflammatory and anti-inflammatory cytokines in pathophysiology of bacterial meningitis and effect of adjunctive therapy. Infect Immun 64(12):4883

Vishwanath S, Shenoy PA, Gupta A, Menon G, Chawla K (2016) Brain abscess with anaerobic Gram negative bacilli: case series. J Case Rep 6(4):467–474

Wagner V, Dullaart A, Bock A-K, Zweck A (2006) The emerging nanomedicine landscape. Nat Biotechnol 24(10):1211–1217

Walker M, Kublin JG, Zunt JR (2006) Parasitic central nervous system infections in immunocompromised hosts: malaria, microsporidiosis, leishmaniasis, and African trypanosomiasis. Clin Infect Dis 42(1):115–125

Wang H, Xu K, Liu L, Tan JP, Chen Y, Li Y et al (2010) The efficacy of self-assembled cationic antimicrobial peptide nanoparticles against Cryptococcus neoformans for the treatment of meningitis. Biomaterials 31(10):2874–2881

Willing BP, Russell SL, Finlay BB (2011) Shifting the balance: antibiotic effects on host-microbiota mutualism. Nat Rev Microbiol 9(4):233–243

Xiang HB, Liu C, Liu TT, Xiong J (2014) Central circuits regulating the sympathetic outflow to lumbar muscles in spinally transected mice by retrograde transsynaptic transport. Int J Clin Exp Pathol 7(6):2987–2997

Yin H, Xu Z, Wang M, Zhang X, Ai S (2013) An electrochemical biosensor for assay of DNA methyltransferase activity and screening of inhibitor. Electrochim Acta 89:530–536

Zaidi S, Misba L, Khan AU (2017) Nano-therapeutics: a revolution in infection control in post antibiotic era. Nanomedicine 13(7):2281–2301

Zhang J-R, Tuomanen E (1999) Molecular and cellular mechanisms for microbial entry into the CNS. J Neurovirol 5(6):591–603

Zhang W, Yang T, Zhuang X, Guo Z, Jiao K (2009) An ionic liquid supported CeO_2 nanoshuttles-carbon nanotubes composite as a platform for impedance DNA hybridization sensing. Biosens Bioelectron 24(8):2417–2422

Zhang S, Chen Y, Liang J, Wang S, Liu L, Liu S (2017) Nanoformulated antimicrobial agents for central nervous system infections. J Nanosci Nanotechnol 17(12):8683–8698(16)

Zhou L, Qiu T, Lv F, Liu L, Ying J, Wang S (2018) Self-assembled nanomedicines for anticancer and antibacterial applications. Adv Healthc Mater 7(20):1800670

Zong J, Cobb SL, Cameron NR (2017) Peptide-functionalized gold nanoparticles: versatile biomaterials for diagnostic and therapeutic applications. Biomater Sci 5:872–886

Zou L-l, Ma J-L, Wang T, Yang T-B, Liu C-B (2013) Cell-penetrating peptide-mediated therapeutic molecule delivery into the central nervous system. Curr Neuropharmacol 11(2):197–208

Antiviral Potency of Small Interfering RNA Molecules

27

Alesia A. Levanova

Abstract

Small interfering RNA (siRNA) are short (19–25 bp) double-stranded (ds) RNA molecules that in cytoplasm of eukaryotic cells triggers posttranscriptional silencing of target genes, a process known as RNA interference (RNAi). RNAi is mediated via the activity of a multiprotein RNA-induced silencing complex (RISC), guided by the siRNA sequence to a cognate sequence on mRNA, which is subsequently degraded or becomes inaccessible for translation machinery. In plants, fungi and invertebrates siRNAs are generated by dicing of exogenous long dsRNAs of viral origin, which interact with viral genomic RNA or mRNAs, thus restricting infection. In mammalian cells, natural antiviral RNAi is apparently observed only in the cells with impaired interferon responses such as embryonic stem cells. Nevertheless, exogenously produced siRNAs can be efficiently incorporated into RISC and specifically inhibit replication of multiple pathogenic viruses with RNA or DNA genomes. Antiviral siRNAs can be delivered to the cytoplasm of the target cells using viral vectors or nanocarriers based on lipids, polymers, DNA nanostructures, or dendrimers. The chapter summarizes 20 years of research of the properties and activities of antiviral siRNAs, their production and delivery to the target tissues and cells.

Keywords

Antiviral siRNA · RNAi · siRNA production · siRNA delivery

A. A. Levanova (✉)
Molecular and Integrative Biosciences Research Programme, Faculty of Biological and Environmental Sciences, University of Helsinki, Helsinki, Finland
e-mail: alesia.levanova@helsinki.fi

S. Hameed, S. Rehman (eds.), *Nanotechnology for Infectious Diseases*,
https://doi.org/10.1007/978-981-16-9190-4_27

27.1 Introduction

Approximately 98% transcripts in humans are not translated into proteins but represent non-coding RNAs (ncRNA) derived from the introns or exons of non-protein coding genes (Mattick 2001; Dennis 2002; Rodriguez et al. 2004). Initially these transcripts were considered useless, a type of "transcriptional noise" (Dennis 2002). However, the discovery of a special class of ncRNA, microRNAs (miRNA), has changed this view. The first genes coding for miRNAs, *lin-4* (Lee et al. 1993; Wightman et al. 1993) and *let-7* (Reinhart et al. 2000), were identified in a worm *Caenorhabditis elegans,* where they are involved in temporal regulation of larva-to-adult development. Later miRNA-coding sequences were found in animals, plants, insects, and viruses (Mattick and Makunin 2005; Brosnan and Voinnet 2009; Tycowski et al. 2015). It is estimated that the human genome encodes more than 1000 miRNAs, which participate in the regulation of numerous genes (Rajewsky 2006) and play essential role in cell differentiation and development (Erson and Petty 2008; Friedman and Jones 2009; Sayed and Abdellatif 2011).

Initially, miRNA mechanism of action was explained simply by a complementary interaction of antisense RNA strand with its target mRNA (Lee et al. 1993). Exogenous antisense single-stranded (ss) RNAs were often used to silence genes of interest (Izant and Weintraub 1984; Nellen and Lichtenstein 1993). However, the observation that double-stranded (ds) RNA molecules are much more potent in inducing gene silencing in *C. elegans* compared to antisense ssRNAs led to a conclusion that an alternative mechanism must be responsible for dsRNA-induced silencing or interference (RNAi; Fire et al. 1998). Notably, in some cases neither sense- nor antisense strands alone can be biologically active. Thus, inhibition of human immunodeficiency virus (HIV) was detected only with short dsRNAs targeting gag or pol mRNAs, but not with their sense- or antisense strands (Hu et al. 2002). Shortly after finding, RNAi has become a powerful tool for the investigation of gene function and demonstrated potential for the development of therapeutic agents. The discovery of RNAi (Fire et al. 1998) has been recognized by the Nobel Prize in Physiology or Medicine already eight years later in 2006. Below we will discuss in details molecular mechanisms of RNAi, production of dsRNAs for RNAi applications, delivery to the target tissues and cells, and RNAi potential as an antiviral therapy.

27.2 Molecular Mechanisms of RNA Interference

RNAi is an evolutionary conserved pathway in eukaryotes initiated by short (19–27 bp) dsRNA molecules that guide sequence-specific degradation of mRNA molecules. There are two main classes of dsRNA molecules involved in RNAi mechanism, miRNAs, and small interfering (si)RNA, which differ in their origin and type of interaction with the target mRNA, hence, determining mRNA degradation pathway. **MiRNA** are endogenous triggers of RNAi encoded in genome, whereas **siRNA** has exogenous synthetic or viral origin. SiRNAs are fully complementary to its target sequence, while miRNAs need complementarity only in a

conserved seed region at positions 2–7 nt from the miRNA 5′ end (Wilson and Doudna 2013).

In mammals, miRNAs are encoded in genome and normally transcribed by RNA polymerase II into at least 1000 nt long primary miRNA transcript (**pri-miRNA**), which is capped and has a poly-A tail (Cai et al. 2004). A subset of miRNAs interspersed among Alu repeats is transcribed with polymerase III (Borchert et al. 2006). The generated pri-miRNA represents a single or clustered dsRNA hairpins with long single-stranded overhangs at their 5′ and 3′ ends and ~10 nt distal loop (Fig. 27.1). This pri-miRNA is further processed by the nuclear **microprocessor complex** consisting of the RNAse III class enzyme Drosha and dsRNA-binding protein that is called DiGeorge syndrome critical region gene 8 (DGCR8). DGCR8 helps to position Drosha for the endonucleolytic cleavage, which occurs ~11 bp from the junction of ssRNA termini and a hairpin stem (Han et al. 2006). Thereby, one end of a mature miRNA is produced. The resulting ~70 nt **pre-miRNA** gets translocated into cytosol by exportin 5, which requires RanGTP for binding of its cargo (Bohnsack et al. 2004). In cytosol, miRNA and siRNA processing pathways converge (Fig. 27.1). Pre-miRNA is further processed into an approximately 21–22 nt long dsRNA by the RNaseIII enzyme Dicer. Similarly, Dicer can process exogenous dsRNAs or Dicer-substrate siRNAs (D-siRNAs). The D-siRNAs are shorter than 30 nt but slightly longer than typical Dicer products and they are more potent than canonical-size siRNA, when introduced to mammalian cells (Kim et al. 2005; Romanovskaya et al. 2012).

Dicing and subsequent RISC loading requires Dicer association with one of the two dsRNA-binding proteins: trans-activation response RNA-binding protein (TRBP) or protein activator of protein kinase R (PACT), which determine cleavage and substrate specificity of the enzyme. In contrast to Dicer-TRBP complex, Dicer in complex with PACT does not process dsRNA into siRNA molecules and provides different isomiRNAs (Lee et al. 2013). Dicer-processed miRNAs or siRNAs bear a 2-nt overhang at the 3′ termini and a phosphate group at 5′ termini (Schwarz et al. 2003). One of the miRNA or siRNA strands (**guide strand**) is loaded onto Argonaute (MacRae et al. 2008), where its 3′ terminus binds to the PAZ domain and 5′ terminus—to the MID domain generating "programmed" RNA-induced silencing complex (**RISC**). It has been shown that the ssRNA strand with less thermodynamically stable 5′ terminus is preferentially selected as a guide strand, while the other "**passenger" strand** with more stable, i.e., GC-rich, 5′-terminus is discarded from RISC (Hutvagner 2005). This is consistent with the observation that binding affinities of UMP and AMP to MID domain of Argonaute are 30-fold lower than those measured for CMP and GMP (Frank et al. 2010). After RISC programming, RNA strand guides Argonaute protein to a target mRNA (Wilson and Doudna 2013; Dueck and Meister 2014; Jonas and Izaurralde 2015). There are four types of Argonaute proteins in humans (Ago1–Ago4), but only Ago2 is catalytically active (Liu et al. 2004). In case of perfect complementarity and catalytic activity of Argonaute, mRNA is cleaved (Liu et al. 2004). In case of partial complementarity (most human miRNAs) and/or catalytically inactive Argonaute, translational repression is induced, which is followed by mRNA deadenylation and degradation

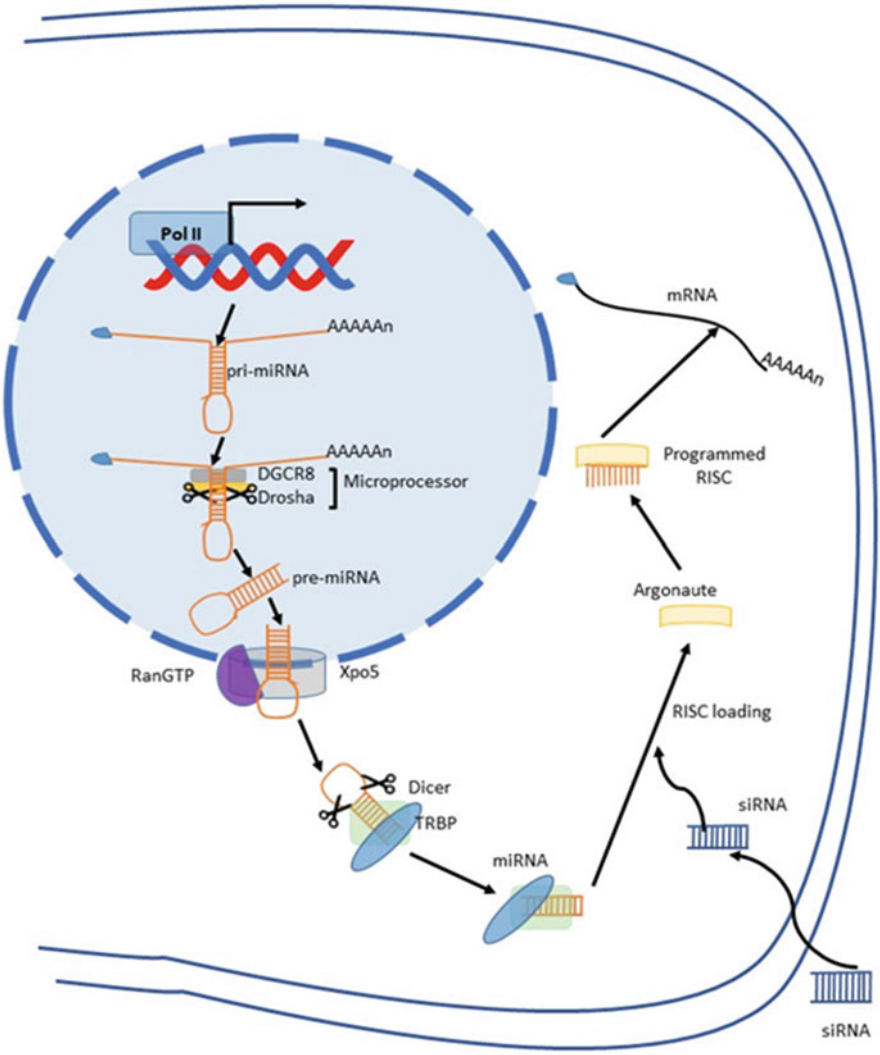

Fig. 27.1 Molecular mechanism of RNAi in mammalian cells. A capped and polyadenylated primary miRNA transcript (pri-miRNA) is generated by RNA polymerase II followed by a cleavage of free ends of pri-miRNA with the Microprocessor complex comprised of Drosha and DGCR8 proteins. The obtained precursor (pre-miRNA) is transported into cytoplasm via exportin 5 (Xpo5), where the hairpin of pre-miRNA is cut out with the Dicer enzyme. One strand of miRNA (or exogenous siRNA delivered into the cell) is incorporated into multiprotein RNA-induced silencing complex (RISC) to guide the RISC to a target mRNA resulting in its translational repression or degradation

(Wilson and Doudna 2013). The guide strand is not affected by this process, and the same programmed RISC can undergo multiple turnovers (Meister and Tuschl 2004), thus providing very efficient silencing of a target gene. In rapidly dividing cells, this

effect can last 3–7 days until siRNAs are too diluted in the cellular population to register the RNAi activity. In slowly dividing cells, RNAi can exist longer than 3 weeks and ends with siRNA degradation (Clayton 2004; Bartlett and Davis 2006). The discovery that exogenously produced siRNAs can be easily incorporated into RNAi pathway causing the suppression of endogenous and heterologous genes (Elbashir et al. 2001) resulted in explosion of research on different siRNA applications, including extensive studies of antiviral potential of siRNAs (see below).

