Malay K. Das
Yashwant V. Pathak *Editors*

Nano Medicine and Nano Safety

Recent Trends and Clinical Evidences

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ISBN 978-981-15-6254-9 ISBN 978-981-15-6255-6 (eBook)
https://doi.org/10.1007/978-981-15-6255-6

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

For All the Gentle and Wonderful Readers

Preface

Nanomedicine—the application of nanotechnology to human health—is a promising field of research at the interface of physical, chemical, biological, and medical science. Nanomedicine is an interdisciplinary field in which nanotechnology, nanoscience, and nanoengineering interact with life sciences and biotechnology. Furthermore, nanoparticles are an attractive vehicle for drug targeting and long-term drug releasing to a targeted site.

Nanomedicine and Nanosafety: Recent Trends and Clinical Evidences describes a broad area of nanomedicine which mainly focuses on concept, development, and clinical application of nanomedicine including regulatory, safety, and marketing aspects of nanomedicine. The present book has been divided into three parts: The first part contains applications of nanobiotechnology in the development of nanomedicine. The second part is devoted to concept, development, clinical applications, and evidences of nanomedicine. The third part discusses regulatory, safety, and marketing aspects of nanomedicine. This book presents a broad spectrum of topics on nanomedicine drug delivery/drug targeting, nanobiotechnology in clinical diagnosis, nanomaterials for alternative antibiotic therapy, herbal nanomedicine, pulmonary nanomedicine, transdermal nanomedicine, nanotheranostics in healthcare, nanomedicine safety/clinical toxicity, and commercial/business perspectives of nanomedicine.

This book would be useful as a reference and guide for students, academics, researchers, chemists, biologists, pharmaceutical scientists, nanoscientists, nanobiotechnologists, biomedical engineers, clinicians, and healthcare professionals.

Dibrugarh, India — Malay K. Das
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Part I

Applications of Nano Biotechnology in the Development of Nano Medicine

Nanobiotechnology and Its Application in Nanomedicine: An Overview

1

Trinayan Deka, Malay K. Das, Sanjoy Das, L. Ronibala Singha, and Punamjyoti Das

Abstract

Nanomedicine is the application of nanobiotechnologies to medicine. This chapter highlights the recent trends and applications of nanobiotechnology in emerging fields of nanodiagnostics, nanotherapeutics, and nanotheranostics including clinical nanomedicine.

Keywords

Applied nanobiotechnology · Nanomedicine · Nanodiagnostics · Nanotherapeutics · Nanooncology · Nanocardiology

1.1 Introduction

The term nanotechnology is derived from the Greek word "nano" that means "dwarf" (short man). The term "Nano" means very tiny in size, the scale 10^{-9} m or less. All natural materials and systems have their roots at the nanoscale. The basic material for any living organism, i.e., DNA itself has a nano size. Nanotechnology is the science that deals with materials of nano size range. The most emerging field of science and technology is nanobiotechnology that brings together biology, chemistry, physics, and many areas of engineering, biotechnology, and medicine [1]. Nanobiotechnology has been evolved as an entirely new scientific and technological area from the fusion of nanotechnology and biotechnology. It reflects the demanding importance of nanoscience and nanotools in the generation of novel biomaterials for use in tissue engineering, nanosensors used in diagnostics, nanopores that facilitate the passage of single molecules for DNA sequencing,

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M. K. Das, Y. V Pathak (eds.), *Nano Medicine and Nano Safety*,
https://doi.org/10.1007/978-981-15-6255-6_1

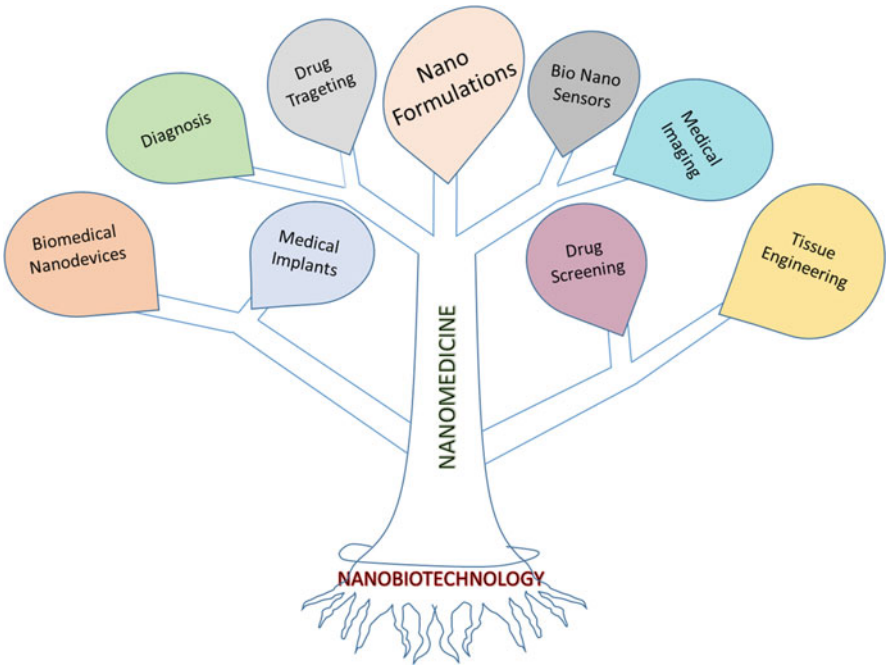

Fig. 1.1 Emerging of nanomedicine by application of nanobiotechnology

nanomaterials for application in imaging single molecules or cells, and devices for therapeutic application [2–5]. By integrating innovative applications of nanotechnology into modern biological issues, many approaches of life sciences are being developed by nanobiotechnology [6].

One of the most elegant emerging fields of applied nanobiotechnology is nanomedicine. Nanobiotechnology has its vast application in different branches of medical science. Tissue engineering, advance medical imaging, clinical diagnosis, nano drug formulation, nanobiosensors are some of the important areas where nanobiotechnology plays a leading role (Fig. 1.1).

The use of nanobiotechnology to revolutionize the medical fields is being highly focused by the scientist. The basic physiological mechanisms of an organism occur at molecular level, i.e., in the nano scale. Understanding different molecular level mechanisms related to physiological changes leads to development of new ideas in the medical perspectives. Drug targeting at molecular level, use of nanosensors, nanopores, quantum dots are some of the significant examples of recent development in nanomedicine [7–10]. It plays a vital role in advanced biology and medical analysis notably within the development of potential targeted delivery systems with lower drug toxicities and higher efficiencies. It has applications in almost each medical branch like neurological disorders (nanoneurology), eye diseases (nanoophthalmology), cardiovascular disorders (nanocardiology), cancer (nanooncology), diseases of skeletal system (nanoorthopedics), and infectious

diseases. Although the application of engineering to drugs seems to be a comparatively recent trend, the basic nanotechnology approaches for medical application dates back to several decades [11–15]. Nanomedicine can likewise be viewed as a refinement of sub-atomic medication and coordinates in genomics and proteomics to encourage the improvement of customized medication. Nanobiotechnology affects the advancement of nanomedicine both legitimately just as by improving different trains, for example, the delivery of nanopharmaceuticals and atomic diagnostics. Similar advancements encourage the improvement of personalized medication corresponding to nanomedicine [7]. Nanotechnologies can expand the limits of current molecular diagnostics and empower purpose of care diagnostics, theranostics, and advancement of personalized medication [16, 17].

1.1.1 Advantages of Nanomedicine

Nanomedicine is being applied to design site specific drug delivery, new techniques for diagnosis and imaging. The advantages of nanomedicine can be categorized into the benefits of nanotherapeutics, nanodiagnostics, and nanotheranostics [16–19] (Table 1.1). This chapter highlights the application of nanobiotechnology in different fields of nanomedicines. Diagnostic, therapeutic, and clinical application of nanomedicines including recent patents are also discussed.

Table 1.1 Advantages of nanomedicine

Advantages of nanomedicine		
Nanotherapeutics	Nanodiagnostics	Nanotheranostics
• Increases drug absorption for effective treatment • Increases drug retention time for higher efficacy • Useful to minimize the amount of active drug for treatment • Provides site specific drug delivery • Helps to deliver drug across blood–brain barrier • Reduced drug toxicity	• Greater optical and magnetic features facilitate effective color coding and labeling of biomarkers for diagnosis • Detection of disease at very early stage made possible by molecular diagnostics • Fast and more accurate detection • Helps to identify the target site for therapy	• Capable of diagnosis and delivery of therapy to the diseased cells • Provides the capacity for personalized medicine

1.2 Application of Nanobiotechnology in Nano Medicine

1.2.1 Diagnosis

Conventional diagnoses for most of the diseases are done by physical examination of symptoms. As the symptom may take time to appear, treatment for those illnesses may have lost their effectiveness. It would be convenient and effective if a disease can be detected at the earliest state of its occurrence. Molecular diagnosis plays a notable role to identify pathogens and diseased cells at very early stage of disease with no symptoms. Nanobiotechnology alters the way of diagnosis by improving sensitivity and better efficacy. Major nanodiagnostics application of nanobiotechnology includes nanobiosensors, biochips and microarrays, nanopore technology, biobarcode, nanoparticle based imaging and labeling, nanoproteomic based diagnosis [20]. Most of the nanodiagnostics technologies are in clinical use and still many are at their development phase.

1.2.1.1 Nanobiosensors

It is one of the most hopeful, concise systems consisting of a biological element (responsible for sampling), and a physical element or transducer (transmitting sampling results for further processing). Detecting an analyte using a transducer by utilizing biochemical reaction to quantify the amount of analyte is the working principle of biosensors [21]. For instance, carbon nanotubes (CNTs) show appropriate electrochemical properties for label-free and multiplexed point-of-care biosensitivity. These were effectively utilized to identify ions, metabolites, and protein biomarkers [22, 23]. They had been used for detection of prostate cancer [24]. CNT-based optical nanobiosensors have been effectively utilized for the determination of immunoglobulins, surface-enhanced Raman spectroscopy (SERS) based biomedical imaging, and phototherapy [25, 26]. Aptamer-AuNPs hybrid frameworks have been showed useful for the identification of specific tumor cells [27, 28]. Comparable hybrid frameworks have been used for combined in vitro imaging and photothermal treatment in oral cancer epithelial cells [29]. Quantum dots (QDs) based lab-on-chip, multiplexed sandwiched immunoassay has been utilized to recognize various lung cancer related biomarkers, for example, carcinoembryonic antigen (CEA), cytokeratin 19 pieces (CYFRA21-1), and neuron-explicit enolase (NSE) in biological fluid [30]. QD based nanosensor was in a nuclease-enzyme-based amplification approach for fluorescence resonance energy transfer (FRET)-based detection of femtomolar concentrations of miRNA [31]. Electrochemical molecularly bioimprinted siloxane biosensor has been utilized for ultra-sensing of gemcitabine as a lung cancer chemotherapy medication [32]. AuNRs modified by ssDNA probes of cadF gene have been developed for precise detection of *Campylobacter jejuni* and *Campylobacter coli* [33]. AuNPs conjugated mesoporous silica-graphene oxide nanoconstructs were effectively utilized for optical bioimaging in colorimetric tumor cell diagnosis [34]. Silicon nanowires (SiNWs) were utilized in sensors as field effect transistors (FETs).