27.3 Development of siRNAs for In Vivo Applications

27.3.1 Rational Design of siRNA Sequences

Promptly after the onset of the experiments with the exogenously produced siRNAs, researchers realized the significance of a careful design of dsRNA molecules for RNAi (Reynolds et al. 2004). A number of tools have been developed to assist in smart siRNA design, for instance, OligoWalk (Mathews 2010) and DSIR (Vert et al. 2006) software. In general, to develop siRNAs, the next issues should be taken into account:

1. Since mRNA is prone to adopt various secondary structures, such as loops, stems, and hairpins, fragments of mRNA located in stem structures might be difficult to target with siRNAs. Therefore, viral genomes must be analyzed for structural features and siRNA must be designed to target accessible regions (Kretschmer-Kazemi Far and Sczakiel 2003; Luo and Chang 2004; Yoshinari et al. 2004). Probability of binding efficiency (PBE) between siRNA and its cognate RNA can be calculated based on the ssRNA structural information, and siRNA candidates with the highest scores can be tested experimentally (Wu and Luo 2021)
2. The target regions in the viral genome must be highly conserved (Naito et al. 2007; von Eije et al. 2008; Rangan et al. 2020) to cover as many strains and variants of a particular virus as possible and to reduce the probability of escape mutants. Furthermore, GC content should preferably be below 65% since high GC content hinders RNAi (Müller and Günther 2007).
3. Possible mRNA modifications might reduce the efficiency of RNAi process, and, therefore, viral transcriptome must be examined to exclude modified mRNA sites from siRNA sequence.
4. Off-target activity of siRNAs might originate either from the non-specific silencing of cellular mRNAs or from the activation of the innate immune responses. The absence of the seed sequences in the human transcriptome must be confirmed to prevent the former off-target activity. Furthermore, siRNAs should not demonstrate observable immunostimulatory activity when introduced into human macrophages.

5. A pool of several siRNAs (at least 3–4 sequences) targeting various regions in viral mRNAs has an advantage over a single-site targeting siRNA since it allows reducing the emergence of escape mutants.

27.3.2 Production of siRNAs

There are currently three main methods for production of siRNAs: **chemical synthesis**, **enzymatic production**, and construction of **shRNA expressing plasmid** or **viral vector**. All these approaches have been extensively used for the generation of antiviral siRNAs (Table 27.1). Chemical solid-phase synthesis of short oligonucleotides (Beaucage 2008) followed by the annealing of sense- and antisense strands to obtain siRNA molecules is a well-controlled process, which allow to synthesize any siRNA sequence containing specific chemical modifications in certain positions. Chemical modifications of sugars, phosphate backbone, or bases of siRNA molecules help to improve their chemical stability and pharmacokinetic properties, reduce off-target effects, and achieve delivery into difficult-to-transfect cells (Watts et al. 2008; Selvam et al. 2017; Hassler et al. 2018; Taniguchi et al. 2020). The main disadvantage of the chemical synthesis is its rather high cost.

Enzymatic synthesis is much more affordable, and, virtually, any laboratory can easily generate milligram amounts of desired siRNAs in a matter of days. Viral RNA polymerases from bacteriophages T7, SP6, phi6 can be applied to produce individual siRNAs (Sohail et al. 2003) or long dsRNA molecules, which can be subsequently diced into a pool of siRNAs (Levanova and Poranen 2018). The latter approach mimics natural RNAi-based antiviral response discovered in plants, fungi, and invertebrates (Ding and Voinnet 2007; Dang et al. 2011) and was successfully realized in siRNA pools against HIV-1 (Konstantinova et al. 2006), caxsakievirus B3 (Nygardas et al. 2009), influenza virus (Jiang et al. 2019), and herpes simplex virus 1 (HSV-1; Romanovskaya et al. 2012). Application of siRNA pool reduces the probability of viral escape mutants and lowers the likelihood of "off-target" effects since each individual sequence represents only a small fraction in the siRNA pool.

The size of siRNAs slightly varies between different organisms. Thus, human Dicer generates siRNAs of about 21 nt (Zhang et al. 2004a), while Dicer from *Giardia intestinalis* cuts dsRNA into 25–27 nt species (Macrae et al. 2006). The 25–27 nt siRNAs are referred to as D-siRNAs because they can serve as a substrate for human Dicer upon their introduction into a human cell. D-siRNAs have enhanced RNAi potency compared to canonical 21 nt siRNA due to more efficient incorporation into RISC (Kim et al. 2005). The main drawback of the enzymatic production of siRNA pools is a limited ability to control and modify siRNA sequences. Although modified bases can be incorporated by viral polymerases (Levanova et al. 2020) into sense-, antisense- or both strands, the specific position of modifications is currently not possible to control. Viral polymerases leave triphosphate at the 5′ end of the produced RNA molecules, which are recognized by cytoplasmic helicase proteins RIG-I causing type I interferon (IFN-I) expression (Hornung et al. 2006; Pichlmair et al. 2006). Hence, enzymatically produced RNA

Table 27.1 Antiviral siRNAs

Virus	Target	siRNA characteristics and delivery	Effects of siRNAs	References
Viruses with dsDNA genome (group I according to Baltimore classification)				
Human papillomavirus (HPV, *Papillomaviridae*)	mRNAs of E6 and E7 oncoproteins	1. siRNAs contained 2-nt overhang of 2′-deoxy thymidine (dTdT) to enhance siRNA stability. Alternatively, siRNA was synthesized using proprietary stabilization chemistry (Dharmacon). Oligofectamine was used for transfection 2. HPV pseudovirions expressing shRNAs 3. lentiviral vectors expressing shRNAs	Human cervical carcinoma cell lines CaSKi and SiHa, which were positive for HPV16, and negative for HPV C33-A cells were used to evaluate antiviral siRNAs. HPV-specific siRNAs induced significant decrease in E6 and E7 mRNAs as confirmed by Northern Blotting and semi-qPCR C57BL6 mice were subcutaneously inoculated with TC1 solid tumor cells previously transfected with pseudovirions, a lentivirus, or shRNA with Lipofectamine. TC1 cells treated with pseudovirions or lentivirus showed retarded tumor formation at much higher levels than those observed in mice treated with shRNA-Lipofectamine CaSki cells were injected s.c. into *nu/nu* athymic mice, followed by siRNA injection into tumor. Treatment with E6/E7 siRNAs almost completely eradicated tumors in 70% of the animals	Jiang and Milner (2002); Bousarghin et al. (2009); Jonson et al. (2008)

(continued)

Table 27.1 (continued)

Virus	Target	siRNA characteristics and delivery	Effects of siRNAs	References
Human herpes virus 1 and 2 (HSV-1 and HSV-2; *Herpesviridae*)	mRNA of ssDNA-binding protein ICP8 encoded by **UL29**, glycoprotein B encoded by **UL27**, and regulatory protein ICP27 encoded by **UL54** gene	Single-site synthetic siRNAs were complexed with TransIT-TKO or with TransIT-siQuest for cell transfection. For animal experiments, siRNA was complexed with Oligofectamine and administered intravaginally Enzymatically created siRNA pools were transfected to the cells in complexes with lipofectamine 2000 or applied into eyes of experimental mice in PBS	Anti-UL27 and UL29 siRNAs were mixed with lipid carriers and applied intravaginally. The siRNAs were efficiently internalized by epithelial cells, where they did not induce IFN-I stimulating genes or cause inflammation. The anti-HSV-1 siRNAs protected mice from the lethal HSV-2 challenge, when given both prophylactically or therapeutically Anti-HSV-1 D-siRNA pools targeting UL27, UL29, and UL54 mRNAs effectively decreased titers of laboratory and clinical HSV-1 strains in glioblastoma U373-MG, retinal pigment epithelial (RPE), and epithelial HaCaT cells. The effect was sequence-specific and did not depend on the activation of innate immune response. UL29 siRNA pool had the most prominent antiviral activity and was used in BALB/c mice to treat	Palliser et al. (2006); Romanovskaya et al. (2012); Paavilainen et al. (2015); Paavilainen et al. (2016); Paavilainen et al. (2017); Levanova et al. (2020)

			experimental eye infection, where it alleviated HSV-1 infection symptoms, inhibited viral replication and shedding. Random incorporation of 2′-Fluoro-modified NTPs into UL29 siRNAs enhanced antiviral potency of the modified Ul29-siRNA pool compared to the unmodified one	
Human polyomavirus BK (BKV, *Polyomaviridae*)	T-antigen oncogen mRNA	shRNA in adenovirus	T-antigen was suppressed in pRPc cell line, a malignant BKV-transformed cell line derived from normal Balb/c mouse fibroblasts. This suppression resulted in the induction of apoptosis	Sabbioni et al. (2007)
Vaccinia virus (VACV, *Poxviridae*)	D5R, B1R and G7L genes	Synthetic siRNAs containing dTdT overhangs at both 3′ ends complexed with lipofectamine 2000	VACV replication was inhibited up to 90% when antiviral siRNAs were used either prophylactically or therapeutically in human lung carcinoma A549 cells. Synergistic effects were detected when anti-VACV siRNAs were administered together with cidofovir	Vigne et al. (2008); Vigne et al. (2009)
Viruses with dsRNA genome (group III according to Baltimore classification)				
Reovirus (*Reoviridae*)	M1, M3, or S3 genome segment encoding core	pSUPER RNAi system was used to generate plasmid-	Stable expression of siRNAs specific for any one of	Kobayashi et al. (2006)

(continued)

Table 27.1 (continued)

Virus	Target	siRNA characteristics and delivery	Effects of siRNAs	References
	protein μ2, non-structural proteins μNS and σNS	based vectors expressing anti-reovirus siRNAs	reovirus M1, M3, or S3 genome segment substantially diminished viral dsRNA, protein synthesis, and inclusion formation in human embryonic kidney 293T (HEK293T) cells	
Rotavirus (*Reoviridae*)	NSP5 (non-structural protein)	Synthetic siRNAs complexed with lipofectamine	Silencing of NSP5 correlated with the reduction of genomic dsRNA and viral protein synthesis, as well as with a marked reduction in the production of infectious viral progeny in infected monkey kidney epithelial cell line MA104	López et al. (2005)
Viruses with +ss RNA genome (group IV according to Baltimore classification)				
Poliovirus (*Picornaviridae*)	Polymerase (3Dpol), capsid protein mRNAs, 5′-UTR	SiRNA electroporation or transfection in complex with Lipofectamine 2000 was used	Although HeLa S3 cells were infected at high multiplicity of infection (MOI = 10) prior to siRNA transfection, accumulation of poliovirus mRNA was markedly reduced. Transfection of anti-poliovirus capsid siRNA before infection with poliovirus completely inhibited virus plaque	Gitlin et al. (2002); Saulnier et al. (2006)

			formation in HeLa cells. Pre-treatment of mouse embryonic fibroblasts with anti-poliovirus siRNAs substantially decreases the virus titer and results in clearance of the virus from the infected cells The persistent infection established in human epithelial tumor cell line HEp-2 cells was completely cured with a mixture of two siRNAs targeting 3Dpol and 5′-UTR in the majority of persistently infected cultures, and no escape mutants emerged	
Severe acute respiratory syndrome coronavirus 1 (SARS-CoV1, *Coronaviridae*)	RdRp (nsp12); nsp13; nsp16; leader sequence; transcription-regulating sequence (TRS); 3′-UTR; spike (S) protein coding sequence	1. Transfection with synthetic siRNAs complexed with Oligofectamine or lipofectamin2000; 2. Lipofectamine transfection with pSUPER. retro vector expressing shRNAs	Anti-SARS-CoV 1 siRNAs caused inhibition of infection in fetal rhesus kidney cells (FRhk-4), African green monkey kidney Vero or VeroE6 cells, HEK293T cells as confirmed by reduction of cytopathic effects (CPE), titer assay, Western blotting, and decrease in viral genomic RNA copies (qRT-PCR) S-specific siRNAs reduced amounts of RNA transcripts and viral antigens, 3′-UTR	He et al. (2003); Wang et al. (2004); Wu et al. (2005); Li et al. (2005a); Li et al. (2005b); Zheng et al. (2004); Zhang et al. (2004b)

(continued)

Table 27.1 (continued)