FET-SiNWs have been appeared to detect numerous prostate cancer biomarkers, for example, PSA (prostate-specific antigen) at very early stage [35].

1.2.1.2 Biochips/Microarray

These are nanoscale devices (normally made of glass or silicon base) to coordinate various processes for DNA/protein analysis. These chips are highly sensitive to interact with cellular constituents. Receptor-functionalized nanomotors are capable to isolate biological targets, for example, pancreatic cancer cells and *E. coli* from biological samples [36, 37]. Protein microarrays have been used in analysis of protein level in colon carcinoma cells with exposure to ionizing radiation [38, 39]. This protein analysis helps in the differentiation of the protein level in normal cells compared to premature and metastatic cancer cells [40]. Protein microarray-based analyses of protein–protein interaction and IgE immunoassay for allergy diagnosis have been reported [38, 41, 42].

1.2.1.3 Nanopore Technology

A nanopore is a pore of nanometer size. It comprises a pore-forming protein or as a pore in silicon or graphene. It has been well reported to be used in DNA sequencing. Working principle is the detection of the ionic current passing through it as a voltage is applied across the membrane [43, 44]. Label-free detection of post-translational modifications of protein has been achieved by using single-cell biological nanopore and has tremendous potential for disease diagnosis and cell biology [45]. Bacterial lower respiratory tract infections using nanopore sequencing has been reported as rapid and potential clinical diagnosis tool to replace culture diagnosis [46]. It is applied for cancer diagnosis and treatment through the identification and accurate estimation of MicroRNA (miRNA—cancer biomarkers) and the determination of aberrant DNA methylation as a robust biomarker in cancer. It offers, utilizing MinION stage (Oxford Nanopore Technologies), a viable technique for quick, genome-wide screening of salmonoid RNA virus, with significant potential applications for diagnostics and details investigation concerning the origins and spread of disease outbreaks [47].

1.2.1.4 Biobarcode

Nanoparticle based biobarcode assay is an ultrasensitive and powerful strategy for the determination of biological targets, for example, proteins and nucleic acids. It works on two target specific probes: Magnetic micro beads (MMB) bearing biological probe to identify the target and the second gold nanoparticles (AuNPs) bearing target binding molecule, called biobarcode (an oligonucleotide) [48–51]. The biobarcode method has been successfully used for rapid and reliable detection of amyloid-derived diffusible ligands (ADDL) in cerebrospinal liquid (CSF) for the clinical diagnosis of Alzheimer's disease [52], *E. coli* O157:H7 microbes by means of AuNP labeling and inductively coupled plasma mass spectrometry (ICP-MS) [53], hepatitis C virus (HCV) core antibodies utilizing a TaqMan probe [54]. This method was reported to be potential diagnostic tool for the detection

of PSA [55], the *Vibrio cholerae* O1 OmpW gene [56], and *Staphylococcus aureus* protein A [57].

1.2.1.5 Nanoparticle Based Imaging and Labeling

Nanotechnologies offer various opportunities for improving existing and designing of new imaging methods. Nanoparticles of perfluorohydrocarbons coated with a lipid layer have been reported as an ultrasonic contrast agent [58]. Iron oxide NPs have been clinically applied as MRI contrast agent, for example, superparamagnetic nanoparticles [59], ultra-small SPIO improves MRI for imaging cerebral ischemic injuries and dextran-coated iron oxide nanoparticle improves MRI visualization of intracranial tumors [60, 61]. The fluorescent in situ hybridization (FISH) combined with conventional fluorescence microscopy and fluorescence confocal microscopy have been successfully used to localize abnormal gene related to a disease and to diagnose and differentiate infected erythrocytes from normal erythrocytes [9, 62, 63]. Surface-enhanced Raman scattering (SERS) is generally applied in the detection of small quantity of circulating tumor cells, RNA, nucleic acid, lipids, and proteins present in blood samples [64–69]. It is also used in cancer diagnosis. The single-photon emission computed tomography (SPECT) and positron-emission tomography (PET) have been reported for radiotracer-based targeted in vivo imaging [70].

1.2.1.6 Nanoproteomic Based Diagnosis

Nanoproteomics can reveal critical information related to rare cell populations, hard-to-obtain clinical specimens, the cellular heterogeneity of pathological tissues, and disease biomarkers. These information help in early diagnosis of a disease and monitoring of disease progression. Magnetic nanospherical probes functionalized with antibodies were utilized to recognize anti-HSA antibody [71]. Identification of target autoantibody GDC glutamate decarboxylase (Type 1 diabetes) was successfully accomplished by utilizing supramolecular nanoprobes [72]. AuNP or europium NP-based bio-barcode identification approach utilized for signal intensification of HIV-1 p24 immunoassay [73]. Sol–gel immobilized nanostructure zinc film was utilized for *Neisseria gonorrhoeae* identification [74]. Serum small extra vesicles proteome of tuberculosis patients showed typical deregulation and consequently, could be helpful for designing alternate host-directed therapeutic interventions [75]. The sputum proteomics study helps to separate active TB from non-TB patients with moderate accuracy [76].

1.2.2 Therapeutics

Today nanoformulation plays a leading role in drug delivery and development. Due to several advantages like high efficacy, site specific delivery over conventional drug therapy, nanotherapeutics is now highly focused for achieving health benefits. Polymeric nanoparticles, liposomes, nanogels, *si*RNA, dendrimers, and gene drug delivery are some of the highly anticipated nanobiotechnology used in therapeutic nanomedicine application. Over the past few decades, the US FDA has approved

100 nanomedicine formulations [77]. This shows that nanotechnology is playing an immense role in today's biomedical science [78, 79].

1.2.2.1 Polymeric Nanoformulation

The polymeric nanoparticles are fabricated from synthetic and/or natural polymers. The synthetic polymers are preferred over natural one due to their good availability with higher purity, batch to batch reproducibility, and controlled release behavior for the entrapped drug(s) [80]. Some examples of biodegradable and non-biodegradable polymers commonly used in the preparation of polymeric nanoparticles are polylactide (PLA), poly lactide-co-glycolide, copolymers (PLGA) and poly (-ε-caprolactone), polyacrylates, and poly (methyl methacrylate) [81]. Both hydrophilic and hydrophobic drugs can be encapsulated into polymeric nanoparticles by emulsion solvent evaporation, double emulsion solvent evaporation technique, or other suitable methods. A few of the popularly marketed polymeric nanoparticles are Decapeptyl®, Gonapeptyl Depot®, Enantone Depot®, and Abraxane [82, 83].

1.2.2.2 Liposomes

Liposome based drug delivery systems enhance the therapeutic indices of various drugs through alterations in their pharmacokinetics and pharmacodynamics. A few liposome products have become commercially available for the management of various cancer and fungal infections. For examples, Doxil® for the treatment of ovarian cancer and AIDS-related Kaposi's sarcoma [84], DaunoXome® for the management of advanced HIV-associated Kaposi's sarcoma [85]. A few more commercial liposome products are Depocyt® by SkyPharma Inc., Myocet® by Elan Pharmaceuticals, Mepact® by Takeda Pharmaceutical, Marqibo® by Talon Therapeutics [86–88], and Onivyde™ by Merrimack Pharmaceuticals, Inc. [86–89]. For fungal infections, the US FDA approved Amphotec® and Ambisome® in 1996 and 1997, respectively [90, 91].

1.2.2.3 Nanogels

A nanogel is a nanoparticle composed of a hydrogel—a crosslinked hydrophilic polymer network. Various bioactive compounds such as DNA, proteins, and drugs can be encapsulated in polymeric mesh for drug delivery for various biomedical applications [92, 93]. The preparation methods include micro-molding and photolithographic methods, continuous microfluidics, and free radical polymerization techniques [94]. Chitin nanogel based clobetasol (anti-psoriatic drug) exhibited strong cytotoxicity towards THP-1 and HaCaT cell lines by MTT assay [95]. Sane Care Nanogel, Zyflex Nanogel, Augen Nanogel Eye-care Gel, Skin Perfect Brightening Nanogel, and Oxalgin Nanogel formulation are commercially available [96].

1.2.2.4 siRNA

Small interfering RNA (siRNA), sometimes known as short interfering RNA or silencing RNA, is a class of double-stranded RNA. It is an exciting new tool in molecular biology and the next frontier in molecular medicine [97–99]. The therapeutic advantages of siRNAs for treatment of viral infection, dominant disorders,

cancer, and neurological disorders show great promise. Gold nanorod and trimer of N-acetylgalactosamine (GalNAc) have been reported as carrier for the delivery of siRNA [100–103]. In another study, siRNA targeting Beclin1 was conjugated to ferric–cobalt electro-magnetic nanomaterial (CoFe2O4@ BaTiO3; MENP-siBeclin1) to deliver siRNA into the brain. This novel drug delivery system was effective against HIV-1 infection following on-demand release of siRNA using an in vitro human BBB model [104]. Anticancer siRNA therapeutics has no or negligible side effects as compared to chemotherapeutics. Scientists have tried to target undruggable oncogenes like k-RAS or c-MYC siRNA in mouse model to develop RNAi based therapeutics [105]. siRNAs have been used against BCR/ABL transcripts induced apoptosis [106]. siRNAs have also been used to target K-RAS transcripts carrying the valine-112 oncogenic mutation (K-RASV112) [107]. SphK1 siRNA or JSI-124 showed strong pro-inflammatory effects on the progression of ulcerative colitis, which may be the therapeutic target for its treatment [108].

1.2.2.5 Dendrimers

Dendrimers are three-dimensional, globular hyper branched polymeric nanoarchistructures. Arginine terminated peptide dendrimers, along with sonophoresis improved the transdermal penetration of ketoprofen [109]. The most widely used dendrimers for pharmaceutical uses are poly-propyleneimine (PPI, AstromolR, DAB) [110] and polyamidoamine (PAMAM; Starburst) dendrimers [111]. The multifunctional dendrimers can carry cancer cell specific molecule, anticancer drug and molecule that recognizes the signals of cell death. Dendrimers are also capable for on-demand drug release within the cancer microenvironment [112–114].

1.2.2.6 Gene Drug Delivery

Gene delivery is the process of introducing foreign genetic material, such as DNA or RNA, into host cells. Genetic material must reach the nucleus of the host cell to induce gene expression. It is essential in gene therapy of human genetic diseases. The gene therapy is a promising therapy for inherited disorders, viral infection, and cancers. DNA-based gene delivery systems have been carried out for lentivirus, poxvirus, adenovirus, adeno-related virus, retrovirus, human foamy virus (HFV), and herpes virus [115]. RNA-based gene delivery systems have been done for HIV with lenti-viral vectors-adjusted CD 34(+) cells in patients experiencing transplantation for AIDS-related lymphoma [116]. In case of cancer, the cytokine immune-gene therapy is a promising strategy [117–119]. The previous literatures report on the development of gene delivery carriers. For examples, the cationic non-viral lipid-based gene carriers “lipoplexes” [120, 121], biodegradable poly (ethyleneimine) for plasmid DNA delivery [122], branched poly (ethyleneimine)-cholesterol water-soluble lipo-polymers [123], and polyethylene glycol-grafted poly (L-lysine) as polymeric gene carriers [124, 125] have been developed.