Virus	Target	siRNA characteristics and delivery	Effects of siRNAs	References
			targeting siRNAs were less effective, and those against the leader sequence and TRS were not effective in the study of Wu et al. (2005) SiRNAs targeting S protein and nsp12 coding region were effective against SARS-CoV1 in Rhesus macaque (*Macaca mulatta*), when administered either before or after infection. They decreased viral load and diffuse alveolar damage (Li et al. 2005a)	
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV2; *Coronaviridae*)	Helicase, RdRp, 5′-UTR, nsp8, nsp9, 7a-E, S, nucleocapsid (N), and membrane (M) protein coding sequence	1. Delivery to mice using i.v. injection of sLNPs and dmLNPs formulated with siRNAs 2. Inhalation of dendrimers complexed with siRNAs containing locked nucleic acids 3. Lipofectamine 2000 was used for cell transfection with siRNAs	Human umbilical vascular endothelial cells (HUVECs), human lung carcinoma A549 cells, and HEp-2 cells were used for initial screening of siRNA antiviral activity Mice of K18-hACE2 lineage were infected with SARS-CoV2 and subsequently treated with a number of antiviral siRNA duplexes. After smart siRNA design, out of 17 candidate molecules, only two siRNAs targeting helicase gene, one siRNA	Idris et al. (2021); Khaitov et al. (2021); Wu and Luo (2021)

			targeting RdRp gene, and one siRNA directed to the 5′-UTR were proved to be potent in repression of viral infection. The effect was transient and disappeared about 48h after siRNA injection Complexed with dendrimers, antiviral siRNAs targeting RdRp significantly reduced virus titer and lung inflammation in infected Syrian hamsters	
Middle East respiratory syndrome coronavirus (MERS-CoV, *Coronaviridae*)	ORF1ab	Lipofectamine 2000 was used for transfection of synthetic siRNAs	In silico designed siRNAs were without off-target effects, minimal cytotoxicity, high efficiency, and improved specificity. These siRNAs significantly lessened the reproduction of MERS-CoV in HEK-293T cells	Sohrab et al. (2021)
Hepatitis C virus (HCV, *Flaviviridae*)	NS3 (protease and RNA helicase), NS5B (RdRp), 5′-UTRs, NS4B, NS5A, 3′X region, internal ribosome entry site (IRES)	1. Synthetic siRNAs complexed with Oligofectamine; 2. DNA-based shRNA expressing vectors complexed with lipofectamine 2000; 3. Electroporation of synthetic siRNAs; 4. siRNA pool obtained after digestion of long dsRNA	Human hepatoma cell line Huh7, its derivative Huh-7.5, and the human embryonic kidney cell line 293T were used in the studies. Inhibition of HCV infection was confirmed by luciferase assay, Northern hybridization, Western blotting, and qRT-PCR (reduction in	Kapadia et al. (2003); Seo et al. (2003); Yokota et al. (2003); Randall et al. (2003); Kronke et al. (2004); Watanabe et al. (2006); Ma et al. (2014)

(continued)

Table 27.1 (continued)

Virus	Target	siRNA characteristics and delivery	Effects of siRNAs	References
		with RNaseIII; 5. Retroviral vectors encoding shRNAs; 6. Short synthetic small-hairpin RNAS (sshRNAs) formulated into LNPs were administered i.v. to mice with livers repopulated with human hepatocytes (humanized livers)	genomic RNA) Weekly administration of sshRNA for 2 weeks resulted in a significant decrease in viral load, which remained reduced by more than 90% at 14 days after the last dose of sshRNA was given	
Japanese encephalitis virus (JEV, *Flaviviridae*)	NS1, envelope (E) protein coding sequence	1. DNA vectors expressing shRNAs were transfected into cells with lipofectamine 2000; 2. Lentiviral vectors expressing shRNAs; 3. Synthetic siRNAs complexed with iFect were injected intracranially to efficiently deliver siRNA to the mouse brain and neurons	Vero, baby hamster kidney (BHK21), and mouse neuronal (Neuro 2a) cell lines were used to demonstrate antiviral activity of siRNA. Vero cells were co-transfected with pNS1-EGFP to evaluate the activity of shRNAs by flow cytometry, western blot, and RT-PCR BALB/c mice were protected with siRNAs from lethal infection with JEV	Kumar et al. (2006); Liu et al. (2006)
West Nile Virus (WNV, *Flaviviridae*)	Envelope (E) protein	1. Lentiviral vectors expressing shRNAs; 2. Synthetic siRNAs complexed with iFect or JetSI/DOPE and injected	Vero, baby hamster kidney (BHK21), and mouse neuronal (Neuro 2a) cell lines were initially used to confirm the antiviral effects of siRNAs	Kumar et al. (2006); Bai et al. (2005)

		intracranially; 3. Hydrodynamic injection was used to deliver naked siRNAs to mice	BALB/c mice were protected post-infection from lethal infection with WNV (Kumar et al. 2006) or partially protected from death in prophylactic treatment (Bai et al. 2005)	
Dengue Virus (DENV, *Flaviviridae*)	NS4B, NS5	Synthetic siRNAs complexed with Dharmafect1	A cocktail of four siRNAs inhibited all four serotypes of DENV both in Vero (mammalian) and C6/36 (insect) cells	Villegas et al. (2018)
Chikungunya virus (CHIKV, *Togaviridae*)	nsp3, E1	1. siPORT Amine reagent was used for transfection of siRNA into Vero cells; 2. Combination of two amiRNAs in a single vector construct was delivered using Lipofectamine 2000	CHIKV titer was reduced 99.6% 24 h after transfection of Vero cells, at 48 h the effect was decreased to 65% AmiRNAs inhibited CHIKV replication in Vero cells at both RNA and protein levels as shown by qRT-PCR, immunoblotting and immunofluorescence techniques. A pool of two amiRNA provided higher inhibition of CHIKV compared to individual amiRNAs	Dash et al. (2008); Saha et al. (2016)
Viruses with -ssRNA genome (group V according to Baltimore classification)				
Rabies virus (*Rhabdoviridae*)	Nucleoprotein (N) gene	Pool of three siRNAs targeting N gene was	A fivefold decrease in virus titer was observed in infected BHK-21 cells treated with	Brandao et al. (2007)

(continued)

Table 27.1 (continued)

Virus	Target	siRNA characteristics and delivery	Effects of siRNAs	References
		transfected with lipofectamine 2000	anti-rabies siRNA pool compared to the control infected and untreated cells	
Marburg (MARV) and Ravn virus (RAVV) (*Filoviridae*)	Nucleoprotein (NP)	Daily i.v. injections of siRNAs encapsulated into LNPs	Rhesus macaques were infected with a lethal dose of MARV or RAVV, followed by the i.v. injection of siRNA–LNP complexes 4 days post-infection (dpi) for MARV and 3 dpi for RAVV, which resulted in survival of all animals treated	Thi et al. (2017)
Ebolavirus (EBOV, *Filoviridae*)	L (RdRp), VP35, VP24	Stable nucleic acid-lipid particles (SNALPs) containing siRNAs modified by 2′-*O*-methyl guanosine or uridines at selective positions in both strands to eliminate the immune stimulatory capacity of the siRNA duplex. The SNALPs were administered via i.v. infusion 30 min after the EBOV challenge, and additional treatments were on days 1, 3, and 5 dpi or on days 1, 2, 3, 4, 5, and 6 dpi	SNALPs containing EBOV siRNAs substantially reduced virus production in supernatants of Vero E6 cells 2 dpi SNALPs containing modified EBOV siRNAs did not induce interferon α or interleukin 6 in the plasma of CD1 ICR and B6C3F1 mice Chinese rhesus macaques (*Macaca mulatta*) were used for the non-human primate studies. Two of three animals treated with four doses of the pooled anti-EBOV siRNAs	Geisbert et al. (2010); Thi et al. (2015)

			survived the lethal challenge with EBOV, and all macaques who got seven doses were protected	
Respiratory syncytial virus (RSV, *Paramyxoviridae*)	Phosphoprotein (P), fusion (F) protein	1. dTdT overhangs were used to provide stability of synthetic siRNAs against RNases. Cell transfection with siRNAs was carried out with OligofectAMINE 2. siRNAs in complex with TransIT-TKO reagent was administered intranasally	Translation of RSV was inhibited in human lung carcinoma epithelial cell line A549, without induction of IFN responses Intranasal siRNAs inhibited RSV replication in lungs of BALB/c mice	Bitko and Barik (2001); Bitko et al. (2005)
Parainfluenza virus (PIV, *Paramyxoviridae*)	Phosphoprotein (P) mRNA	Both 3′ ends contained dTdT siRNAs in complex with TransIT-TKO reagent was administered intranasally	Intranasal siRNAs inhibited PIV replication in lungs of the BALB/c mice	Bitko et al. (2005)
Human metapneumovirus (hPMV, *Pneumoviridae*)	nucleoprotein (*N*) and phosphoprotein (*P*) mRNAs	HiPerFect Transfection Reagent was used for cell transfection	Prophylactic application of siRNAs to A549 cells inhibited virus replication as measured by plaque assay	Nitschinsk et al. (2018)
Influenza virus A (IVA, *Orthomyxoviridae*)	nucleoprotein (NP), acidic polymerase (PA), or basic polymerase (PB1), chimeric siRNAs containing multiple conserved sequences from IVA genome	1. Synthetic siRNAs or DNA vectors for shRNA expression were injected i.v. in PBS or complexed with polyethyleneimine (PEI). 2. siRNAs diluted in 1 mL PBS were administered by hydrodynamic i.v. injection; 16–24 h post-injection, the mice were infected with IVA	BALB/cAnNCR mice were specifically protected from the lethal challenge with a seasonal influenza virus H1N1 and highly pathogenic avian influenza A viruses of the H5 and H7 subtypes. SiRNA was effective in both prophylactic and therapeutic settings	Ge et al. (2004); Tompkins et al. (2004); Jiang et al. (2019)

(continued)

Table 27.1 (continued)

Virus	Target	siRNA characteristics and delivery	Effects of siRNAs	References
		and given a second dose of siRNA/oligofectamine intranasally	D-siRNA pool was effective at suppressing IAV replication in human primary monocyte-derived macrophages and dendritic cells, when administered prior the infection. A substantial inhibition of viral RNA expression was detected, which translated to a significant reduction of IAV protein synthesis and virus titer	
Lymphocytic choriomeningitis virus (LCMV, *Arenaviridae*)	L and Z mRNAs	SiRNA were synthesized by Dharmacon. Alternatively, adenovirus vectors expressing siRNAs were generated	The efficacy of siRNAs against LCMV depended on the delivery method in HEK-293 cells. Thus, transfection of synthetic siRNA pools was ineffective in LCMV inhibition, while delivery with a replication-deficient recombinant adenovirus significantly inhibited LCMV replication. Furthermore, in the latter case persistent LCMV infection was effectively cured	Sánchez et al. (2005)

Lassa virus (*Arenaviridae*)	RNA sequences upstream of NP and L gene	Synthetic siRNAs	SiRNAs reduced the expression of the Lassa virus replicon and inhibited reproduction of a number of Lassa virus strains in Vero cells	Müller and Günther (2007)
Reverse transcribing viruses, ssRNA genome with DNA intermediate (group VI according to Baltimore classification)				
Human Immunodeficiency virus-1 (HIV-1, *Retroviridae*)	*Virus factors*: integrase (*int*), attachment site (*att*) genes, transcription factor *tat* and *rev* (regulatory protein), *gag* (core protein) and *env* (envelope protein), viral long terminal repeats (LTRs), accessory genes *nef* and *vif vpu, pol,* p24 (structural protein in HIV-1 capsid, obtained by proteolytic cleavage of Gag polypeptide) *Host factors:* ALIX, ATG16, TRBP, CD4 (receptor), nuclear enzyme poly (ADP-ribose) polymerase 1 (PARP-1)	1. Transfection with synthetic siRNAs with or without dTdT overhangs complexed with lipofectamine 2000 or Oligofectamine 2. Pool of siRNAs obtained by dicing of long dsRNAs, long hairpin RNA (lhRNA) 3. lentiviral vectors expressing shRNAs	SiRNAs and shRNAs have been developed against almost all HIV-1mRNAs. Typically HIV-1 replication was evaluated with p24 ELISA, Northern blot analysis and qPCR. Inhibition of HIV-1 replication by antiviral siRNAs was confirmed in HEK293T, its derivative 293/EcR, cervix carcinoma C33A, Vero, HeLa, CD4+ HeLa (Magi) and Magi-CCR5 cells, immortalized Jurkat and primary CD4+ T cells Targeting host mRNAs, from which proteins required for HIV-1 life cycle are expressed, worked well in prevention of HIV-1 replication. Thus, siRNAs silencing host CD4 inhibited HIV-1 entry and, hence, reduced HIV-1 replication.	Lee et al. (2002); Naito et al. (2007); Capodici et al. (2002); Coburn and Cullen (2002); Jacque et al. (2002); Park et al. (2002); Eekels et al. (2011); Kameoka et al. (2004); Lee et al. (2005); Konstantinova et al. (2006); ter Brake et al. (2006); Saayman et al. (2008); Nishitsuji et al. (2006); Barichievy et al. (2007); Liu et al. (2007); Chang et al. (2005); Sano et al. (2008); Novina et al. (2002); Hu et al. (2002)

(continued)

Table 27.1 (continued)

Virus	Target	siRNA characteristics and delivery	Effects of siRNAs	References
Reverse transcribing viruses, dsDNA genome with RNA intermediate (group VII according to Baltimore classification)				
Hepatitis B virus (HBV, *Hepadnaviridae*)	Core (HBcAg) antigen, polymerase (P), X transcript, small surface (S) antigen (HBsAg), HBx ORF	1. Calcium phosphate transfection of shRNA expression (pSUPER) vectors into cells; 2. Oligofectamine was used for naked siRNA transfection; 3. Transduction of the target cells with retrovirus-based shRNA expression vectors; 4. lhRNAs; 5. Hydrodynamic-based transfection of shRNA expression plasmids or siRNAs in PBS was used in mice experiments	Human hepatoma Huh-7 cells, HepG2 hepatoblastoma cell line, and its derivative HepG2.2.15 cells were used in the studies. Immunocompetent (BALB/c or C57BL/6J) and immunocompromised (NOD/LtSz-*Prkdc*^{scid}/J) mice were used in animal experiments. Northern and Southern blots demonstrated substantially reduced levels of HBV RNAs and DNAs in mouse liver. Immunohistochemistry showed reduced levels of HBsAg after HBV-specific siRNA treatment, and also significant decrease in amounts of secreted HBsAg and HBcAg. LhRNA targeting HBx ORF lessened markers of virus replication by 70–90%, without the induction of IFN-I response	McCaffrey et al. (2003); Shlomai and Shaul (2003); Jia et al. (2007); Giladi et al. (2003); Weinberg et al. (2007); Kong et al. (2012); Fu et al. (2008)

molecules must be dephosphorylated, for instance, with alkaline calf intestinal phosphatase. SiRNAs produced either by chemical or by enzymatic synthesis must be purified from contaminants for safe application to mammalian cells. The most widely used purification method is anion-exchange or ion-pair reverse-phase chromatography (McCarthy et al. 2009; Romanovskaya et al. 2013; Kanwal and Lu 2019).

Small hairpin RNA (**shRNA**) are analogous to pre-miRNAs. The shRNAs are transcribed in the nucleus from a **plasmid or viral vector** containing a polymerase III promoter (U6 or H1), an inverted repeat sequence of shRNA, and a stop signal for transcription (Brummelkamp et al. 2002). The constructs provide constitutive expression of shRNAs and stable knockdown of a target gene. Thus, a retrovirus-based shRNA system provided efficient expression of hepatitis B virus (HBV)-specific shRNAs in HepG2.2.15 cells, which resulted in substantial decrease in the levels of HBV mRNAs and proteins, and suppressed HBV replication (Jia et al. 2007). Alternatively, shRNA plasmids can be delivered inside pseudovirions, which mimic virus in DNA delivery to the nucleus. For instance, human papillomavirus (HPV) pseudovirions containing shRNAs against E6 and E7 oncoproteins of HPV caused efficient silencing of these genes in infected cells (Bousarghin et al. 2009).

Long hairpin RNA (**lhRNA**) contains hairpin RNA sequences longer than 50 nt (Akashi et al. 2005) and multiple siRNAs are generated upon Dicer cleavage of lhRNA transcript. This approach has been introduced to limit viral escape via targeting a variety of sequences found in different virus genotypes. In mammalian cells, use of dsRNAs longer than 30 bp may induce type I IFN response (Stark et al. 1998), which ultimately results in translational repression and mRNA degradation. Interestingly, endogenously expressed lhRNAs do not activate the type I IFN-inducible genes, probably, due to their fast processing with Dicer (Akashi et al. 2005). The lhRNAs have been successfully applied for the inhibition of the hepatitis C virus (HCV), HBV, and HIV-1 replication (Konstantinova et al. 2006; Watanabe et al. 2006; Weinberg et al. 2007). However, there is a certain limitation in the useful length of lhRNA constructs and already the third siRNA sequence from the stem to the loop is hardly processed (Saayman et al. 2008; Sano et al. 2008). Nevertheless, lhRNA construct targeting tat/rev region of HIV-1 and producing two siRNAs provided a significant advantage over individual siRNAs in duration of the antiviral activity and in inhibition of the reproduction of the virus with two point mutations (Sano et al. 2008). An important limitation of both shRNA and lhRNA-based vectors is their possible impact on microRNA pathway via competition for critical RNAi components. This competition is related to the saturation of exportin 5. To mitigate this limitation artificial miRNAs (**amiRNAs**) have been developed. AmiRNAs are expressed from exogenous plasmid construct by RNA polymerase II resulting in transcripts analogous to pri-miRNAs, which are incorporated into natural miRNA maturation pathway (see above). AmiRNAs are safer compared to shRNAs both in vitro and in vivo (Boudreau et al. 2009), probably, because they are expressed at lower levels than shRNAs (Boudreau et al. 2008). AmiRNAs have been successfully tested as an antiviral treatment against chikungunya virus (Saha et al. 2016). An alternative strategy to reduce shRNA toxicity is to decrease their length.

Short synthetic hairpin RNAs (**sshRNAs**) are shRNAs of ≤19 bp, which are too short to be processed with Dicer (Dallas et al. 2012). Therefore, intact hairpins are loaded into RISC and are activated by Ago2 cleavage of a passenger sequence. Since sshRNAs are a single molecular entity, the off-target activity of the passenger strand is less probable compared to siRNAs (Dallas et al. 2012). This approach was successfully applied to inhibit HCV replication in mice with humanized liver (Ma et al. 2014).

The potential competition of pooled siRNAs with each other and cellular miRNAs is a problem since particular sequences seem to be preferably loaded into RISC, thus compromising effectiveness of other siRNAs. DsRNA-binding protein TRBP is a sensor for selection and incorporation of a guide siRNA strand into RISC, and its down-regulation results in mitigation of sequence-specific competition between siRNAs and miRNAs (Castanotto et al. 2007). Furthermore, by finding combinations of siRNAs that work at the lowest possible concentrations it should be feasible to diminish the potential competition with endogenous miRNAs for RNAi machinery.

27.4 Delivery of siRNAs

Preclinical experiments showed that antiviral siRNAs are very effective in suppression of viral replication (Sect. 27.5). However, the efficient RNAi delivery systems are of crucial importance to avoid reservoirs of cells, where virus can propagate and mutate (Lundstrom 2020). In principal, siRNAs can be delivered to the target cells in (1) viral vectors or (2) nanocarriers, such as nanoparticles, DNA nanostructures, lipid or polymeric carriers, and dendrimers (Lundstrom 2020; Ullah et al. 2020; Dong et al. 2021; Idris et al. 2021; Khaitov et al. 2021). Vectors based on adenoviruses, adeno-associated viruses, retroviruses, conditionally replicating lentiviruses, self-replicating rhabdoviruses, and alphaviruses have been applied to achieve RNAi (reviewed by Lundstrom 2020). The lentiviral vectors are approved for clinical use in humans, and those expressing shRNAs to suppress HIV-1 replication are currently in clinical trials (Levanova and Poranen 2018). However, most viral infections are acute, and, thus, do not need prolonged expression of shRNAs. Furthermore, lentivirus-based delivery might not be an optimal clinical approach because of the possible immune response against the administered delivery means and difficulty to predict long-term effects of lentiviral integration (Ullah et al. 2020). Moreover, the quantity of shRNA vectors needed to be administered might be a limiting factor especially with high loads of replicating virus. SiRNA dose can be easily regulated, and the molecules might be less toxic compared to shRNAs because of their transient nature. One more advantage of siRNAs over shRNAs and similar structures is the fast onset of the antiviral activity of siRNAs since they do not need to be expressed in the first place. Therefore, siRNAs seem to be preferable therapeutic modality, and development of delivery strategies for exogenous siRNAs is of great importance.

If target tissue or organ is readily accessible, for instance eye, skin, or lungs, a topical siRNA delivery can be used, even without conjugation or complexing with lipids. Early studies demonstrated that CD4+ T cells can be effectively treated with siRNAs, where CTPs and UTPs are replaced by analogs with a fluoro group instead of OH-group at the second carbon atom of ribose, 2′-F-dCTP and 2′-F-dUTP. These siRNAs can be delivered to the cells without complexing with lipofectin in the presence of serum (Capodici et al. 2002). The first siRNA in clinical trials ALN-RSV01 contained two (2′-deoxy) thymidine overhangs on 3′ termini to enhance resistance to RNases (Alvarez et al. 2009). The ALN-RSV01 was safe and effective against respiratory syncytial virus (RSV) in the form of nasal spray (DeVincenzo et al. 2008). Intranasal delivery is feasible for siRNAs against infections caused by other respiratory viruses, including influenza virus, human metapneumovirus, coronaviruses, and parainfluenza (Barik 2011). Topical ocular or intravaginal administration of "naked" siRNAs or siRNA-lipid complexes against HSV 1 and 2 has been proved to be effective in mice (Wu et al. 2009b; Paavilainen et al. 2017). SiRNAs are complexed with cationic lipids or polymers in order to enhance siRNA delivery to the cells. Dendrimers are cationic polymers representing radially symmetric branched 3D nanostructures, in which central core is surrounded by positively charged groups responsible for binding to siRNAs (Khaitov et al. 2021). Inhalation of (SARS-CoV-2)-specific siRNAs formulated with a peptide dendrimer resulted in transfer of antiviral siRNAs into infected cells and demonstrated a potential to treat coronavirus-induced inflammation in Syrian hamsters (Khaitov et al. 2021). However, thick mucosa associated with many respiratory viruses might hinder the delivery of intranasally administered therapeutics to infected tissue. To overcome this, intravenous (i.v.) administration of siRNAs to lungs has been proposed (Wu et al. 2009a; McCaskill et al. 2013; Idris et al. 2021).

SiRNAs represent an A-form helix with a diameter of approximately 2.4 nm (Lipfert et al. 2014). Taking into account that a mean length increase per base pair of a dsRNA is approximately 0.28 nm (Abels et al. 2005; Lipfert et al. 2014), 21–25 bp siRNA duplexes are about 6–7 nm in length and their molecular weight is approximately 13–17 kDa. With the kidney glomerular filtration barrier pore size of about 8 nm, naked siRNAs can pass into urine and be cleared from the circulatory system in less than 10 min (Soutschek et al. 2004; Sarisozen et al. 2016). Hence, siRNAs should be formulated with carriers to have a larger size in order to avoid renal clearance. However, in this case the opsonization of the resulting complexes with subsequent phagocytosis can be observed. To eliminate this possibility, polyethylene glycol (PEG) is used to form a steric barrier around the siRNA-carrier complex (Torchilin and Trubetskoy 1995). It should be taken into account that PEG also impedes the interaction between complexed siRNA and a target cell (Sarisozen et al. 2016). After siRNA leaves the bloodstream, the net negative charge and hydrophilic nature prevent siRNAs from diffusion through the cell membrane consisting of negatively charged bilayer of phospholipids (Sarisozen et al. 2016). Cell-penetrating peptides conjugated to chitosan can enhance siRNA uptake (Layek et al. 2015). Extracellular vesicles can be also engaged for delivery of cholesterol-conjugated siRNA molecules (O'Loughlin et al. 2017). However, internalization does not

guarantee an effective gene silencing, since siRNA must escape endosomal encapsulation and lysosomal degradation and be released into cytoplasm (Oliveira et al. 2007).

Liposome delivery platform based on the "stealth" lipid nanoparticles (sLNPs) for siRNA delivery to the lungs (Wu et al. 2009a; McCaskill et al. 2013) has been used for the development of siRNA therapy for SARS-CoV-2 (Idris et al. 2021). The sLNPs contained 50% 1,2-dioleoyl-3-thrimethylammonium-propane (DOTAP) and they targeted lungs (~35%), liver (~55%), and spleen (~10%) (Wu et al. 2009a; McCaskill et al. 2013). It has been shown that high concentrations of DOTAP (50%) in combination with ionizable amino lipid DLin-MC3-DMA (MC3, 25%) in LNPs results in enhanced delivery to the lungs (Cheng et al. 2020), while lower content of DOTAP is required to reduce LNP-siRNAs immune stimulation. Therefore, the formulation of sLNPs was modified to contain 40% DOTAP with MC3 to generate dmLNPs (Idris et al. 2021). However, the new formulation of LNP did not translate into better delivery into the lungs. When injected into mice, dmLNP-siRNA were detected in lungs (~21%), liver (~67%), and spleen (~12%) (Idris et al. 2021). Both formulations (sLNPs and dmLNPs) carrying antiviral siRNA duplexes were similarly effective in inhibition of SARS-CoV-2 replication (Idris et al. 2021). Since LNPs accumulate mostly in liver, they can be naturally used to deliver siRNAs, which inhibit replication of hepatotropic viruses, such as HBV, HCV, Marburg virus (MARV), and Ravn virus (RAVV). Cationic lipid-coated gold nanoparticles were employed to target HBV in hepatocytes (Kong et al. 2012). A mixture of cationic lipids (JetSI) and a fusogenic lipid dioleoylphosphatidyl-ethanolamine (DOPE) was successfully applied for siRNA delivery into mouse brain without toxicity, where it reduced replication of flaviviruses (Kumar et al. 2006).

DNA nanocarriers seem to be a promising delivery means since they are inherently biocompatible and biodegradable, sufficiently stable in physiological environment and capable of penetrating cellular membranes via endocytosis (Patra et al. 2018; Dong et al. 2021). SiRNAs can be loaded simply by hybridization with complementary sequences of a DNA nanocarrier. The recent proof-of-principal study (Dong et al. 2021) demonstrated a delivery into mammalian cells of siRNAs targeting classical swine fever virus (*Flaviviridae*) using tetrahedral framework nucleic acids.

27.5 Antiviral Potential of siRNAs

Viruses are obligate intracellular parasites with either RNA or DNA genome enclosed within a protein core. Viruses are fully dependent on cells for their propagation and some of them cause disease in infected organisms. They are generally classified based on the mode of mRNA synthesis, which is determined by the virus genome (Baltimore classification). According to the Baltimore classification, there are seven groups of viruses: viruses with (1) dsDNA genome; (2) ssDNA genome; (3) dsRNA genome; (4) positive-sense single-stranded RNA viruses (+ssRNA); (5) negative-sense single-stranded RNA viruses (-ssRNA);

(6) ssRNA viruses with a DNA intermediate; (7) dsDNA viruses with a DNA intermediate. All these groups, with the only exception of viruses with ssDNA genome, include notorious human pathogens. Despite the fact that more than a hundred antivirals have been approved by now (De Clercq and Li 2016), there is still a need for the development of more efficient antiviral therapies, especially when new viruses are emerging (Khan et al. 2020; Rehman et al. 2021). The development of novel antiviral drugs and vaccines might take years, which is unacceptably long, especially at times of pandemic. In relation to the recent pandemic caused by SARS-CoV-2, RNAi-based antiviral strategies have become in focus of many research group and companies since RNAi is a promising drug modality with a well-understood mechanism of action. Both acute and persistent infections can be cured with antiviral siRNAs (Saulnier et al. 2006, Table 27.1).