1.2.2.7 Other Nanoformulations

Nanoparticulate drug delivery systems can alter the PK/PD of poorly soluble drugs by increasing their solubility and bioavailability. Drugs loaded in NPs can be protected against external environment making them less sensitive to physical/chemical changes due to photo-oxidation [126–128]. US FDA approved nanocrystal drug formulations for target specific delivery, dose reduction, and enhanced safety profile. For examples, Tricor (fenofibrate, AbbVie), Emend (Aprepitant, Merck), and MAT2501 nanocrystal (Amikacin) [129–131]. Drugs can also be encapsulated into lipid and/or polymer core to alter their PK/PD properties [132, 133]. For examples, resveratrol loaded lipid-core nanocapsules (RSV-LNC) for targeting colon cancer [134] and ciprofloxacin loaded SLNs for better antibacterial activity [135]. Various delivery routes such as oral, dermal, pulmonary, ocular, and rectal routes have been investigated for the administration of nanocapsules [136–141]. Some iron oxide nanodrugs have been approved by US FDA for iron replacement therapies. For examples, Venofer (iron sucrose infusion, American Regent, Inc.), Ferrlecit (sodium ferric gluconate complex in sucrose infusion, Sanofi-Aventis U.S.), Infed (iron dextran infusion, Actavis Pharma), and Dexferrum (iron dextran infusion, American Regent, Inc.) indicated for anemia associated with chronic kidney disease [130]. There are also colloidal gold bound tumor necrosis factor and TNF-bound colloidal gold for anticancer effects [131].

1.2.2.8 Nano Surgery

Nanobiotechnology has significant application in the field of surgery. The development of surgical nanorobot is a significant achievement in the field of surgery. It can act as a semi-autonomous on-site surgeon inside the body guided by a human surgeon. Surgical procedures are performed through various functions such as pathology, diagnosing and correcting lesions by nanomanipulation via coded ultrasound signals, coordinated by an on-board computer and a human surgeon [142]. In femtosecond laser surgery, femtolaser is considered as a pair of nanoscissors by vaporizing tissue locally while leaving adjacent tissue unharmed [143]. Proteolytic liposomal NPs of collagenase were reported to enhance periodontal remodeling of the oral connective tissues that replaced surgical blades [144]. Nanorobotic microbivores have been developed for spying and removing unwanted pathogens from bloodstream [145].

1.2.2.9 Medical Implants

Medical implants are devices or tissues that are placed inside or on the surface of the body as prosthetics or for drug delivery or to control physiological functions or for giving support to body parts. Previous literatures showed that a significant development has been done in the field of medical implants using nanobiotechnology. Titanium spinal implants with surface modification through the addition of titanium oxide/zirconium nanoparticles have shown increased bone formation compared to conventional smooth implants [146]. Cervical cages modified with silicon nitride nanoparticles have shown multiple biomechanical advantages and commercially available [147]. The nanoLOCK™ by Titan Spine technology has been found to

induce a higher osteogenic and angiogenic growth factors than with traditional titanium polyether-ether-ketone cages [148]. Additionally, arthroplasty implants [149], orthodontic implants [150], dental nanorobots [151], and dental implants [152] have also been reported in the literatures. Some commercially available medical implants include Nano TiteTM (Bicon LLC, Boston, USA), Nano TiteTM (Biomet 3i, Palm Beach Gardens, USA), OSSEANTM (IntraLock International, Boca Raton, FL, USA), and Osseo SpeedTM (Astra Tech, AB, Mölndal, Sweden) [153].

1.2.3 Clinical Advances and Patents

Currently, the approval process for nanomedicines in humans is regulated by the US FDA, and is essentially the same as that for any other regulated drug, device, or biologic [77]. As of October 2019, 68 clinical trials including the term "nano" were listed as "recruiting" or "active" on ClinicalTrials.gov. Likewise, 165 clinical trials including the term "liposome" were listed [154]. Both diagnostic and therapeutic nanomedicine those are in clinical trials or recently patented are listed in Tables 1.2 and 1.3.

1.3 Challenges for Nanobiotechnology in Nanomedicine

The use of nanobiotechnology to nanomedicines is being expanding and developed day by day. However, numerous difficulties have also been faced by the researcher, industry, and regulators for doing as such [156]. Characterization of novel nanocompounds for their safety and toxicity is one of the major difficulties in nanomedicine development. Huge efforts have been given to discover how structure of nanoparticles and their properties like charge, size, shape, surface coats, and so on interact with living body system [129]. Lack of specific protocols for assessing of nanomedicines at the physicochemical and biological level influence their development [157]. US FDA has published guidelines regarding the importance of nanomaterial characterization [129]. The Nanotechnology Characterization Laboratory (NCL), set up by the National Cancer Institute, has additionally published guidelines about innovative platforms for the development of nanodrugs for cancer treatment [156]. Reports have been released in regard to the tendency of certain NPs to show toxicity at molecular, cellular, and tissue level [158]. Biological toxicities of NPs include oxidative stress, inflammation, immunotoxicity, genotoxicity, neurotoxicity, and carcinogenicity [159]. The harmful properties of NPs might be used positively for surgical removal of diseased tissues and cancer immunotherapy. Additionally, the toxicity of NPs can be reduced through surface coating with hydrophilic polymers to improve cell viability [160]. Cost for the development and regulatory approval of nanoformulations is other challenge, which is difficult to compensate for low selling nanomedicine. The withdrawal of nanomedicines from the market post FDA approval may be due to the toxicity issues. For examples,

Table 1.2 Recent nanomedicines in clinical development [154]

Name	Description	Condition/disease	Phase	Clinical trial no	Sponsor
Zinc oxide nanoparticles	Antibacterial effect of laser diode and zinc oxide nano particles in dental cavity disinfection	Dental caries	–	NCT03478150	Cairo University
Nano-crystalline hydroxyapatite silica gel	Clinical and radiographic evaluation of nano-crystalline hydroxyapatite silica gel in comparison with open flap debridement for management of periodontal intrabony defects	Chronic periodontitis	–	NCT02507596	Cairo University
Nano polymer-free sirolimus-eluting stents	Evaluation of the safety, efficacy, and deliverability of the combo bio-engineered sirolimus-eluting stent versus the nano polymer-free sirolimus-eluting stents in the treatment of patients with de novo stenotic lesions of native coronary artery	Coronary arteriosclerosis	–	NCT02542007	OrbusNeich
BCMA nano antibody CAR-T	Evaluation of the safety and efficacy of BCMA nano antibody CAR-T in the treatment of multiple myeloma	Relapsed and refractory multiple myeloma	I	NCT03661554	The Pregene (ShenZhen) Biotechnology Company, Ltd
Aminolevulinic acid nano emulsion	Comparison of three photosensitizers, hexylaminolevulinate (HAL), and aminolevulinic acid nano emulsion (BF-200 ALA) to methylaminolevulinate (MAL) in photodynamic therapy of superficially growing basal cell carcinomas	Carcinoma, basal cell	II	NCT02367547	Joint Authority for Päijät-Häme Social and Health Care
Ultrasmall superparamagnetic iron oxides (USPIO)of ferumoxtran-10	Evaluation of the diagnostic accuracy of an USPIO contrast agent (ferumoxtran-10) in combination with 7 tesla MRI to detect lymph node metastases in rectal and breast cancer	Rectal neoplasms Breast neoplasms	III	NCT02751606	Radboud University

(continued)

Table 1.2 (continued)

Name	Description	Condition/disease	Phase	Clinical trial no	Sponsor
Nano-crystalline hydroxyapatite	Evaluation of the histomorphometric study of nano-crystalline hydroxyapatite (nano bone) with lovastatin in the preservation of the tooth socket	Bone loss	II	NCT03981601	Islamic Azad University, Tehran
USPIO nanoparticles	Investigation of inflammation of cranial and meningeal arteries during pharmacologically induced migraine attacks, using ultrasmall superparamagnetic iron oxide (USPIO) nanoparticles and black blood imaging (BBI) MRI	Migraine headache Migraine without aura	–	NCT02549898	Danish headache center
Anti-CD19 CAR-T cells injection	Evaluation of the safety and clinical activity of anti-CD19 chimeric antigen receptor T cells (KD-019 CAR-T) infusion in the treatment of relapsed/refractory B-cell lymphoma and B-cell acute lymphoblastic leukemia	B-cell lymphoma B-cell acute lymphoblastic leukemia	I	NCT03854994	Yan'an Affiliated Hospital of Kunming Medical University
Ceramide nanoliposome	A dose escalation study of ceramide nanoliposome in patients with advanced solid tumors	Carcinoma solid tumors	I	NCT02834611	Keystone Nano, Inc
Nano-albumin bound paclitaxel	Testing of a combination of chemotherapy of carboplatin, nano-albumin bound paclitaxel, and durvalumab against surgically resectable squamous cell carcinoma of the head and neck	Carcinoma, squamous cell Oral cancer Oropharynx cancer Larynx cancer Lip cancer Esophageal cancer	II	NCT03174275	UNC Lineberger Comprehensive Cancer Center

NanoKnife LEDC system	Evaluation of irreversible electroporation (IRE) therapy works in treating patients with breast cancer. IRE kills tumor cells by electrical impulses creating nanopore on the cell membrane and inducing target cell death	Breast cancer	–	NCT02340858	Fuda Cancer Hospital, Guangzhou
Nanomicellar curcumin	Determining the effects of supplementation of nanomicellar curcumin on glycemic control, serum lipid profile, blood pressure, and anthropometric measurements in patients with metabolic syndrome	Metabolic syndrome	–	NCT03534024	National Nutrition and food technology institute
Nanostructured titanium dental implant "KONTACT N"	Evaluating the clinical outcome of dental implants "Kontact N"; and the effects of its nanostructured surface on the osseointegration and secondary stability without increasing the rate of peri-implantitis	Implant-supported fixed prosthesis	–	NCT03582657	Biotech dental
Liposomal amphotericin B	Comparison of the pharmacokinetic exposure to liposomal amphotericin B between critically ill patients and non-critically ill (hematology) patients in an early and late exposure day	Hematological patients	IV	NCT03529617	Universitaire Ziekenhuizen Leuven
Irinotecan hydrochloride liposome injection (LY01610)	Evaluation of the safety and tolerability, the maximum tolerated dose (MTD), and the dose limited toxicity (DLT) of LY01610 monotherapy and combine with 5-Fu in patients with advanced solid tumors	Advanced solid tumor	I	NCT04088604	Luye Pharma Group Ltd.