RNAi machinery is present in mammals (Schuster et al. 2019), and some mammalian viruses encode protein suppressors of RNAi (Li et al. 2013; Ding et al. 2018; Maillard et al. 2019; Qiu et al. 2020; Zhang et al. 2020). However, the induction of natural antiviral RNAi has been reported only in cells with inferior interferon responses, such as oocytes, embryonic stem cells, and embryonal carcinoma cell lines (for review see Maillard et al. 2019). In general, antiviral RNAi is masked in mammalian cells by a more evolutionary advanced protein-based innate immune response, which is activated by dsRNA, a hallmark of a viral infection (tenOever 2017). Nevertheless, antiviral RNAi can be initiated by the delivery of exogenous siRNAs, and these short dsRNAs, if properly designed, do not induce interferon response. RSV was the first human virus targeted by RNAi in 2001 (Bitko and Barik 2001). Since then multiple research teams have been exploring antiviral potency of exogenous siRNAs (Table 27.1). In all these studies, RNAi was efficiently induced in a dose-dependent and sequence-specific manner and was not related to the non-specific activation of IFN-I responses (see, for instance, Bitko and Barik 2001; Gitlin et al. 2002; Jacque et al. 2002). The cells transfected with siRNAs cognate to viral genome demonstrated a clear reduction in production of new virions with a decrease in virus titer of 10–1000-fold. Furthermore, accumulation of viral mRNAs in infected cells was significantly reduced by antiviral siRNAs, indicating their efficient cleavage with RNAi machinery. At the same time, control sequences, which are unrelated to viral or host proteins, failed to inhibit virus reproduction.

Viral RNA genomes are not always an easy target for siRNAs. For instance, the genomes of dsRNA viruses are enclosed in a proteinaceous core, ssRNA genomes are protected during replication either by nucleoprotein (-ssRNA) or by association with cellular membranes (+ssRNA viruses). Additionally, synthesized RNA is often swiftly encapsidated in a newly formed virus particle. Nevertheless, viral mRNAs are accessible for RNAi, as confirmed by multiple studies (Table 27.1). Thus, encapsidated genomic and antigenomic RNAs of RSV were not affected by RNAi. However, amounts of specific viral mRNAs diminished along with viral proteins expression (Bitko and Barik 2001). HIV-1 genome was inaccessible for HIV-1 specific siRNAs, but expression of HIV RNA from proviral DNA was effectively inhibited (Hu et al. 2002). Alternative siRNA duplexes directed against exposed target sequences can induce degradation of HIV-1 genome (Capodici et al. 2002;

Coburn and Cullen 2002; Jacque et al. 2002). Viruses with RNA genomes or reverse transcribing viruses are replicated with a specific viral enzyme RNA-dependent RNA polymerase (RdRp) or reverse transcriptase (RT), respectively, which are ideal targets for RNAi because similar proteins in mammalian cells were not discovered. A specific and nearly complete loss of viral RNA synthesis can be achieved with siRNAs targeting mRNAs expressing these enzymes, as demonstrated in a number of research works (Table 27.1). Viral RdRps and RTs are highly prone to the incorporation of wrong nucleotides into viral genomes (Bebenek et al. 1989; Duffy 2018), thus generating a population of mutant viruses. It is challenging to design siRNAs that efficiently target all quasispecies of a virus population. These viruses can easily generate escape mutants in the presence of shRNA or siRNA (Chang et al. 2005; Nishitsuji et al. 2006). Effective solution to this problem is to use a pool of siRNAs (at least three siRNAs) to target multiple sites in viral genome and to decrease the possibility of escape mutants. Furthermore, host factors involved in virus replication, but not essential for normal cell physiology can be potentially targeted. Thus, for instance, it was not possible to obtain an escape mutant of HIV-1 when a combination of siRNAs targeting three HIV-1 co-factors, ATG16, ALIX, and TRBP was used (Eekels et al. 2011). An enzymatically produced siRNA pool can cover long target sequences, and it seems very unlikely that a viral genome can acquire enough point mutations to escape the antiviral activity of siRNAs covering viral genome sequence of several hundred nucleotides. Furthermore, it is possible to generate a chimeric dsRNA comprising selected most conservative fragments from an entire viral genome, which upon dicing provides very efficacious siRNA pool targeting multiple viral strains, as was demonstrated in the case of influenza virus A (IVA; Jiang et al. 2019).

RNAi is effective not only against RNA viruses but also against viruses with DNA genomes, such as herpesviruses, papillomaviruses, poxviruses, and polyomaviruses (Table 27.1). The DNA viruses are least likely to escape from siRNAs due to their lower mutation potential. A striking difference of antiviral RNAi from innate and adaptive immune responses is that the viral infection is cleared without causing any visible harm to the infected cell (Gitlin et al. 2002).

Despite multiple tools developed for rational siRNA design, the most potent siRNA sequences are impossible to predict and they need to be verified experimentally. The specificity, with which siRNAs bind to the target sequences, is also an important parameter for efficient silencing. RNAi escape mutants have been reported in vitro for poliovirus (Gitlin et al. 2002), HIV-1 (Boden et al. 2003; Das et al. 2004; von Eije et al. 2008), and HCV (Wilson and Richardson 2005). Even a single nucleotide mismatch can abolish antiviral activity of siRNA (Chiu and Rana 2002; Hamada et al. 2002; Amarzguioui et al. 2003; Nishitsuji et al. 2006; Pusch et al. 2003; Sabariegos et al. 2006). The central 13 nucleotides of the siRNA are critical for RNAi activity, while mismatches at four nucleotides on each terminus can be tolerated (Martinez and Tuschl 2004). Thus, a single siRNA against a conserved sequence of the gene encoding flavivirus envelope protein inhibited replication of both Japanese encephalitis (JEV) and West Nile (WNV) viruses despite a single

mismatch at positions 3 and 21 in JEV and WNV sequences, respectively (Kumar et al. 2006).

Antiviral siRNAs have certain advantages over conventional antivirals and vaccines. First, the siRNAs can be quickly modified to keep up with rapidly evolving viruses. There is a great advance in rational design of siRNAs molecules, which is flexible, and allows swiftly estimate the silencing potential of siRNAs, for instance, based on probability of binding efficiency (Wu and Luo 2021). VIRsiRNAdb database accumulated information on experimentally validated antiviral siRNAs and shRNAs (Thakur et al. 2012), potent sequences from which can be modified to tailor them to emerging viruses. Broad-spectrum antiviral siRNA pool can be developed to target multiple viruses or their variants by a single formulation. Thus, a combination of two siRNAs effectively inhibited replication of RSV and parainfluenza virus (PIV) in the lungs of BALB/c mice with mixed infection (Bitko et al. 2005). A single siRNA targeting conservative sequence in flavivirus envelope protein was active in vivo against related WNV and JEV (Kumar et al. 2006). SiRNAs were broadly affective and protected mice against lethal challenges with IVA, including seasonal and highly pathogenic avian influenza strains of the H5 and H7 subtypes (Tompkins et al. 2004). A broad-spectrum protection against Marburg viruses has been demonstrated for siRNAs with a single mismatch, which prevented death of the rhesus macaques from the lethal challenge with RAVV or MARV, when they were applied at least 1 day before the death of the control animals, i.e., 3–4 days after infection (Thi et al. 2017). SiRNAs act rapidly, specifically, and typically any stage of a virus cycle can be targeted. Only sub-nanomolar amounts of siRNAs are enough to significantly decrease viral titers and RNA amounts. Topical noninvasive administration of siRNAs works efficiently without introducing severe side effects. Efficient i.v. delivery of siRNAs to target different organs and tissues is rapidly developing. Recent massive clinical application of LNP formulations (Moderna and Pfizer SARS-CoV-2 vaccines) is a great advance in nanomedicine, supporting the view of LNPs as safe delivery systems for siRNAs. Antiviral siRNAs have been extensively verified in cell lines, mice, non-human primates (Table 27.1), and they proved their high potency to inhibit replication of target viruses in a dose-dependent sequence-specific manner. Clinical trials, although limited, also provided promising results (Levanova and Poranen 2018). Infections caused by respiratory viruses seem to be ideal targets for siRNAs because of relatively easy delivery to the lungs and upper respiratory tract.

References

Abels JA, Moreno-Herrero F, van der Heijden T, Dekker C, Dekker NH (2005) Single-molecule measurements of the persistence length of double-stranded RNA. Biophys J 88:2737–2744. https://doi.org/10.1529/biophysj.104.052811

Akashi H, Miyagishi M, Yokota T, Watanabe T, Hino T, Nishina K, Kohara M, Taira K (2005) Escape from the interferon response associated with RNA interference using vectors that encode long modified hairpin-RNA. Mol BioSyst 1:382–390. https://doi.org/10.1039/b510159j

Alvarez R, Elbashir S, Borland T, Toudjarska I, Hadwiger P, John M, Roehl I, Morskaya SS, Martinello R et al (2009) RNA interference-mediated silencing of the respiratory syncytial virus nucleocapsid defines a potent antiviral strategy. Antimicrob Agents Chemother 53:3952–3962. https://doi.org/10.1128/AAC.00014-09

Amarzguioui M, Holen T, Babaie E, Prydz H (2003) Tolerance for mutations and chemical modifications in a siRNA. Nucleic Acids Res 31:589–595. https://doi.org/10.1093/nar/gkg147

Bai F, Wang T, Pal U, Bao F, Gould LH, Fikrig E (2005) Use of RNA interference to prevent lethal murine west nile virus infection. J Infect Dis 191:1148–1154. https://doi.org/10.1086/428507

Barichievy S, Saayman S, von Eije KJ, Morris KV, Arbuthnot P, Weinberg MS (2007) The inhibitory efficacy of RNA POL III-expressed long hairpin RNAs targeted to untranslated regions of the HIV-1 5′ long terminal repeat. Oligonucleotides 17:419–431. https://doi.org/10.1089/oli.2007.0095

Barik S (2011) Intranasal delivery of antiviral siRNA. Methods Mol Biol 721:333–338. https://doi.org/10.1007/978-1-61779-037-9_20

Bartlett DW, Davis ME (2006) Insights into the kinetics of siRNA-mediated gene silencing from live-cell and live-animal bioluminescent imaging. Nucleic Acids Res 34:322–333. https://doi.org/10.1093/nar/gkj439

Beaucage SL (2008) Solid-phase synthesis of siRNA oligonucleotides. Curr Opin Drug Discov Dev 11:203–216

Bebenek K, Abbotts J, Roberts JD, Wilson SH, Kunkel TA (1989) Specificity and mechanism of error-prone replication by human immunodeficiency virus-1 reverse transcriptase. J Biol Chem 264:16948–16956

Bitko V, Barik S (2001) Phenotypic silencing of cytoplasmic genes using sequence-specific double-stranded short interfering RNA and its application in the reverse genetics of wild type negative-strand RNA viruses. BMC Microbiol 1:34. https://doi.org/10.1186/1471-2180-1-34

Bitko V, Musiyenko A, Shulyayeva O, Barik S (2005) Inhibition of respiratory viruses by nasally administered siRNA. Nat Med 11:50–55. https://doi.org/10.1038/nm1164

Boden D, Pusch O, Lee F, Tucker L, Ramratnam B (2003) Human immunodeficiency virus type 1 escape from RNA interference. J Virol 77:11531–11535. https://doi.org/10.1128/jvi.77.21.11531-11535.2003

Bohnsack MT, Czaplinski K, Gorlich D (2004) Exportin 5 is a RanGTP-dependent dsRNA-binding protein that mediates nuclear export of pre-miRNAs. RNA 10:185–191. https://doi.org/10.1261/rna.5167604

Borchert GM, Lanier W, Davidson BL (2006) RNA polymerase III transcribes human microRNAs. Nat Struct Mol Biol 13:1097–1101. https://doi.org/10.1038/nsmb1167

Boudreau RL, Monteys AM, Davidson BL (2008) Minimizing variables among hairpin-based RNAi vectors reveals the potency of shRNAs. RNA 14:1834–1844. https://doi.org/10.1261/rna.1062908

Boudreau RL, Martins I, Davidson BL (2009) Artificial microRNAs as siRNA shuttles: improved safety as compared to shRNAs in vitro and in vivo. Mol Ther 17:169–175. https://doi.org/10.1038/mt.2008.231

Bousarghin L, Touze A, Gaud G, Iochmann S, Alvarez E, Reverdiau P, Gaitan J, Jourdan ML, Sizaret PY, Coursaget PL (2009) Inhibition of cervical cancer cell growth by human papillomavirus virus-like particles packaged with human papillomavirus oncoprotein short hairpin RNAs. Mol Cancer Ther 8:357–365. https://doi.org/10.1158/1535-7163.MCT-08-0626

Brandao PE, Castilho JG, Fahl W, Carnieli P Jr, Oliveira Rde N, Macedo CI, Carrieri ML, Kotait I (2007) Short-interfering RNAs as antivirals against rabies. Braz J Infect Dis 11:224–225. https://doi.org/10.1590/s1413-86702007000200011

Brosnan CA, Voinnet O (2009) The long and the short of noncoding RNAs. Curr Opin Cell Biol 21:416–425. https://doi.org/10.1016/j.ceb.2009.04.001

Brummelkamp TR, Bernards R, Agami R (2002) A system for stable expression of short interfering RNAs in mammalian cells. Science 296:550–553. https://doi.org/10.1126/science.1068999

Cai X, Hagedorn CH, Cullen BR (2004) Human microRNAs are processed from capped, polyadenylated transcripts that can also function as mRNAs. RNA 10:1957–1966. https://doi.org/10.1261/rna.7135204

Capodici J, Kariko K, Weissman D (2002) Inhibition of HIV-1 infection by small interfering RNA-mediated RNA interference. J Immunol 169:5196–5201. https://doi.org/10.4049/jimmunol.169.9.5196

Castanotto D, Sakurai K, Lingeman R, Li H, Shively L, Aagaard L, Soifer H, Gatignol A, Riggs A, Rossi JJ (2007) Combinatorial delivery of small interfering RNAs reduces RNAi efficacy by selective incorporation into RISC. Nucleic Acids Res 35:5154–5164. https://doi.org/10.1093/nar/gkm543

Chang LJ, Liu X, He J (2005) Lentiviral siRNAs targeting multiple highly conserved RNA sequences of human immunodeficiency virus type 1. Gene Ther 12:1133–1144. https://doi.org/10.1038/sj.gt.3302509

Cheng Q, Wei T, Farbiak L, Johnson LT, Dilliard SA, Siegwart DJ (2020) Selective organ targeting (SORT) nanoparticles for tissue-specific mRNA delivery and CRISPR-Cas gene editing. Nat Nanotechnol 15:313–320. https://doi.org/10.1038/s41565-020-0669-6

Chiu YL, Rana TM (2002) RNAi in human cells: basic structural and functional features of small interfering RNA. Mol Cell 10:549–561. https://doi.org/10.1016/s1097-2765(02)00652-4

Clayton J (2004) RNA interference: the silent treatment. Nature 431:599–605. https://doi.org/10.1038/431599a

Coburn GA, Cullen BR (2002) Potent and specific inhibition of human immunodeficiency virus type 1 replication by RNA interference. J Virol 76:9225–9231. https://doi.org/10.1128/jvi.76.18.9225-9231.2002

Dallas A, Ilves H, Ge Q, Kumar P, Shorenstein J, Kazakov SA, Cuellar TL, McManus MT, Behlke MA, Johnston BH (2012) Right- and left-loop short shRNAs have distinct and unusual mechanisms of gene silencing. Nucleic Acids Res 40:9255–9271. https://doi.org/10.1093/nar/gks662

Dang Y, Yang Q, Xue Z, Liu Y (2011) RNA interference in fungi: pathways, functions, and applications. Eukaryot Cell 10:1148–1155. https://doi.org/10.1128/EC.05109-11

Das AT, Brummelkamp TR, Westerhout EM, Vink M, Madiredjo M, Bernards R, Berkhout B (2004) Human immunodeficiency virus type 1 escapes from RNA interference-mediated inhibition. J Virol 78:2601–2605. https://doi.org/10.1128/jvi.78.5.2601-2605.2004

Dash PK, Tiwari M, Santhosh SR, Parida M, Lakshmana Rao PV (2008) RNA interference mediated inhibition of Chikungunya virus replication in mammalian cells. Biochem Biophys Res Commun 376:718–722. https://doi.org/10.1016/j.bbrc.2008.09.040

Dennis C (2002) The brave new world of RNA. Nature 418:122–124. https://doi.org/10.1038/418122a

De Clercq E, Li G (2016) Approved antiviral drugs over the past 50 years. Clin Microbiol Rev 29:695–747. https://doi.org/10.1128/CMR.00102-15

DeVincenzo J, Cehelsky JE, Alvarez R, Elbashir S, Harborth J, Toudjarska I, Nechev L, Murugaiah V, Van Vliet A, Vaishnaw AK et al (2008) Evaluation of the safety, tolerability and pharmacokinetics of ALN-RSV01, a novel RNAi antiviral therapeutic directed against respiratory syncytial virus (RSV). Antivir Res 77:225–231. https://doi.org/10.1016/j.antiviral.2007.11.009

Ding SW, Voinnet O (2007) Antiviral immunity directed by small RNAs. Cell 130:413–426. https://doi.org/10.1016/j.cell.2007.07.039

Ding SW, Han Q, Wang J, Li WX (2018) Antiviral RNA interference in mammals. Curr Opin Immunol 54:109–114. https://doi.org/10.1016/j.coi.2018.06.010

Dong H, Song G, Ma D, Wang T, Jing S, Yang H, Tao Y, Tang Y, Shi Y, Dai Z et al (2021) Improved antiviral activity of classical swine fever virus-targeted siRNA by tetrahedral framework nucleic acid-enhanced delivery. ACS Appl Mater Interfaces 13:29416–29423. https://doi.org/10.1021/acsami.1c08143

Dueck A, Meister G (2014) Assembly and function of small RNA—argonaute protein complexes. Biol Chem 395:611–629. https://doi.org/10.1515/hsz-2014-0116

Duffy S (2018) Why are RNA virus mutation rates so damn high? PLoS Biol 16:e3000003. https://doi.org/10.1371/journal.pbio.3000003

Eekels JJ, Geerts D, Jeeninga RE, Berkhout B (2011) Long-term inhibition of HIV-1 replication with RNA interference against cellular co-factors. Antivir Res 89:43–53. https://doi.org/10.1016/j.antiviral.2010.11.005

Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T (2001) Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature 411:494–498. https://doi.org/10.1038/35078107

Erson AE, Petty EM (2008) MicroRNAs in development and disease. Clin Genet 74:296–306. https://doi.org/10.1111/j.1399-0004.2008.01076.x

Fire A, Xu S, Montgomery MK, Kostas SA, Driver SE, Mello CC (1998) Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. Nature 391:806–811. https://doi.org/10.1038/35888

Frank F, Sonenberg N, Nagar B (2010) Structural basis for 5′-nucleotide base-specific recognition of guide RNA by human AGO2. Nature 465:818–822. https://doi.org/10.1038/nature09039

Friedman JM, Jones PA (2009) MicroRNAs: critical mediators of differentiation, development and disease. Swiss Med Wkly 139:466–472

Fu J, Tang ZM, Gao X, Zhao F, Zhong H, Wen MR, Sun X, Song HF, Qian XH (2008) Optimal design and validation of antiviral siRNA for targeting hepatitis B virus. Acta Pharmacol Sin 29:1522–1528. https://doi.org/10.1111/j.1745-7254.2008.00891.x

Ge Q, Filip L, Bai A, Nguyen T, Eisen HN, Chen J (2004) Inhibition of influenza virus production in virus-infected mice by RNA interference. Proc Natl Acad Sci U S A 101:8676–8681. https://doi.org/10.1073/pnas.0402486101

Geisbert TW, Lee AC, Robbins M, Geisbert JB, Honko AN, Sood V, Johnson JC, de Jong S, Tavakoli I, Judge A, Hensley LE, Maclachlan I (2010) Postexposure protection of non-human primates against a lethal Ebola virus challenge with RNA interference: a proof-of-concept study. Lancet 375:1896–1905. https://doi.org/10.1016/S0140-6736(10)60357-1

Giladi H, Ketzinel-Gilad M, Rivkin L, Felig Y, Nussbaum O, Galun E (2003) Small interfering RNA inhibits hepatitis B virus replication in mice. Mol Ther 8:769–776. https://doi.org/10.1016/s1525-0016(03)00244-2

Gitlin L, Karelsky S, Andino R (2002) Short interfering RNA confers intracellular antiviral immunity in human cells. Nature 418:430–434. https://doi.org/10.1038/nature00873

Hamada M, Ohtsuka T, Kawaida R, Koizumi M, Morita K, Furukawa H, Imanishi T, Miyagishi M, Taira K (2002) Effects on RNA interference in gene expression (RNAi) in cultured mammalian cells of mismatches and the introduction of chemical modifications at the 3′-ends of siRNAs. Antisense Nucleic Acid Drug Dev 12:301–309. https://doi.org/10.1089/108729002761381285

Han J, Lee Y, Yeom KH, Nam JW, Heo I, Rhee JK, Sohn SY, Cho Y, Zhang BT, Kim VN (2006) Molecular basis for the recognition of primary microRNAs by the Drosha-DGCR8 complex. Cell 125:887–901. https://doi.org/10.1016/j.cell.2006.03.043

Hassler MR, Turanov AA, Alterman JF, Haraszti RA, Coles AH, Osborn MF, Echeverria D, Nikan M, Salomon WE, Roux L et al (2018) Comparison of partially and fully chemically-modified siRNA in conjugate-mediated delivery in vivo. Nucleic Acids Res 46:2185–2196. https://doi.org/10.1093/nar/gky037

He ML, Zheng B, Peng Y, Peiris JS, Poon LL, Yuen KY, Lin MC, Kung HF, Guan Y (2003) Inhibition of SARS-associated coronavirus infection and replication by RNA interference. JAMA 290:2665–2666. https://doi.org/10.1001/jama.290.20.2665

Hornung V, Ellegast J, Kim S, Brzozka K, Jung A, Kato H, Poeck H, Akira S, Conzelmann KK, Schlee M et al (2006) 5′-Triphosphate RNA is the ligand for RIG-I. Science 314:994–997. https://doi.org/10.1126/science.1132505

Hu WY, Myers CP, Kilzer JM, Pfaff SL, Bushman FD (2002) Inhibition of retroviral pathogenesis by RNA interference. Curr Biol 12:1301–1311. https://doi.org/10.1016/s0960-9822(02)00975-2

Hutvagner G (2005) Small RNA asymmetry in RNAi: function in RISC assembly and gene regulation. FEBS Lett 579:5850–5857. https://doi.org/10.1016/j.febslet.2005.08.071

Idris A, Davis A, Supramaniam A, Acharya D, Kelly G, Tayyar Y, West N, Zhang P, McMillan CLD, Soemardy C et al (2021) A SARS-CoV-2 targeted siRNA-nanoparticle therapy for COVID-19. Mol Ther 29:2219–2226. https://doi.org/10.1016/j.ymthe.2021.05.004

Izant JG, Weintraub H (1984) Inhibition of thymidine kinase gene expression by anti-sense RNA: a molecular approach to genetic analysis. Cell 36:1007–1015. https://doi.org/10.1016/0092-8674(84)90050-3

Jacque JM, Triques K, Stevenson M (2002) Modulation of HIV-1 replication by RNA interference. Nature 418:435–438. https://doi.org/10.1038/nature00896

Jia F, Zhang YZ, Liu CM (2007) Stable inhibition of hepatitis B virus expression and replication in HepG2.2.15 cells by RNA interference based on retrovirus delivery. J Biotechnol 128:32–40. https://doi.org/10.1016/j.jbiotec.2006.09.007

Jiang M, Milner J (2002) Selective silencing of viral gene expression in HPV-positive human cervical carcinoma cells treated with siRNA, a primer of RNA interference. Oncogene 21:6041–6048. https://doi.org/10.1038/sj.onc.1205878

Jiang M, Österlund P, Westenius V, Guo D, Poranen MM, Bamford DH, Julkunen I (2019) Efficient inhibition of avian and seasonal influenza A viruses by a virus-specific Dicer-substrate small interfering RNA swarm in human monocyte-derived macrophages and dendritic cells. J Virol 93:e01916–e01918. https://doi.org/10.1128/JVI.01916-18

Jonas S, Izaurralde E (2015) Towards a molecular understanding of microRNA-mediated gene silencing. Nat Rev Genet 16:421–433. https://doi.org/10.1038/nrg3965

Jonson AL, Rogers LM, Ramakrishnan S, Downs LS Jr (2008) Gene silencing with siRNA targeting E6/E7 as a therapeutic intervention in a mouse model of cervical cancer. Gynecol Oncol 111:356–364. https://doi.org/10.1016/j.ygyno.2008.06.033

Kameoka M, Nukuzuma S, Itaya A, Tanaka Y, Ota K, Ikuta K, Yoshihara K (2004) RNA interference directed against Poly(ADP-Ribose) polymerase 1 efficiently suppresses human immunodeficiency virus type 1 replication in human cells. J Virol 78:8931–8934. https://doi.org/10.1128/JVI.78.16.8931-8934.2004

Kanwal F, Lu C (2019) A review on native and denaturing purification methods for non-coding RNA (ncRNA). J Chromatogr B Anal Technol Biomed Life Sci 1120:71–79. https://doi.org/10.1016/j.jchromb.2019.04.034

Kapadia SB, Brideau-Andersen A, Chisari FV (2003) Interference of hepatitis C virus RNA replication by short interfering RNAs. Proc Natl Acad Sci U S A 100:2014–2018. https://doi.org/10.1073/pnas.252783999

Khaitov M, Nikonova A, Shilovskiy I, Kozhikhova K, Kofiadi I, Vishnyakova L, Nikolskii A, Gattinger P, Kovchina V, Barvinskaia E et al (2021) Silencing of SARS-CoV-2 with modified siRNA-peptide dendrimer formulation. Allergy 76:2840–2854. https://doi.org/10.1111/all.14850

Khan S, Tombuloglu H, Hassanein SE, Rehman S, Bozkurt A, Cevik E, Abdel-Ghany S, Nabi G, Ali A, Sabit H (2020) Coronavirus diseases 2019 (2020): current biological situation and potential therapeutic perspective. Eur J Pharmacol 886:173447. https://www.sciencedirect.com/science/article/pii/S0014299920305392khan

Kim DH, Behlke MA, Rose SD, Chang MS, Choi S, Rossi JJ (2005) Synthetic dsRNA Dicer substrates enhance RNAi potency and efficacy. Nat Biotechnol 23:222–226. https://doi.org/10.1038/nbt1051

Kobayashi T, Chappell JD, Danthi P, Dermody TS (2006) Gene-specific inhibition of reovirus replication by RNA interference. J Virol 80:9053–9063. https://doi.org/10.1128/JVI.00276-06

Konstantinova P, de Vries W, Haasnoot J, ter Brake O, de Haan P, Berkhout B (2006) Inhibition of human immunodeficiency virus type 1 by RNA interference using long-hairpin RNA. Gene Ther 13:1403–1413. https://doi.org/10.1038/sj.gt.3302786

Kong WH, Bae KH, Jo SD, Kim JS, Park TG (2012) Cationic lipid-coated gold nanoparticles as efficient and non-cytotoxic intracellular siRNA delivery vehicles. Pharm Res 29:362–374. https://doi.org/10.1007/s11095-011-0554-y

Kretschmer-Kazemi Far R, Sczakiel G (2003) The activity of siRNA in mammalian cells is related to structural target accessibility: a comparison with antisense oligonucleotides. Nucleic Acids Res 31:4417–4424. https://doi.org/10.1093/nar/gkg649

Kronke J, Kittler R, Buchholz F, Windisch MP, Pietschmann T, Bartenschlager R, Frese M (2004) Alternative approaches for efficient inhibition of hepatitis C virus RNA replication by small interfering RNAs. J Virol 78:3436–3446. https://doi.org/10.1128/jvi.78.7.3436-3446.2004

Kumar P, Lee SK, Shankar P, Manjunath N (2006) A single siRNA suppresses fatal encephalitis induced by two different flaviviruses. PLoS Med 3:e96. https://doi.org/10.1371/journal.pmed.0030096