(continued)

Table 1.2 (continued)

Name	Description	Condition/disease	Phase	Clinical trial no	Sponsor
Polyethylene glycol liposome doxorubicin	Clinical application of polyethylene glycol liposome doxorubicin (PLD) in primary lymphoma	Lymphoma, non-Hodgkin; Hodgkin disease	IV	NCT02526823	Shandong Provincial Hospital
MPER-656 liposome vaccine	Evaluation of the safety and immunogenicity of an HIV-1 gp41 MPER-656 liposome vaccine in healthy, HIV-uninfected adult participants	HIV infections	I	NCT03934541	National Institute of Allergy and Infectious Diseases (NIAID)
Liposomal bupivacaine	Enhanced recovery with liposomal bupivacaine in orthognathic surgery	Pain, postoperative	IV	NCT03844451	University of Texas at Austin
Ethosomal and liposomal preparations of anthralin	Formulation and clinical evaluation of ethosomal and liposomal preparations of anthralin in psoriasis	Psoriasis vulgaris	IV	NCT03348462	Assiut University
Oxiconazole nitrate SLNs loaded gel	Clinical assessment of oxiconazole nitrate solid lipid nanoparticles loaded gel	Tinea fungal diseases	I	NCT03823040	Minia University
Docetaxel-polymeric micelles	Evaluation of the effects and safety of first line docetaxel-PM and oxaliplatin weekly administration chemotherapy for the participants with inoperable or metastatic esophageal squamous cell carcinoma	Esophagus squamous cell carcinoma (SCC) Metastatic cancer	II	NCT03585673	Sung Yong Oh

Table 1.3 Recent US FDA approved nanomedicines [155]

Name	Type	Indication	Year
Esperoct	Antihemophilic factor (recombinant), glycopegylated-exei	Use to treat and control bleeding in adults and children with hemophilia A	2019
Jivi	Antihemophilic factor [recombinant] PEGylated-aucl	Use in previously treated adults and adolescents (12 years of age and older) with hemophilia A (congenital factor VIII deficiency)	2018
Vyxeos	Liposomal combination of daunorubicin, and cytarabine	Treatment of adults with newly-diagnosed therapy-related acute myeloid leukemia (t-AML) or AML with myelodysplasia-related changes (AML-MRC)	2017
Onivyde	Irinotecan liposome injection	Combination with fluorouracil and leucovorin, for the treatment of patients with metastatic adenocarcinoma of the pancreas after disease progression following gemcitabine-based therapy	2015
Marqibo	Vincristine encapsulated in sphingomyelin/cholesterol liposomes	Treatment of adolescent young adult with Philadelphia chromosome-negative (Ph-) acute lymphoblastic leukemia	2012
Exparel	Bupivacaine liposome injectable suspension	Administration into the surgical site to produce postsurgical analgesia	2011
Ozurdex	Intravitreal implant containing dexamethasone in the Novadur solid polymer sustained-release drug delivery system	Treatment of macular edema following branch retinal vein occlusion (BRVO) or central retinal vein occlusion (CRVO)	2009

Feruglose and Resovist. Hence, phase 4 post-marketing pharmacovigilance is the major concern to further assess the safety of nanomedicine [161].

1.4 Conclusion and Future Aspects

Nanotechnology provides innovative nanodevices and nanosystems those are much smaller than a human cell. Such tools can be used at molecular and cellular levels to kill cancer cells or take over the function of subcellular organelles. Nanodiagnostics will enable routine detection of single particles of viruses or bacteria in minuscule samples. Nanobiotechnology will give nanodevices to look at tissues in minute details. Biosensors those are smaller than a cell would provide us an interior check out of cellular operation. With lab-on-chip utility using nanobiochips, routine check of diagnostic parameters of patients will come to a precise and fast effective theranostic measure. Research is increasing daily to find out newer drug delivery options, newer targeting strategies for medicinal products by the use of nanobiotechnology. Successful implementation of liposomal carrier system, Doxil^{R},

in drug market is a land mark of nanomedicine that inspires industry and regulators to bring forward many more nanodrug formulations for human use. Increasing scenario of the clinical trials and FDA approved nanoformulation represent the growth of awareness utility and knowledge in this area. Such trends in nanoverse will lead to hand to hand use of medical application in the near future.

Nanomedicines have shown nice potential to handle clinical needs in various diseases. However, toxicity and ethical problems with nanomedicine are the major challenges. Fortunately, with the outburst of public and scientific awareness of nanobiotechnology, there is a detail discussion on these ethical and toxicological issues. The potential applications provided by nanotechnology for diagnosis, prevention, and treatment of diseases are presently terribly broad. Therefore, to pursue the sensible application of nanomedicine, there is a demand of straightforward approaches, and systematic development in conjunction with creativeness and visionary power.

Acknowledgement The authors are grateful to the Indian Council of Medical Research (ICMR), Government of India, for offering Senior Research Fellowship to Trinayan Deka to pursue his research works in the Department of Pharmaceutical Sciences, Dibrugarh University, India. S. D acknowledges the Dibrugarh University Research Fellowship (DURF) to carry out this research works.

Conflict of Interest The authors declare there is no conflict of interest. All the tables and figures are original and self-made.

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Nanobiotechnology for Therapeutic Targeting of Circulating Tumor Cells in the Blood

2

Nikhil Biswas, Bhanu P. Sahu, and Malay K. Das

Abstract

Cancer-associated mortality and morbidity is linked to tumor metastasis in more than 90% cases. Primary tumor sheds millions of circulating tumor cells (CTCs) in the blood circulation as single or clusters of cells every day, which may initiate the metastases in the presence of metastatic precursors. Very little is known about the biology of CTCs and their role in cancer metastasis. An increased understanding of the biology and heterogeneity of CTCs and their interaction with other cells can help us to understand the metastatic process in details so that we can identify novel drug targets. Conventional cancer therapeutics, the most common mode of cancer treatment currently employed in the clinic, have a relatively short circulation time in the blood, which may render the killing of CTCs inefficient due to reduced exposure of CTCs to drugs. So therapeutic targeting of CTCs in the bloodstream and neutralization may be a good approach to prevent the metastasis even before its initiation and increase survival outcomes. Recent developments in the field of nanoscale-material science and nanobiotechnology allow the researchers to continuously explore new nanoplatforms for therapeutic targeting and eliminating CTCs efficiently and thereby inhibiting tumor metastasis. In this chapter, we discuss the relation of CTCs with metastatic progression. We also talk about the recent advances in CTC-targeted cancer therapy exploiting the unique properties of the nanomaterials. We conclude by introducing developments in CTC-directed nanosystems and other advanced technologies currently in (pre) clinical research.

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M. K. Das, Y. V Pathak (eds.), *Nano Medicine and Nano Safety*,
https://doi.org/10.1007/978-981-15-6255-6_2

Keywords

Circulating tumor cells (CTCs) · Nanobiotechnology · Therapeutic targeting · Cancer metastasis · Biology of CTCs

2.1 Introduction

Cancer is the second leading cause of death worldwide after heart disease and is responsible for an estimated 9.6 million deaths in 2018 of which 70% was reported from low- and middle-income countries [1]. Chemotherapy, the most common mode of cancer treatment currently employed in the clinic, on one side has severe side effects due to nonspecific damage to normal cells and on the other side shows limited and individually different therapeutic responses [2]. With the hope to overcome those existing problems, new possibilities are constantly being explored for more active and less toxic (to normal cell) treatment alternatives.

The vast majority of research works in the field of cancer blames cancer metastasis as the key cause behind the cancer-related deaths, which accounts for almost 90% of the total mortality [3, 4]. Though it is not completely understood, roughly speaking, cancer metastasis is a complex sequential process, which involves the shedding of cells from the primary tumor, invasion through the surrounding tissues, penetration of basement membranes, entry into the blood vessels, survival within the blood, exit the bloodstream, extravasation and premetastatic niche formation, and ultimately growth into a fully developed metastatic lesion in distant organs [5].

Circulating tumor cells (CTCs) are cells (single or clusters) that have shed into the vasculature or lymphatic from a primary tumor and are carried around the body in the blood circulation. CTCs in the blood are very common because a fully matured primary tumor sheds millions of cells every day, but metastasis is very rare, which indicates that the process of metastasis is very inefficient and very specific in time. So early detection and neutralization of CTCs in the blood may be a good therapeutic approach for arresting tumor metastasis.

Till date, FDA has not approved any drug that can prevent cancer metastasis by targeting it [6]. The recent knowledge of molecular profile, isolation techniques of CTCs, and their pivotal role in metastatic progression provides sufficient reason to target CTCs to eliminate cancer. It is an interesting idea to isolate CTCs from the blood sample of the deceased and then profile these CTCs to consider the best therapeutic treatment options. With the fast technological progress in the field of nanomaterials and its applications in biomedical, the scientists are designing and formulating different types of therapeutic systems for eliminating CTCs and arrest metastasis. In this chapter, we discuss the relation of CTCs with metastatic progression. We also talk about the recent advances in CTC-targeted cancer therapy exploiting the unique properties of nanomaterials.

2.2 Biology of Circulating Tumor Cells

Metastasis is a process by which primary tumor or cancer spreads to distant organs through the circulatory or lymphatic system and was found responsible for 90% cancer-related deaths. This is a complex process that demands the cancer cells to acquire diversity at precise times. The primary tumor sheds millions of circulating tumor cells every day (CTC) in the bloodstream among which a very few successfully colonize in the distant organs and transform into a detectable lesion following few sequential steps. These are a detachment of CTCs from the primary tumor body, invasion through tissues surrounding the initial lesion, cell migration, survival within the blood, migration through the lymphatic system, arrest at secondary and primary sites, extravasation at distant organs like lungs, liver, brain, bones, engraftment at distant sites, and colonization [7, 8]. During this complete process, these circulating cancer cells (CTC) must employ some type of camouflage to avoid being detected and eventually get destroyed by the natural defense system of the body.

2.2.1 Survival of CTCs in Blood Circulation

When CTCs enter the blood circulation system, they are presented with many challenges, which eventually lead to their apoptotic death. So, in order to survive, they need to rectify incorrect cellular or extracellular matrix binding and overcome the resistance provided by NK cells, macrophages, oxidative stress, and shear force [9, 10]. Only a few very selective CTCs adapt to conceal and evade the innate immune system to transform into a successful distant metastasis [11]. CTCs migrate successfully during stress by redesigning the expression profile of integrins and activating the protein kinase B/akt signaling pathway by a distinct context-sensitive mechanism [12]. CTCs escape phagocytic death by properly attaching with macrophages through the upregulation of surface-associated proteins like CD47, PDL1, and vascular cell adhesion molecule 1 (VCAM-1). To circumvent the oxidative stress in the blood, CTCs reduce NADPH production by interfering with DHFR enzyme in the folate metabolic pathway [13]. Recent reports suggest that the tissue factor proteins (TF) present on the surface of CTCs have the propensity to attract and attach with platelets, which, in turn, counter the oxidative stress by triggering the reversible metabolic changes in CTCs and protect itself in the blood circulation by forming microclots after binding with CD11b+macrophages [14].

2.2.2 Entry of CTCs in the Bloodstream

CTCs enter the bloodstream mainly by two known mechanisms—direct intrvasation through the blood vessels and indirectly via lymphatic system. Active and passive transport may be involved in both cases. The American Joint Committee on Cancer (AJCC) have indicated to use the lymphatic node spread as a marker for the diagnosis of most of the stage IV cancer in breast, colon, liver, and lungs. Though

according to the AJCC, the status of the lymph node is one of the key determinants in the metastatic spread, there is not much practical evidence, which proves that the CTCs necessarily need to be carried out by the lymphatic system prior to developing distant metastasis. Till date, it is not clear whether lymphatic spread truly contributes to the development of a distant tumor or just a marker of invasive disease.

It is hard to develop an in vitro model of CTC entry into the blood vessel and to access it in vivo. It may be possible that the direct intrvasation of CTCs into the bloodstream takes place through the compromised blood vessel or hemorrhage into the tumor. There is not much evidence of this passive shedding of CTCs into the bloodstream and how often it occurs. In the active transport process, shaded CTCs move from primary tumor surrounding stroma to the blood vessel due to the nutrient and growth factor gradients and then penetrate the vessel wall. Tumor cell-intrinsic factors like N-WASP protein regulate the development process of invadopodium, which, in turn, helps the CTCs to rupture the endothelial basement membrane surrounding the primary tumor and enter into the bloodstream. Recent studies on rat and mouse breast cancer model show that the silencing of N-WASP by either a dominant negative construct or shRNA prevents the formation of invadopodia and stops the entry of CTCs into the bloodstream by this mechanism [15].

Tumor microenvironment and vasculature also plays an important role in the development of CTCs. The blood vessels connected to the primary tumor becomes fragile and prone to increased permeability, which helps in the easy access of CTCs into the bloodstream. The reason behind this alteration in vascular function of the tumor may be due to the unregulated angiogenic signal FGF and VEGF and inflammatory signal endothelin B and PDL1. A recent study reveals that the decrease in PHD2 expression, an oxygen-sensing molecule, which targets HIF transcription factor responsible for the degradation of vasculature, and thus controls the entry into bloodstream and develops CTCs [16]. It was observed that the injection of PHD2+/+ tumor cells into PHD2+/− heterozygous deficient mice leads to the development of tumor of similar growth rate but with decreased metastatic potential. But the direct introduction of same cells into the bloodstream resulted in an aggressive metastatic lesion. This study essentially showed that vasculature plays an important role in the penetration of the cells and generation of CTCs (Fig. 2.1).

Primary tumor sheds CTCs in the blood circulation as a single cell or as a cluster of two or more cells. Single CTCs enter bloodstream actively by EMT process and Clusters by breaking of as cell clump. CTCs flow through the blood and get arrested eventually at the secondary distant site or stuck in the capillaries. Their fate at the secondary site depends on many factors, for example, the microenvironment. Extravasation from blood or intrvasation to the new location may be supported by mesenchymal to epithelial transition. At the new location, CTCs remain in the state of dormancy for even a decade and make necessary metabolic changes to adjust and live in the new environment. These modified CTCs colonize and grow to a clinically detectable metastasis.

Temporarily and spatially localized dynamic interactions in and between micro-environment of CTCs and tumor-related macrophages play an essential role in the migration of CTCs through the blood vessel [17]. Recent studies on TIE2-expressing

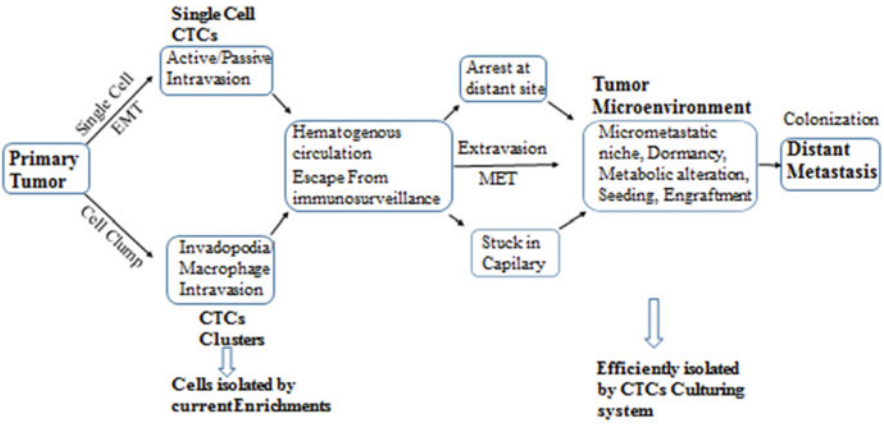

Fig. 2.1 Steps involved in the progression of metastasis

macrophages localized in the perivascular region showed that they enter into the blood circulation by producing VEGFA, which destroys vascular junction, reduces vascular permeability, and promotes direct entry through the junction between tumor cells, macrophages, and blood vessels [18].

2.2.3 CTC Single Cell Vs. CTC Clusters

Primary tumors release CTCs in the blood circulation as a single cell or as a cluster of two or more cells (microemboli). CTC clusters show a significantly shorter half-life of 6–10 min in the circulation, whereas for single cells it is 25–30 min. Recently, these clusters have been identified and isolated from the single CTCs with the help of multiple microfluidic devices without altering their conformation [19, 20]. Recently, researchers have been trying to find out the importance of clusters in the metastatic developments. The beginning of the formation of a metastatic lesion may be facilitated by the crosstalk within the network of migrating clusters of homotypic or heterotypic origin [21]. Two types of clusters are identified, homotypic clusters and heterotypic clusters. Some important traits of cellular heterogeneity in homotypic clusters like EMT vs epithelial and differentiated vs undifferentiated probably play an important role in distant metastasis. Heterotypic clusters remain undetected by the immune monitoring system by interacting with nontumor cells like immune cells or stromal cells and colonize at distant sites. Recent findings point toward the involvement of the immune cells in tumor-promoting or -arresting effect [22]. So the identification of tumor-associated immune cells and their interaction within the clusters will provide important information on their biological function and clinical significance in distant metastatic development [22]. Newly developed technologies like single-cell molecular profiling may be used to identify each nontumor cell in the cluster and their specific activity in CTC clusters. Recently, it

was found that CTCs show an important trait, a state of nonproliferation or dormancy, and remain completely inactive or undetected, evade the cytotoxic treatments, and colonize in the distant sites [23, 24]. CTC clusters are detected in the blood of some cancer survivors even after 22 years of primary treatment and cure. We still don't know anything about this nonproliferative state of CTC clusters and their role in the relapse of cancer. Recently, it has been suggested that the cancers with unexplained primary origin may be explained with the help of dormant CTC clusters in the blood, which remain inactive for a long time and certainly transformed into a metastatic lesion without any sign of development in the primary tumor site [25]. So it is very important to know the role of dormant CTC clusters in promotion of metastatic cancer growth as well as their interaction at the distant site to initiate the metastatic cascade.

2.2.4 Epithelial Plasticity of CTCs

Today epithelial-mesenchymal plasticity is believed to be a key factor in the metastatic cascade, which enables CTCs to adapt to different microenvironments starting from the separation from the primary tumor to distant colony formation [26–28]. CTCs survive the hurdles of the microenvironments probably due to the time-specific dynamic interconversions between epithelial and mesenchymal states in the metastatic cascade. Though it is confirmed that the signals from EMT are associated with cancer cells spread, the exact contribution of EMT in metastases is very unclear [29, 30]. Studies of CTCs revealed the presence of biphenotypic cells that expresses the epithelial and mesenchymal cell lineage with distinct heterogeneity in their marker expression [31]. As an example, the multiplexed RNA-ISH analysis of CTCs of the metastatic breast cancer patient shows epithelial and mesenchymal marker expression, which indicates the continuity of EMT [11]. A direct relation was noted between mesenchymal marker expression, triple-negative/Her2-positive breast cancer, and therapeutic resistance. The heterogeneity in epithelial and mesenchymal markers of CTCs was shown by some researchers in prostate cancer [32] and pancreatic cancer [33] mouse models. The advancement of the disease and its clinical outcomes were even correlated with the EMT markers in the CTCs of breast, liver, lung, and colon cancers [11, 34].

2.2.5 CTC Response to Reactive Oxygen Species (ROS)

CTCs encounter a substantial biochemical and physical stress in the bloodstream, which wipes out most of their population from the circulation. The level of ROS in CTCs increased due to the loss of matrix adhesion property, increased oxygen tension, and some other components of blood. The adaptive behavior of CTCs to the oxidative stress was recently observed in one of the studies on melanoma, which shows that CTCs undergo more oxidative stress in the bloodstream and distant organs rather than in the subcutaneous tissue. The study also claimed that the cells

in the metastatic cascade made some necessary modifications in the metabolism to minimize the effect of oxidative stress [35]. A distant metastasis may be prevented by raising the level of oxidative stress in melanoma cells by knocking down two important enzymes involved in the folate pathway, MTHFD1 or ALDH1L2.

Another study reported that the CTCs tolerate oxidative stress by upregulation of a gene called β-hemoglobin (HBB) [36]. The single-cell RNA-seq analysis of CTCs from lung, breast, and prostate cancer patients shows the indication of HBB gene expression. So the ectopic expression of HBB in the CTCs makes them more sensitive toward ROS and revokes their ability to form distant metastatic colonies. HBB-expressed CTCs resist the oxidative stress, survive in the blood circulation, and begin the metastases process in the mouse model. In summary, it can be said that the oxidative stress presents one of the biggest challenges against survival and metastases of CTCs and CTCs in response try to take different protective measures to evade the raised level of ROS.

2.2.6 CTC Interaction with Platelets

It is reported that the platelets are one of the key components in cancer metastases; inhibition of platelet activation initiates CTC destruction in the bloodstream and interferes with the metastatic cascade. In the bloodstream, CTCs experience major resistance from the immune system and fluid shear stress. It is reported that the shear stress in colon and prostate cancer cells begins the apoptosis pathway by activating TNF-related apoptosis-inducing ligand TRAIL [37]. It is speculated that platelets protect CTCs in the circulation by adhering on its surface, prevent them from being recognized by the immune system, and reduce the shear stress [38].

TGF- β, a cytokine secreted by the platelets, directly interacts with CTCs at their surface and enhances the probability of metastatic development. When platelets are cultured together with the cell lines from colon /breast cancer, TGF- β pathway gets activated and initiates EMT process by up- and downregulation of mesenchymal and epithelial marker expression, respectively.

The interaction between platelets and CTCs also activates NF-κB and TGF-β/Smad pathway and facilitates the beginning of EMT, which, in response, enhances the chance of CTCs survival [39, 40]. Platelets may act indirectly by forming a duct, which entraps the CTCs in the wall of the blood vessel. The selectins found on the wall of the platelets may initiate this type of interactions and anticoagulants prevent it [41]. Platelets release lysophosphatidic acid in bone metastasis, which helps in the proliferation of CTCs and activates osteoclast activity in the metastatic site by releasing the factors like IL-6 and IL-8 [42].