Layek B, Lipp L, Singh J (2015) Cell penetrating peptide conjugated chitosan for enhanced delivery of nucleic acid. Int J Mol Sci 16:28912–28930. https://doi.org/10.3390/ijms161226142

Lee RC, Feinbaum RL, Ambros V (1993) The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 75:843–854. https://doi.org/10.1016/0092-8674(93)90529-y

Lee NS, Dohjima T, Bauer G, Li H, Li MJ, Ehsani A, Salvaterra P, Rossi J (2002) Expression of small interfering RNAs targeted against HIV-1 rev transcripts in human cells. Nat Biotechnol 20:500–505. https://doi.org/10.1038/nbt0502-500

Lee SK, Dykxhoorn DM, Kumar P, Ranjbar S, Song E, Maliszewski LE, Francois-Bongarcon V, Goldfeld A, Swamy NM, Lieberman J et al (2005) Lentiviral delivery of short hairpin RNAs protects CD4 T cells from multiple clades and primary isolates of HIV. Blood 106:818–826. https://doi.org/10.1182/blood-2004-10-3959

Lee HY, Zhou K, Smith AM, Noland CL, Doudna JA (2013) Differential roles of human Dicer-binding proteins TRBP and PACT in small RNA processing. Nucleic Acids Res 41:6568–6576. https://doi.org/10.1093/nar/gkt361

Levanova A, Poranen MM (2018) RNA interference as a prospective tool for the control of human viral infections. Front Microbiol 9:2151. https://doi.org/10.3389/fmicb.2018.02151

Levanova AA, Kalke KM, Lund LM, Sipari N, Sadeghi M, Nyman MC, Paavilainen H, Hukkanen V, Poranen MM (2020) Enzymatically synthesized 2′-fluoro-modified Dicer-substrate siRNA swarms against herpes simplex virus demonstrate enhanced antiviral efficacy and low cytotoxicity. Antivir Res 182:104916. https://doi.org/10.1016/j.antiviral.2020.104916

Li BJ, Tang Q, Cheng D, Qin C, Xie FY, Wei Q, Xu J, Liu Y, Zheng BJ, Woodle MC et al (2005a) Using siRNA in prophylactic and therapeutic regimens against SARS coronavirus in Rhesus macaque. Nat Med 11:944–951. https://doi.org/10.1038/nm1280

Li T, Zhang Y, Fu L, Yu C, Li X, Li Y, Zhang X, Rong Z, Wang Y, Ning H et al (2005b) siRNA targeting the leader sequence of SARS-CoV inhibits virus replication. Gene Ther 12:751–761. https://doi.org/10.1038/sj.gt.3302479

Li Y, Lu J, Han Y, Fan X, Ding SW (2013) RNA interference functions as an antiviral immunity mechanism in mammals. Science 342:231–234. https://doi.org/10.1126/science.1241911

Lipfert J, Skinner GM, Keegstra JM, Hensgens T, Jager T, Dulin D, Kober M, Yu Z, Donkers SP, Chou FC et al (2014) Double-stranded RNA under force and torque: similarities to and striking differences from double-stranded DNA. Proc Natl Acad Sci U S A 111:15408–15413. https://doi.org/10.1073/pnas.1407197111

Liu J, Carmell MA, Rivas FV, Marsden CG, Thomson JM, Song JJ, Hammond SM, Joshua-Tor L, Hannon GJ (2004) Argonaute2 is the catalytic engine of mammalian RNAi. Science 305:1437–1441. https://doi.org/10.1126/science.1102513

Liu X, Cao S, Zhou R, Xu G, Xiao S, Yang Y, Sun M, Li Y, Chen H (2006) Inhibition of Japanese encephalitis virus NS1 protein expression in cell by small interfering RNAs. Virus Genes 33: 69–75. https://doi.org/10.1007/s11262-005-0039-3

Liu YP, Haasnoot J, Berkhout B (2007) Design of extended short hairpin RNAs for HIV-1 inhibition. Nucleic Acids Res 35:5683–5693. https://doi.org/10.1093/nar/gkm596

López T, Rojas M, Ayala-Bretón C, López S, Arias CF (2005) Reduced expression of the rotavirus NSP5 gene has a pleiotropic effect on virus replication. J Gen Virol 86:1609–1617. https://doi.org/10.1099/vir.0.80827-0

Lundstrom K (2020) Viral vectors applied for RNAi-based antiviral therapy. Viruses 12:924. https://doi.org/10.3390/v12090924

Luo KQ, Chang DC (2004) The gene-silencing efficiency of siRNA is strongly dependent on the local structure of mRNA at the targeted region. Biochem Biophys Res Commun 318:303–310. https://doi.org/10.1016/j.bbrc.2004.04.027

Ma H, Dallas A, Ilves H, Shorenstein J, MacLachlan I, Klumpp K, Johnston BH (2014) Formulated minimal-length synthetic small hairpin RNAs are potent inhibitors of hepatitis C virus in mice with humanized livers. Gastroenterology 146:63–6.e5. https://doi.org/10.1053/j.gastro.2013.09.049

Macrae IJ, Li F, Zhou K, Cande WZ, Doudna JA (2006) Structure of Dicer and mechanistic implications for RNAi. Cold Spring Harb Symp Quant Biol 71:73–80. https://doi.org/10.1101/sqb.2006.71.042

MacRae IJ, Ma E, Zhou M, Robinson CV, Doudna JA (2008) In vitro reconstitution of the human RISC-loading complex. Proc Natl Acad Sci U S A 105:512–517. https://doi.org/10.1073/pnas.0710869105

Maillard PV, van der Veen AG, Poirier EZ, Reis e Sousa C (2019) Slicing and dicing viruses: antiviral RNA interference in mammals. EMBO J 38:e100941. https://doi.org/10.15252/embj.2018100941

Martinez J, Tuschl T (2004) RISC is a 5′ phosphomonoester-producing RNA endonuclease. Genes Dev 18:975–980. https://doi.org/10.1101/gad.1187904

Mathews DH (2010) Using OligoWalk to identify efficient siRNA sequences. Methods Mol Biol 629:109–121. https://doi.org/10.1007/978-1-60761-657-3_8

Mattick JS (2001) Non-coding RNAs: the architects of eukaryotic complexity. EMBO Rep 2:986–991. https://doi.org/10.1093/embo-reports/kve230

Mattick JS, Makunin IV (2005) Small regulatory RNAs in mammals. Hum Mol Genet 14:R121–R132. https://doi.org/10.1093/hmg/ddi101

McCaffrey AP, Nakai H, Pandey K, Huang Z, Salazar FH, Xu H, Wieland SF, Marion PL, Kay MA (2003) Inhibition of hepatitis B virus in mice by RNA interference. Nat Biotechnol 21:639–644. https://doi.org/10.1038/nbt824

McCarthy SM, Gilar M, Gebler J (2009) Reversed-phase ion-pair liquid chromatography analysis and purification of small interfering RNA. Anal Biochem 390:181–188. https://doi.org/10.1016/j.ab.2009.03.042

McCaskill J, Singhania R, Burgess M, Allavena R, Wu S, Blumenthal A, McMillan NA (2013) Efficient biodistribution and gene silencing in the lung epithelium via intravenous liposomal delivery of siRNA. Mol Ther Nucleic Acids 2:e96. https://doi.org/10.1038/mtna.2013.22

Meister G, Tuschl T (2004) Mechanisms of gene silencing by double-stranded RNA. Nature 431: 343–349. https://doi.org/10.1038/nature02873

Müller S, Günther S (2007) Broad-spectrum antiviral activity of small interfering RNA targeting the conserved RNA termini of Lassa virus. Antimicrob Agents Chemother 51:2215–2218. https://doi.org/10.1128/AAC.01368-06

Naito Y, Nohtomi K, Onogi T, Uenishi R, Ui-Tei K, Saigo K, Takebe Y (2007) Optimal design and validation of antiviral siRNA for targeting HIV-1. Retrovirology 4:80. https://doi.org/10.1186/1742-4690-4-80

Nellen W, Lichtenstein C (1993) What makes an mRNA anti-sense-itive? Trends Biochem Sci 18: 419–423. https://doi.org/10.1016/0968-0004(93)90137-c

Nishitsuji H, Kohara M, Kannagi M, Masuda T (2006) Effective suppression of human immunodeficiency virus type 1 through a combination of short- or long-hairpin RNAs targeting essential sequences for retroviral integration. J Virol 80:7658–7666. https://doi.org/10.1128/JVI.00078-06

Nitschinsk KM, Clarke DT, Idris A, McMillan NA (2018) RNAi targeting of human metapneumovirus P and N genes inhibits viral growth. Intervirology 61:149–154. https://doi.org/10.1159/000491927

Novina CD, Murray MF, Dykxhoorn DM, Beresford PJ, Riess J, Lee SK, Collman RG, Lieberman J, Shankar P, Sharp PA (2002) siRNA-directed inhibition of HIV-1 infection. Nat Med 8:681–686. https://doi.org/10.1038/nm725

Nygardas M, Vuorinen T, Aalto AP, Bamford DH, Hukkanen V (2009) Inhibition of coxsackievirus B3 and related enteroviruses by antiviral short interfering RNA pools produced using phi6 RNA-dependent RNA polymerase. J Gen Virol 90:2468–2473. https://doi.org/10.1099/vir.0.011338-0

Oliveira S, van Rooy I, Kranenburg O, Storm G, Schiffelers RM (2007) Fusogenic peptides enhance endosomal escape improving siRNA-induced silencing of oncogenes. Int J Pharm 331:211–214. https://doi.org/10.1016/j.ijpharm.2006.11.050

O'Loughlin AJ, Mäger I, de Jong OG, Varela MA, Schiffelers RM, El Andaloussi S, Wood MJA, Vader P (2017) Functional delivery of lipid-conjugated siRNA by extracellular vesicles. Mol Ther 25:1580–1587. https://doi.org/10.1016/j.ymthe.2017.03.021

Paavilainen H, Romanovskaya A, Nygårdas M, Bamford DH, Poranen MM, Hukkanen V (2015) Innate responses to small interfering RNA pools inhibiting herpes simplex virus infection in astrocytoid and epithelial cells. Innate Immun 21(4):349–357. https://doi.org/10.1177/1753425914537921

Paavilainen H, Lehtinen J, Romanovskaya A, Nygårdas M, Bamford DH, Poranen MM, Hukkanen V (2016) Inhibition of clinical pathogenic herpes simplex virus 1 strains with enzymatically created siRNA pools. J Med Virol 88:2196–2205. https://doi.org/10.1002/jmv.24578

Paavilainen H, Lehtinen J, Romanovskaya A, Nygardas M, Bamford DH, Poranen MM, Hukkanen V (2017) Topical treatment of herpes simplex virus infection with enzymatically created siRNA swarm. Antivir Ther 22:631–637. https://doi.org/10.3851/IMP3153

Palliser D, Chowdhury D, Wang QY, Lee SJ, Bronson RT, Knipe DM, Lieberman J (2006) An siRNA-based microbicide protects mice from lethal herpes simplex virus 2 infection. Nature 439:89–94. https://doi.org/10.1038/nature04263

Park WS, Miyano-Kurosaki N, Hayafune M, Nakajima E, Matsuzaki T, Shimada F, Takaku H (2002) Prevention of HIV-1 infection in human peripheral blood mononuclear cells by specific RNA interference. Nucleic Acids Res 30:4830–4835. https://doi.org/10.1093/nar/gkf627

Patra JK, Das G, Fraceto LF, Campos EVR, Rodriguez-Torres MDP, Acosta-Torres LS, Diaz-Torres LA, Grillo R, Swamy MK, Sharma S et al (2018) Nano based drug delivery systems: recent developments and future prospects. J Nanobiotechnol 16:71. https://doi.org/10.1186/s12951-018-0392-8

Pichlmair A, Schulz O, Tan CP, Naslund TI, Liljestrom P, Weber F, Reis e Sousa C (2006) RIG-I-mediated antiviral responses to single-stranded RNA bearing 5′-phosphates. Science 314:997–1001. https://doi.org/10.1126/science.1132998

Pusch O, Boden D, Silbermann R, Lee F, Tucker L, Ramratnam B (2003) Nucleotide sequence homology requirements of HIV-1-specific short hairpin RNA. Nucleic Acids Res 31:6444–6449. https://doi.org/10.1093/nar/gkg876

Qiu Y, Xu YP, Wang M, Miao M, Zhou H, Xu J, Kong J, Zheng D, Li RT, Zhang RR et al (2020) Flavivirus induces and antagonizes antiviral RNA interference in both mammals and mosquitoes. Sci Adv 6:eaax7989. https://doi.org/10.1126/sciadv.aax7989

Rajewsky N (2006) microRNA target predictions in animals. Nat Genet 38(Suppl):S8–S13. https://doi.org/10.1038/ng1798

Randall G, Grakoui A, Rice CM (2003) Clearance of replicating hepatitis C virus replicon RNAs in cell culture by small interfering RNAs. Proc Natl Acad Sci U S A 100:235–240. https://doi.org/10.1073/pnas.0235524100

Rangan R, Zheludev IN, Hagey RJ, Pham EA, Wayment-Steele HK, Glenn JS, Das R (2020) RNA genome conservation and secondary structure in SARS-CoV-2 and SARS-related viruses: a first look. RNA 26:937–959. https://doi.org/10.1261/rna.076141.120

Rehman S, Ravinayagam V, Nahvi I, Aldossary H, Al-Shammari M, Amiri MS, Kishore U, Al-Suhaimi EA (2021) Immunity, sex hormones, and environmental factors as determinants of COVID-19 disparity in women. Front Immunol 2021:3279. https://www.frontiersin.org/articles/10.3389/fimmu.2021.680845/full

Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE, Horvitz HR, Ruvkun G (2000) The 21-nucleotide let-7 RNA regulates developmental timing in Caenorhabditis elegans. Nature 403:901–906. https://doi.org/10.1038/35002607

Reynolds A, Leake D, Boese Q, Scaringe S, Marshall WS, Khvorova A (2004) Rational siRNA design for RNA interference. Nat Biotechnol 22:326–330. https://doi.org/10.1038/nbt936