2.2.7 CTC Interactions with Immune Cells

Recent reports suggest that the immune cells have great impact on limiting or promoting metastatic capabilities of CTCs. When CTCs enter the bloodstream,

leaving behind the immune protection of the primary tumor, they undergo changes that modify their phenotypes, which facilitates their crosstalk with the immune system [11]. In the bloodstream, CTCs loses all the interactions with stromal cells and extracellular matrix and exposed to various cells in the immune system. In this situation, locally effective soluble factors like cytokines that are produced by the immune system may not affect CTCs. NK cells of the immune system obstruct the CTCs flow through the bloodstream, prevent their extravasation, and thus destroy them. Study with human colon cancer in mice models reveals that NK cells kill the CTCs in the blood directly by perforin-mediated pathway and indirectly by inducing apoptosis-generating factors. It is evidenced from the PCR analysis that the direct killing prevents the metastases more efficiently because the process slows down the primary tumor growth significantly and thus cuts down CTC population in the blood by 80%. $CD8^+$ cytotoxic T lymphocytes (CTLs) and $CD4^+$ T-helper (Th) cells together with NK cell interaction with a primary tumor has shown a significant reduction in metastases and relapse and improves the chance of disease-free survival as well as overall survival [43, 44]. The macrophages residing in liver-like Kuepfer cells can directly identify and block CTCs movement in the blood and make hepatic parenchyma free of metastasis. The probable mechanism may be activating adjacent T Cells against CTCs and directly killing them [45]. Tumor-associated macrophages (TAMs), on the other hand, promote the passage of CTCs by releasing paracrine factors, which, in turn, increases the motility of TAM and CTCs in the blood. The whole process takes place like a loop-EGF, one of the activated paracrine factor release colony-stimulating factors (CSF-1), which facilitates TAM movement [46]. TAM may promote the entry of CTCs into the circulation by releasing some other factors like oncogenic miR-22-containing exosomes, the chemokine CCL18, and the chemokine CCL20 [47, 48]. EMT activation is also another way how TAM helps in metastatic migration of CTCs. Studies on neutrophils favoring surveillance against CTC movement through the blood are quite limited except one that states in breast cancer mouse model neutrophils target the premetastatic lungs where they produce hydrogen peroxide, suspend CTCs invasion and metastatic growth [49]. Most of the other studies suggest that neutrophils work for safer passage of CTCs. G-CSF-induced activation of γδ T cells helps in the polarization of neutrophils toward immune suppression and increases CTC mobilization [50]. G-CSF helps in the motility of CTCs and metastases in lung cancer mouse model by guiding the movement of the lymphocytes $Ly6G^+$ $Ly6C^+$ to the metastatic lung where they release Bv8 protein and begin the angiogenesis [51]. There is enough evidence from the *in vivo* imaging studies that reveal that CTCs follow neutrophils and collectively build up in the premetastatic network, which supports that the neutrophils can coexist with CTCs and help in their metastatic colonization. Primary tumors stimulate interleukin (IL)-10, transforming growth factor (TGF)-β, and galectin 1 to release regulatory T-lymphocytes (Tregs), which blocks the immune surveillance and clear the way for metastatic development [52].

2.3 Advanced Nanobiotechnology for Therapeutic Targeting of CTCs

2.3.1 Effect of Nanoparticle Morphology on Their Fate in Blood Circulation

Based on the variation in their biodistribution, different factors like surface charge, composition, particle size, and route of administration are tuned as desired in cancer therapy and diagnosis [53]. Nanoparticles smaller than 10 nm in size can easily pass through the renal filtration of the kidney, whereas the particles larger than 100 nm can be easily washed off by the phagocytic uptake and hepatic filtration [54]. So the therapeutic cargo-loaded adhesive nanoparticles, which are capable of adhering to the blood vessels, may be used to target CTCs entering to the blood circulation through the vessel wall [55].

For spherical particles smaller than 200 nm in size, larger particles exhibit better margination than smaller particles in circulation, which improves their chances to interact with the CTCs during intrvasation or extravasation and target them. Margination is a biological phenomenon, where semirigid cells and Nanoparticles in circulation move away from the center of flows toward the vessel wall depending upon the size of the particle [56].

NPs entering blood circulation encounter continuously active two counteracting forces – adhesive interaction between NPs and cells and hemodynamic forces of flowing blood. These two counteracting forces are the key determinants of their specific targeting and adhering abilities [57] in the vasculature. The behavior of a nanoparticle in blood circulation is strongly attributed to its shape because shape plays a key role in counteracting hemodynamic forces. Nanoparticles formulated in different shapes like rod, spherical, conical, cylindrical, and discoidal showed differences in the flow pattern in the bloodstream [58].

Spherical particles like leukocytes in the bloodstream approach toward the endothelium and interact with them by exhibiting rotational motion [59]. Particles of other shapes may demonstrate tumbling motion and flows by different transport mechanism. For example, biconcave disc or dumbbell-shaped red blood cells (RBCs) of 6–8 μm size flow through the reticular meshwork filtering system of the sinusoidal spleen with an opening size between 200–500 nm, whereas spherical nanoparticles must be smaller than 200 nm to do the same job [58]. The advantages of the biconcave shapes are not only their flexibility to transform into Slipper, cup, or spherical shapes depending upon the changes in flow velocity and shear stress, but also they return to their original discoidal form at reduced blood flow [60]. These unique properties of RBC allow them to cross narrow blood vessels even smaller than 2–3 μm and make them an excellent carrier for the therapeutic cargo to target CTCs. Platelets, another essential component of blood, are generally oblate spheroid in shape, but when exposed in vitro to the adhesive surfaces for a long time they transformed into different shapes. So with the changes in shapes, various factors also change like platelet interaction time, frequency, and area with other platelets, cells,

and vessel wall as well as the magnitude of shear stress and other forces acting on the cells [61].

Based upon the knowledge of how the shape of biological molecules affects their interaction, transport, and fate in the blood, various synthetic particles were designed recently with loaded therapeutic cargo and evaluated as drug delivery system.

In a recent study, scientists tried to understand the relations between shapes, size, and biodistribution of intravascularly injected particles in mouse model [62]. They injected uncoated spherical beads and nonspherical silicon-based particles, with quasihemispherical, cylindrical, and discoidal shapes into tumor-bearing mice and analyzed the distribution of silicon in all major organs and in the tumor. They reported that the particles with discoidal shapes accumulated more in most of the organs in comparison to other shapes except liver where cylindrical particles distributed more and the distribution efficiency of spherical particles was largely dependent on their size. In another study, filomicelles (polymer micelle assemblies) were compared with spherical particles of similar chemistry for the transport of flexible filaments. They reported that the larger filomicelles retained in the blood flow even after 1 week, a stay ten times longer than the spherical particles. Larger filomicelles efficiently entrapped anticancer drug paclitaxel and showed shrinkage of rodent tumors. Spherical particle and smaller filomicelles are easily taken up by the cells in the blood flow and removed from the system [63]. A filament-shaped bacteriophage was modified to a nanowire to deliver anticancer peptides and photosensitive agents [64]. Biodistribution and pharmacokinetics of tobacco mosaic virus, available as spherical or rod due to the unique protein scaffold, was reported in mice model. Though macrophages residing in the liver and spleen eliminated tobacco mosaic virus from the body, rod-shaped particles stayed longer and distributed better than the spherical particles in the circulation [65].

These bioinspired nonspherical particles with various shapes may be designed and use for targeting CTCs in blood circulation. Rod, cylindrical, or filamentous particles show better pharmacokinetic, distribution, and elimination profile than the spherical particles due to their higher flexibility, deformability, and better permeability. Nonspherical particles always exhibit better margination in the circulation, which improve their chances to attack CTCs during their intrvasation. Again, the extended elimination half-life of these particles helps them to have better interaction with CTCs. Though the unique properties of nonspherical nanoparticles make them superior as a carrier for the delivery of therapeutic cargo, still it needs lots of extensive evaluation of its mechanical properties, stability, and polydispersity. A bioinspired nanoparticle with properly tuned shape, size, and mass mimics a biomolecule and interacts with vessel wall and CTCs with higher potential [66].

2.3.2 RBC-Based Nanoplatform for CTC Targeting

In a recent study, Red blood cells were engineered for identifying and isolating circulating tumor cells with high performance [67]. Folic acid and magnetic nanoparticles were coated on the surface of RBC by hydrophobic interaction and

chemical conjugation, respectively. Folic acid acted as a CTC surface receptor targeting entity and magnetic force was applied to isolate the CTCs from the blood. In a short duration of 3 h, almost 90% of the CTCs of more than 75% purity were detected. Conjugated RBC was treated with the lysis buffer, centrifuged, and CTCs were captured. These CTCs were successfully cultured and grown again in vitro. In another study, lipophilic antibody-modified erythrocytes were developed to target and kill the CTCs [68]. Lipophilic ligand painting was employed to modify the RBC in one step into a targeted molecule, which binds with various cells in vitro and in vivo. A characteristic rosette formation occurred when lipophilic anti-EpCAM or anti-CD45 antibodies painted RBC were bound CTCs in vitro. Anti-CD20 (Rituximab)-painted RBCs efficiently (over 90%) depleted CD19 +/CD20 +/CD45 + human lymphoma cells in mantle cell lymphoma (MCL) JeKo-1 model, while the same amount of rituximab-lipid (2 μg/mouse) was much less efficient in lymphoma cell depletion.

2.3.3 Neutrophil-Based Nanoplatform for CTC Targeting

Neutrophils are the important components of the premetastatic niche of metastatic cascade where they reside as bulk. Their movement toward the niche is guided by granulocyte colony-stimulating factor present in the inflammatory microenvironment of the niche. These activated neutrophils target and interact with the CTCs in the blood through the expression of a proinflammatory phenotype and helps in the extravasation process of tumor by forming neutrophil extracellular traps. CTCs and premetastatic niche targeting the movement of neutrophils are mainly controlled by their surface-associated adhesion molecules, which induces the inflammatory process to facilitate the seeding of CTCs in the niche. So neutrophils as delivery cargo may be a good option to target CTCs in blood, but their direct application is prevented due to their short life span of only 7 h. In a recent study, neutrophil-mimicking nanoparticles were designed by concealing the surface of PLGA-based nanoparticles with the inflammatory membrane derived from the neutrophils [69]. The cocktails of many adhesive protein molecules grafted on the surface of these nanoparticles help them behave like a superneutrophil and tirelessly target CTCs in the bloodstream. The researchers confirmed the targeting potential of the NPs and accumulation in the premetastatic niche by flow cytometry and confocal imaging. These nanodevices were loaded with carfilzomib, a proteasome inhibitor, and studied in 4T1 metastatic models labeled both with GFP and luciferase. The nanoparticles selectively targeted CTCs and induced apoptosis in blood.