Rodriguez A, Griffiths-Jones S, Ashurst JL, Bradley A (2004) Identification of mammalian microRNA host genes and transcription units. Genome Res 14:1902–1910. https://doi.org/10.1101/gr.2722704

Romanovskaya A, Paavilainen H, Nygardas M, Bamford DH, Hukkanen V, Poranen MM (2012) Enzymatically produced pools of canonical and Dicer-substrate siRNA molecules display comparable gene silencing and antiviral activities against herpes simplex virus. PLoS One 7:e51019. https://doi.org/10.1371/journal.pone.0051019

Romanovskaya A, Sarin LP, Bamford DH, Poranen MM (2013) High-throughput purification of double-stranded RNA molecules using convective interaction media monolithic anion exchange columns. J Chromatogr A 1278:54–60. https://doi.org/10.1016/j.chroma.2012.12.050

Saayman S, Barichievy S, Capovilla A, Morris KV, Arbuthnot P, Weinberg MS (2008) The efficacy of generating three independent anti-HIV-1 siRNAs from a single U6 RNA Pol III-expressed long hairpin RNA. PLoS One 3:e2602. https://doi.org/10.1371/journal.pone.0002602

Sabariegos R, Gimenez-Barcons M, Tapia N, Clotet B, Martinez MA (2006) Sequence homology required by human immunodeficiency virus type 1 to escape from short interfering RNAs. J Virol 80:571–577. https://doi.org/10.1128/JVI.80.2.571-577.2006

Sabbioni S, Callegari E, Spizzo R, Veronese A, Altavilla G, Corallini A, Negrini M (2007) Anticancer activity of an adenoviral vector expressing short hairpin RNA against BK virus T-ag. Cancer Gene Ther 14:297–305. https://doi.org/10.1038/sj.cgt.7701014

Saha A, Bhagyawant SS, Parida M, Dash PK (2016) Vector-delivered artificial miRNA effectively inhibited replication of Chikungunya virus. Antivir Res 134:42–49. https://doi.org/10.1016/j.antiviral.2016.08.019

Sánchez AB, Perez M, Cornu T, de la Torre JC (2005) RNA interference-mediated virus clearance from cells both acutely and chronically infected with the prototypic arenavirus lymphocytic choriomeningitis virus. J Virol 79:11071–11081. https://doi.org/10.1128/JVI.79.17.11071-11081.2005

Sano M, Li H, Nakanishi M, Rossi JJ (2008) Expression of long anti-HIV-1 hairpin RNAs for the generation of multiple siRNAs: advantages and limitations. Mol Ther 16:170–177. https://doi.org/10.1038/sj.mt.6300298

Sarisozen C, Salzano G, Torchilin VP (2016) Lipid-based siRNA delivery systems: challenges, promises and solutions along the long journey. Curr Pharm Biotechnol 17:728–740. https://doi.org/10.2174/1389201017666160401145319

Saulnier A, Pelletier I, Labadie K, Colbere-Garapin F (2006) Complete cure of persistent virus infections by antiviral siRNAs. Mol Ther 13:142–150. https://doi.org/10.1016/j.ymthe.2005.07.697

Sayed D, Abdellatif M (2011) MicroRNAs in development and disease. Physiol Rev 91:827–887. https://doi.org/10.1152/physrev.00006.2010

Schuster S, Miesen P, van Rij RP (2019) Antiviral RNAi in insects and mammals: parallels and differences. Viruses 11:448. https://doi.org/10.3390/v11050448

Schwarz DS, Hutvagner G, Du T, Xu Z, Aronin N, Zamore PD (2003) Asymmetry in the assembly of the RNAi enzyme complex. Cell 115:199–208. https://doi.org/10.1016/s0092-8674(03)00759-1

Selvam C, Mutisya D, Prakash S, Ranganna K, Thilagavathi R (2017) Therapeutic potential of chemically modified siRNA: recent trends. Chem Biol Drug Des 90:665–678. https://doi.org/10.1111/cbdd.12993

Seo MY, Abrignani S, Houghton M, Han JH (2003) Small interfering RNA-mediated inhibition of hepatitis C virus replication in the human hepatoma cell line Huh-7. J Virol 77:810–812. https://doi.org/10.1128/jvi.77.1.810-812.2003

Shlomai A, Shaul Y (2003) Inhibition of hepatitis B virus expression and replication by RNA interference. Hepatology 37:764–770. https://doi.org/10.1053/jhep.2003.50146

Sohail M, Doran G, Riedemann J, Macaulay V, Southern EM (2003) A simple and cost-effective method for producing small interfering RNAs with high efficacy. Nucleic Acids Res 31:e38. https://doi.org/10.1093/nar/gng038

Sohrab SS, El-Kafrawy SA, Mirza Z, Hassan AM, Alsaqaf F, Azhar EI (2021) Designing and evaluation of MERS-CoV siRNAs in HEK-293 cell line. J Infect Public Health 14:238–243. https://doi.org/10.1016/j.jiph.2020.12.018

Soutschek J, Akinc A, Bramlage B, Charisse K, Constien R, Donoghue M, Elbashir S, Geick A, Hadwiger P, Harborth J et al (2004) Therapeutic silencing of an endogenous gene by systemic administration of modified siRNAs. Nature 432:173–178. https://doi.org/10.1038/nature03121

Stark GR, Kerr IM, Williams BR, Silverman RH, Schreiber RD (1998) How cells respond to interferons. Annu Rev Biochem 67:227–264. https://doi.org/10.1146/annurev.biochem.67.1.227

Taniguchi T, Endo KI, Tanioka H, Sasaoka M, Tashiro K, Kinoshita S, Kageyama M (2020) Novel use of a chemically modified siRNA for robust and sustainable in vivo gene silencing in the retina. Sci Rep 10:22343. https://doi.org/10.1038/s41598-020-79242-w

tenOever BR (2017) Questioning antiviral RNAi in mammals. Nat Microbiol 2:17052. https://doi.org/10.1038/nmicrobiol.2017.52

ter Brake O, Konstantinova P, Ceylan M, Berkhout B (2006) Silencing of HIV-1 with RNA interference: a multiple shRNA approach. Mol Ther 14:883–892. https://doi.org/10.1016/j.ymthe.2006.07.007

Thakur N, Qureshi A, Kumar M (2012) VIRsiRNAdb: a curated database of experimentally validated viral siRNA/shRNA. Nucleic Acids Res 40:D230–D236. https://doi.org/10.1093/nar/gkr1147

Thi EP, Mire CE, Lee AC, Geisbert JB, Zhou JZ, Agans KN, Snead NM, Deer DJ, Barnard TR, Fenton KA, MacLachlan I, Geisbert TW (2015) Lipid nanoparticle siRNA treatment of Ebola-virus-Makona-infected nonhuman primates. Nature 521:362–365. https://doi.org/10.1038/nature14442

Thi EP, Mire CE, Lee AC, Geisbert JB, Ursic-Bedoya R, Agans KN, Robbins M, Deer DJ, Cross RW, Kondratowicz AS, Fenton KA, MacLachlan I, Geisbert TW (2017) siRNA rescues nonhuman primates from advanced Marburg and Ravn virus disease. J Clin Invest 127:4437–4448. https://doi.org/10.1172/JCI96185

Tompkins SM, Lo CY, Tumpey TM, Epstein SL (2004) Protection against lethal influenza virus challenge by RNA interference in vivo. Proc Natl Acad Sci U S A 101:8682–8686. https://doi.org/10.1073/pnas.0402630101

Torchilin VP, Trubetskoy VS (1995) Which polymers can make nanoparticulate drug carriers long-circulating? Adv Drug Deliv Rev 16:141–155. https://doi.org/10.1016/0169-409X(95)00022-Y

Tycowski KT, Guo YE, Lee N, Moss WN, Vallery TK, Xie M, Steitz JA (2015) Viral noncoding RNAs: more surprises. Genes Dev 29:567–584. https://doi.org/10.1101/gad.259077.115

Ullah A, Qazi J, Rahman L, Kanaras AG, Khan WS, Hussain I, Rehman A (2020) Nanoparticles-assisted delivery of antiviral-siRNA as inhalable treatment for human respiratory viruses: a

candidate approach against SARS-COV-2. Nano Sel 12. https://doi.org/10.1002/nano.202000125

Vert JP, Foveau N, Lajaunie C, Vandenbrouck Y (2006) An accurate and interpretable model for siRNA efficacy prediction. BMC Bioinformatics 7:520. https://doi.org/10.1186/1471-2105-7-520

Vigne S, Germi R, Duraffour S, Larrat S, Andrei G, Snoeck R, Garin D, Crance JM (2008) Specific inhibition of orthopoxvirus replication by a small interfering RNA targeting the D5R gene. Antivir Ther 13:357–368

Vigne S, Duraffour S, Andrei G, Snoeck R, Garin D, Crance JM (2009) Inhibition of vaccinia virus replication by two small interfering RNAs targeting B1R and G7L genes and their synergistic combination with cidofovir. Antimicrob Agents Chemother 53:2579–2588. https://doi.org/10.1128/AAC.01626-08

Villegas PM, Ortega E, Villa-Tanaca L, Barrón BL, Torres-Flores J (2018) Inhibition of dengue virus infection by small interfering RNAs that target highly conserved sequences in the NS4B or NS5 coding regions. Arch Virol 163:1331–1335. https://doi.org/10.1007/s00705-018-3757-2

von Eije KJ, ter Brake O, Berkhout B (2008) Human immunodeficiency virus type 1 escape is restricted when conserved genome sequences are targeted by RNA interference. J Virol 82:2895–2903. https://doi.org/10.1128/JVI.02035-07

Wang Z, Ren L, Zhao X, Hung T, Meng A, Wang J, Chen YG (2004) Inhibition of severe acute respiratory syndrome virus replication by small interfering RNAs in mammalian cells. J Virol 78:7523–7527. https://doi.org/10.1128/JVI.78.14.7523-7527.2004

Watanabe T, Sudoh M, Miyagishi M, Akashi H, Arai M, Inoue K, Taira K, Yoshiba M, Kohara M (2006) Intracellular-diced dsRNA has enhanced efficacy for silencing HCV RNA and overcomes variation in the viral genotype. Gene Ther 13:883–892. https://doi.org/10.1038/sj.gt.3302734

Watts JK, Deleavey GF, Damha MJ (2008) Chemically modified siRNA: tools and applications. Drug Discov Today 13:842–855. https://doi.org/10.1016/j.drudis.2008.05.007

Weinberg MS, Ely A, Barichievy S, Crowther C, Mufamadi S, Carmona S, Arbuthnot P (2007) Specific inhibition of HBV replication in vitro and in vivo with expressed long hairpin RNA. Mol Ther 15:534–541. https://doi.org/10.1038/sj.mt.6300077

Wightman B, Ha I, Ruvkun G (1993) Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in C. elegans. Cell 75:855–862. https://doi.org/10.1016/0092-8674(93)90530-4

Wilson RC, Doudna JA (2013) Molecular mechanisms of RNA interference. Annu Rev Biophys 42:217–239. https://doi.org/10.1146/annurev-biophys-083012-130404

Wilson JA, Richardson CD (2005) Hepatitis C virus replicons escape RNA interference induced by a short interfering RNA directed against the NS5b coding region. J Virol 79:7050–7058. https://doi.org/10.1128/JVI.79.11.7050-7058.2005

Wu R, Luo KQ (2021) Developing effective siRNAs to reduce the expression of key viral genes of COVID-19. Int J Biol Sci 17:1521–1529. https://doi.org/10.7150/ijbs.59151

Wu CJ, Huang HW, Liu CY, Hong CF, Chan YL (2005) Inhibition of SARS-CoV replication by siRNA. Antivir Res 65:45–48. https://doi.org/10.1016/j.antiviral.2004.09.005

Wu SY, Putral LN, Liang M, Chang HI, Davies NM, McMillan NA (2009a) Development of a novel method for formulating stable siRNA-loaded lipid particles for in vivo use. Pharm Res 26:512–522. https://doi.org/10.1007/s11095-008-9766-1

Wu Y, Navarro F, Lal A, Basar E, Pandey RK, Manoharan M, Feng Y, Lee SJ, Lieberman J, Palliser D (2009b) Durable protection from Herpes Simplex Virus-2 transmission following intravaginal application of siRNAs targeting both a viral and host gene. Cell Host Microbe 5:84–94. https://doi.org/10.1016/j.chom.2008.12.003

Yokota T, Sakamoto N, Enomoto N, Tanabe Y, Miyagishi M, Maekawa S, Yi L, Kurosaki M, Taira K, Watanabe M et al (2003) Inhibition of intracellular hepatitis C virus replication by synthetic and vector-derived small interfering RNAs. EMBO Rep 4:602–608. https://doi.org/10.1038/sj.embor.embor840

Yoshinari K, Miyagishi M, Taira K (2004) Effects on RNAi of the tight structure, sequence and position of the targeted region. Nucleic Acids Res 32:691–699. https://doi.org/10.1093/nar/gkh221

Zhang H, Kolb FA, Jaskiewicz L, Westhof E, Filipowicz W (2004a) Single processing center models for human Dicer and bacterial RNase III. Cell 118:57–68. https://doi.org/10.1016/j.cell.2004.06.017

Zhang Y, Li T, Fu L, Yu C, Li Y, Xu X, Wang Y, Ning H, Zhang S, Chen W et al (2004b) Silencing SARS-CoV Spike protein expression in cultured cells by RNA interference. FEBS Lett 560:141–146. https://doi.org/10.1016/S0014-5793(04)00087-0

Zhang Y, Li Z, Ye Z, Xu Y, Wang B, Wang C, Dai Y, Lu J, Lu B, Zhang W et al (2020) The activation of antiviral RNA interference not only exists in neural progenitor cells but also in somatic cells in mammals. Emerg Microbes Infect 9:1580–1589. https://doi.org/10.1080/22221751.2020.1787798

Zheng BJ, Guan Y, Tang Q, Du C, Xie FY, He ML, Chan KW, Wong KL, Lader E, Woodle MC et al (2004) Prophylactic and therapeutic effects of small interfering RNA targeting SARS-coronavirus. Antivir Ther 9:365–374