2.3.4 Targeting CTCs with Platelet Membrane-Functionalized Particles

Based on the knowledge that platelets adhere on the surface of CTCs and provide perfect camouflage from immune system has encouraged the designing of many

platelet-based drug delivery system, including natural or genetically engineered platelet membrane-based drug delivery systems and platelet membrane-coated drug delivery systems.

Nanoparticles coated with natural or genetically engineered platelet membranes mimic all the features of natural platelets and targets CTCs in the blood.

In a recent study, engineered monoclonal antibodies against programmed-death ligand 1 (anti-PD-L1) conjugated platelet drug delivery was designed to target CTCs and eliminate them from the blood in an effort to prevent postsurgical recurrence and metastatic spread [70]. Programmed death-ligand 1 (PD-L1), which are overexpressed on the tumor cells, binds to PD-1 receptors on the activated T cells and switched off their cytotoxic activity. This allows tumor cells to pass through the immune surveillance in the blood. Monoclonal antibodies against PD-L1 were designed to inactivate PD1/PD-L1 pathway and allow the activated T cells to attack tumor cells and kill them. Conjugation of platelets with anti PD-L1 resulted in the increase of the half-life of these monoclonal antibodies from 5 to 35 days. Platelets activated by external or internal sources release Platelet-derived microparticles (PMPs) from the plasma membrane, which facilitate the release of attached anti-PD-L1 into the blood circulation. In vivo imaging study confirmed a ten-fold higher accumulation of anti-PD-L1 from the engineered platelets in comparison to free anti-PD-L1. Two highly metastatic mice models, melanoma (B16-F10) and triple-negative breast carcinoma (4 T1), were developed to study tumor recurrence and metastatic spread in response to the treatment. A T-cell-inflamed tumor microenvironment was created by the platelets on activation, leading to increased PDL1 expression at the tumor site. On activation of these intravenously administered engineered platelets, they release Anti-PD-L1, inactivate PD1/PD-L1 pathway, and activate T cells, and kill CTCs in the blood and tumor microenvironment.

Two counteractive forces play an essential role in the survival of CTCs in the bloodstream. Natural killer cells, neutrophils, macrophages, and cytotoxic T cells are always active to eliminate them. On the other hand, activated platelets conceal them from the immune surveillance and assure their survival. Inspired by the fact that the platelets adhere with the CTCs on their surface and get activated, biocompatible silica (Si) particles were functionalized with membrane-derived vesicles from activated platelets [71]. This biomimetic coating allows for targeting of synthetic particles to CTCs. These platelet membrane-coated silica particles were conjugated with TRAIL, a tumor necrosis factor-related apoptosis-inducing ligand, which is upregulated on the surface of most of the cancer-killing cells like natural killer cells, activated neutrophils, and cytotoxic T cells. So the complete system works by adhering with CTCs due to activated platelet membrane and binding with the cancer killer cells through overexpressed TRAIL ligands. These allow cancer killer cells to come in a reacting distance with CTCs and kill them. The device was administered intravenously in lung vasculature of a mouse breast cancer metastasis model. In vivo imaging study confirmed eight-fold reduction in tumor metastasis after 4 weeks following conjugated TRAIL treatment in comparison to free TRAIL solution.

We know that the activated platelets protect CTCs from the immune attack by binding through integrin receptors and providing a camouflage. So preventing their

interaction may be a good option to allow the immune cells to attack and eliminate CTCs from the blood to prevent metastatic development. Fibronectin, a glycoprotein of the extracellular matrix (ECM), is overexpressed in different types of malignant tumors. Alteration in their disposition and upregulation by transforming growth factor-beta (TGF-β) during epithelial-to-mesenchymal transition facilitates premetastatic niche formation. Fibronectin formed a complex with other matrix proteins such as fibrin and allows tumor proliferation and metastasis.

Platelets interact with tumor cells through integrin receptors mainly β1 and β3, which are expressed on their surface. So it was a good idea to target Fibrin-fibronectin complex as well as the integrin receptors to prevent the interactions between platelets and tumor cells. A small linear tumor-homing pentapeptide, CREKA (Cysteine–Arginine–Glutamic acid–Lysine–Alanine), was synthesized to attack fibrin-fibronectin complexes on vessel walls and conjugated with Ticagrelor, a reversible antagonist of the P2Y12 receptor on platelets [72]. In vitro study with CREKA-Ticagrelor confirmed that it prevents platelet-induced migration of tumor cells and tumor-platelet interaction. The *in vivo* study was performed in a 4T1 breast cancer tumor mouse models. After 16 days, it was found that the weight of the lung was reduced by 120 mg and 36 mg for CREKA-Ticagrelor- and Ticagrelor-treated groups, respectively, in comparison to control group. So the system worked perfectly in arresting tumor metastasis.

2.3.5 Liposomes

TNF-related apoptosis-inducing ligand (TRAIL) is a type II transmembrane protein molecule, which induces apoptosis to the cancer cells, with no toxic side effects to most normal cells [73]. Scientists developed liposome-based TRAIL therapy to target CTCs in the blood and eliminate those [74]. Adhesion receptor E-selectin (ES) is known for its recognizing and binding with most of the tumor cells and leukocytes in the blood. E-selectin and TRAIL were conjugated on the surface of liposomes and administered directly into the blood using the intravenous route. Liposomes immediately adhered with the CTCs and leukocytes through the E-selectin receptors on their surface. This allows the TRAIL molecule to come close enough to react with the death receptors present on the surface of CTCs and send the signal to initiate the process of apoptosis.

Results showed that the ES/TRAIL therapy eliminated more than 95% of the CTCs within 2 h of administration of the liposomes. ES/TRAIL liposomes exhibited enhanced therapeutic efficacy in vivo in the blood in comparison to in vitro in buffered media. This is exceptional because normally the efficacy of other synthetic reagents reduced in vivo in the blood due to cellular internalization and nonspecific binding of plasma proteins. They found that ES/TRAIL therapy increased the hematocrit value of blood, which acted as an apoptosis-inducing factor and decreased the number of viable cancer cells. They assumed that the cancer cells and leukocytes encounter constantly acting compressive force in the blood flow, which wipes out the glycocalyx protective layer surrounding the CTCs and exposes

them to bind with the ES/TRAIL liposomes through the ES receptors and allows TRAIL molecules to come in contact with the death receptors to initiate cell apoptosis.

In another work, the researchers tested the same ES/TRAIL liposomes in mouse prostate cancer xenograft model [75]. Half-life of the formulation was indicated by the human TRAIL characteristics in the leukocytes of the mouse and it was almost 30 h. After 6 weeks of treatment with specific dosing schedule, they did not find any metastatic development and more interestingly the volume of the primary tumor also decreased in the treatment group, whereas control group developed a secondary tumor in abdominal cavity, liver, kidney, and spleen. There was 94% variation in the CTC count between the treated and the control groups at the end of the experiment. The formulation was found absolutely safe for the normal cells.

The same research group developed another type of TRAIL-based liposome formulation to target and eliminate lymph node CTCs in an effort to prevent metastatic development [76]. It is very interesting to find that the lymph node is the primary organ for the metastatic spread for most of the cancers though many immune cells reside here. The reason may be that most cancer cells can move past the poor immune surveillance of cancer patients. During the process of metastatic spread, the cells from the primary tumors need to cross the Sentinel lymph nodes (SLN) and the first line of defense, which becomes weaker or inefficient due to alteration in its morphology in cancer. So the researchers tried to prevent the immune suppression of SLN by reviving the immune function of the body by developing TRIL liposome with natural killer cells (NK), which will target CTCs in the Lymph node and eliminate them. NK Cells are the cytotoxic lymphocytes, which continuously bombard toxins to the tumor cells, activate the apoptotic pathways, and kill them. In cancer patients, NK cells show abnormalities like reduced cytotoxicity and lose their tumor cell-infiltrating capacity due to chemotherapy-induced immune suppression.

TRAIL liposomes were thiolated and conjugated with anti-CD57 (an antibody to CD-57), which helped in adhesion of liposomes to NK cells. In vitro studies with MDA-MB- 231, COLO 205, and LNCaP cancer cell lines confirmed no metastatic growth in lymph nodes. The study was continued *in vivo* using subcutaneous mouse xenograft tumor model. In this model, anti-NK1.1 antibody was used in liposomes instead of anti-CD57 to target NK cells in mice. The liposomes were very specific to target only NK cells and remains 28% bound with NK cells even after 72 h posttreatment. After 2 weeks of implanting primary tumor anti-NK1.1-TRAIL-based liposomes were administered subcutaneously. NK cells directed the liposomes toward the inguinal lymph nodes and arrested the lymphatic spread of a subcutaneous tumor in the mice. Moreover, NK cells were found absolutely healthy without loss of cellular activity and cytotoxicity.

2.3.6 DNA-Based Nanodevices

Recently, DNA was used as a core material to design various nanostructures applying the fact that it forms complementary base pairs at base pairs GC/AT. As an ideal nanostructuring material, easy manipulation in its conformation from a double-stranded structure to a 3-dimensional origami provides the advantages like good biocompatibility, easy structural modifications, and programmability. [77].

In an effort to kill CTCs, locally Wang and group proposed an aptamer, which gets activated in a switching mechanism and releases photosensitizer (PS) in presence of CTCs [78]. The strategy was simply to develop a PS-labeled hairpin switch aptamer (HAS) immobilized on the PDMS-glass supporter and to install in the deep tissue near the primary tumor surgically. When CTCs come within the interacting range of the device, aptamer structure is modified to a hairpin conformation and it gets detached from the supporter. During its movement through the superficial blood vessels, if it is supplied with external light energy, PS get activated and switch on the aptamer to release oxygen (O_2), which eliminate CTCs from the blood. The device was designed in such a way that it works in two-step mechanism. The first step involves the separation of PS-labeled aptamer and the supporter in the presence of CTCs, which is achieved by utilizing magnetic beads. To achieve the second goal, to kill CTCs selectively, a vessel simulating microfluidic device and anticancer PS was labeled on the aptamer. When the aptamer probes enter into the CTCs and get activated by external light, it releases CTCs killer O_2. The device was composed of a FAM-labeled hairpin aptamer for targeting, biotin, and a quencher labeled C-DNA for hairpin aptamer hybridization, HAS probe made of aptamer and cDNA.

In an effort to make this device more effective to eliminate CTCs, doxorubicin was encapsulated in the DNA structure. This way the system combines chemotherapy and photodynamic therapy and launches dual attack selectively on CTCs without harming normal cells.

2.3.7 Dendrimers

Dendrimers are highly branched polymers with easy surface manipulations. These kinds of structural conformations make them a good candidate for functionalization and conjugation with other molecular entities.

From our discussion on the biology of CTCs, we now know that the various sequential events like local invasion, intravasation, survival in the circulation, heteroadhesion to vascular endothelial bed of secondary organs, extravasation, micrometastasis formation, and metastatic colonization are always active in the metastatic cascade. EpCAM and SleX are adhesion molecules, which help in epithelial adhesion of CTCs directly by Ca^{2+}-independent homotypic pathway and indirectly by Slex/E-selection interaction, respectively. So the antibodies of these molecules block an important step in metastatic process, adhesion to vascular endothelial bed.

Xie and group developed a nanoplatform composed of a polyamidoamine dendrimer (PAMAM) in its core, which is conjugated with two antibodies (anti-EpCAM) and (anti-SLeX) to access the antiadhesion and termination of CTCs in an effort to arrest metastasis [79–81]. The result showed that the conjugate selectively adhered with SW620 cells responsible for colon cancer and captures them. The attachment of these SW620 cells to the Human umbilical vein endothelial cells (HUVECs) or fibronectin substrate was markedly reduced in the presence of the conjugate in a concentration-dependent mechanism. It was reported that almost 60–70% cell adhesion inhibition from SW620 cells, which was much higher than other two cell lines involved in the study, SW480 and LoVo, and may be due to more EpCAM and Slex receptors on SW620 cell surface.

EpCAM and Slex antibody-conjugated dendrimers were injected along with the human carcinoma cells HT29 in a sequential manner to nude mice to understand the CTC capturing and elimination potential and compared with its single antibody-conjugated counterparts. On injecting red fluorescence protein labeled- HT29 cells, dual antibody-coated dendrimers exhibited higher capacity to detect and isolate CTCs from the leukocytes (RBC) as well as from the blood of mice and human as comparison to its single antibody-coated counterpart. Flow cytometry analysis showed that the conjugates blocked the adhesion of CTCs to the epithelial membrane at the S phase, made them dormant, and arrest the metastatic spread.

In another study, CTC biomarker targeting dual aptamer ring were conjugated with dendrimers to eliminate CTCs. The results showed that the dual aptamer conjugation increased the selectivity to capture and isolate CTCs from even 10^8 cells or blood of human and mice. The aptamer conjugation reduced endogenous nucleases-induced biodegradation, improves the stability, and arrests metastasis *in vivo*.

The present studies constructed the novel dual aptamer ring conjugates to simultaneously recognize and seize two surface biomarkers on one type of CTC. Such unique molecular architecture can significantly withstand degradation by nucleases and precisely capture the target CTCs in the presence of millions of interfering normal cells and in patient and animal blood. The conjugate with its enhanced functionality and biostability provides a more easily scalable and low-cost clinical approach to restraining CTCs and preventing CTCs-based cancer metastasis. The biomarkers, EpCAM and Her2, block two arms of the aptamer conjugates and prevent the endothelial attachments of the cells and promote apoptosis [82].

In an effort to recognize and isolate the variety of CTCs, Zheng et al. proposed the design of barcode particles, which are composed of spherical crystal clusters of colloids and are adorned with dendrimer-amplified aptamer probes [83]. The microfluidic droplet templates were employed to tune the size of these spherical crystals in accordance with the dimensions of the cells. The characteristic reflection peaks arising from these particles due to the photonic bandgap in their structure help in encoding the information and enhance the stability. A particular aptamer in the device identifies and captures a specific type of CTCs in blood, whereas dendrimer works as an amplifier and improves the sensitivity of detection.

2.3.8 Mesoporous Silica Nanoparticles (MSN)

Some unique features of MSN have made it an excellent candidate in the biomedical field like highly ordered pore structures, tunable pore size, large pore volume and surface area, high loading, controlled release of drug molecules, cell specificity and biocompatibility, etc. Jia and group developed an MSN-based nanostructure, which is conjugated with EpCAM antibody and loaded with abortifacient mifepristone (MIF) to serve as a dual target for eliminating the CTCs from the blood and stop the process of metastasis [84]. The flow cytometry assay showed quantitatively that these nanodevices selectively captured the colorectal cancer cells in the cell medium or in the blood through EpCAM-binding sites. The EpCAM-led selective binding downregulates the captured cells and moves them to G0/G1 phase to eliminate them. In the absence of adhesion protein molecule EpCAM, the cancer cells could not adhere to the endothelial cells to be able to form a premetastatic niche. MIF works by interfering with E-selectin pathways. The functionalization helped the MSNs to stay long enough in the blood circulation so that it could efficiently release its MIF load and prevents lung metastasis.

In another study, Jia et al. developed doxorubicin-loaded MSNs and covalently conjugated with two aptamers to selectively target EpCAM and CD44, the common surface biomarkers for colorectal cancer [85]. This nanodevice sensed CTCs in the blood, followed it like a guided missile, and captured it with aptamer probes, and bombarded with doxorubicin to kill it by DOX-dependent pathway. On the other hand, aptamers prevent the CTCs from adhering with the epithelial cells, an important step in the metastatic cascade. When injected in the mice colorectal cancer model, dual aptamer-conjugated DOX-MSNs were found in blood circulation even after 8 h, which was much higher than its single aptamer-conjugated counterpart or DOX-MSNs and efficient enough to chase away and kill the CTCs to prevent lung metastasis.

2.3.9 Polymeric Micelles

Polymeric micelles are nanosized core-shell structured materials, which are formed by the self-assembly of amphiphilic block copolymers in water. Hydrophobic core to minimize aqueous exposure and a hydrophilic shell stabilize the core in the aqueous environment. Some interesting features of polymeric micelles like biocompatibility, low toxicity, tunable core-shell conformation, micellar assembly, nanosize range, and good stability make them an ideal candidate for using as a delivery cargo. The typical core-shell conformation of the micelles ensures better loading of hydrophobic drugs in the core and adds steric protection to the shell. Moreover, electrostatic attraction or chemical conjugations may be employed to load large molecules like nucleic acid and hydrophilic drugs efficiently. Recently, it has been found that the tuning of structural conformation of polymeric micelles allows them to control the release of macromolecules according to the needs of the therapy.

Deng and group developed DOX-loaded Monomethyl poly(ethylene glycol)-poly (ε-caprolactone) (MPEG-PCL) diblock copolymer polymeric micelle with the intent to kill CTCs in the blood [86]. They employed pH-induced self-assembly method to obtain small micelles with narrow size distribution and high entrapment efficiency. In vitro studies with 4T1 cells confirmed that DOX micelles were superior to free DOX because they exhibited better cytotoxic profile, improved cellular uptake, and sustained release behavior. In transgenic Zebrafish model, the formulation showed a longer stay in circulation, lower extravasation to the surrounding organs and prevented CTCs from the development of metastatic niche, and increased the life span of tumor-bearing zebrafish. In vivo imaging in 4T1 tumor-bearing mouse model supported the results found with transgenic Zebrafish, like the formulation induced more apoptosis to the CTCs than the free drugs with minimum toxic effect to the normal cells.

Yao and group [87] developed K237 peptide and Ep23 aptamer-conjugated biodegradable PEG-PLA polymeric micelles with a model drug paclitaxel with the intent to target the primary tumor and eliminate CTCs from the blood simultaneously to obtain a synergistic antitumor therapeutic effect. K237 peptide acts as an ideal ligand for tumor vessel targeting because it interacts with KDR/Flk-1 tyrosine kinase pathway and destroys vasculogenic mimicry channels. Ep23 aptamer interacts with the EpCAM and prevents the CTC attachment with endothelial cells, an important step in metastasis, and stops secondary tumor development. In vitro studies with HUVEC and 4T1 cells confirmed improved cellular uptake, better cytotoxicity profile and apoptosis induction from peptide and aptamer dual-conjugated micelles than their single-conjugated counterparts. Flow cytometry, intravital imaging, and confocal microscopy showed that the dual targeting micelles target and eliminate CTCs effectively from the blood and 4T1-GFP cell-derived lung metastasis mice model.

2.4 Conclusion

Recent cancer research and findings have drawn special interest for circulating tumor cells (CTCs) due to their definite connection with tumor metastasis. The study of the biology of CTCs provides us clinically relevant important insights regarding the metastatic progression and cancer-related mortality. As such, CTCs are investigated as predictive biomarkers and targeting CTCs may be a good therapeutic approach to improve survival outcomes. However, typical characteristics of CTCs like rarity and heterogeneity in morphology and phenotype make them extremely difficult to identify and isolate. Recent developments in the field of nanoscale-material science and nanobiotechnology allow the researchers to continuously explore new nanoplatforms for capture, detection, and elimination of CTCs. CTCs targeting engineered nanomedicines play an important role in early diagnosis, reducing distal recurrence and preventing metastasis before it occurs. So as our understanding regarding CTCs and nanotechnology will grow, we will be identifying new

vulnerabilities to target the lethality of cancer metastasis in more efficient and cost-effective ways in the coming days.

Acknowledgments The authors are grateful to the chief editors for providing the opportunity to write this book chapter. They also acknowledge the central library of Hebrew University for accessing the literature.

Conflict of Interest The author(s) confirm that this article content has no conflict of interest. All the figures and tables are self-made and original.

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Application of Nanobiotechnology in Clinical Diagnosis

3

Jayanta Barman

Abstract

Nanotechnology is the ability to manipulate materials to establish the nanostructures at the desired level. In the present scenario, the field is quickly elaborating, and numbers of work have been done by synthesizing and characterizing the nanomaterial with a suitable design, for application of devices in various fields. In the last few decades, more stress is given on the use of nanofabrication-based medicinal diagnoses as per theory and experiments concerned. In this chapter, the focus has been given on the stress of various nanostructures and nanodevices' fabrication in clinical diagnostics. The chapter starts with introducing some basic properties and prospects, benefits and limitations as well as biodetection in medical diagnostics.

Keywords

Nanobiotechnology · Diagnosis · Cancer · Device

3.1 Introduction

Nanocrystal and quantum dots of various materials have been applied not only in nanoelectronics but has application in different technological areas, including biological labelling, processing and diagnostics, photonic devices, optical waveguide and non-linear optics, catalysis, ceramics, magnetic storage devices, etc. In addition, the applicability of the words like nanograph, nanoscopy and nanosurgery will be the issues of nanoscience and nanotechonology, which are not far reaching as far as theory and experiment are concerned [1–7]. Nanostructures have special relevance to biomedical applications due to their ultra-small size with cell

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M. K. Das, Y. V Pathak (eds.), *Nano Medicine and Nano Safety*,
https://doi.org/10.1007/978-981-15-6255-6_3

(5–50 μm), viruses (10–500 nm), proteins (10–50 nm) and gene (10–100 nm). Nanostructure material has a special capacity that it can act inside the human body without affecting the normal behaviour. Nanostructure material study allows this critical process on a single cell level. Semiconductor and magnetic biomaterials have different challenges for application in biological point of view. Nanostructure-based device application requires strict biocompatibility. The present nanostructure research is largely biased due to the diagnostic and therapeutic from a medicinal point of view. Nowadays, for diagnosis, the highly used technique is magnetic resonance, and in the detection of biomolecules, nanostructure is used as a fluorescent material. For targeted delivery of drugs, nanostructures are highly applied for destroying the cancer cell and repairing the cell [6, 7]. From the application point of view, nanostructures have important value in clinical purpose and drugs delivery technique [8–11].

3.1.1 Classification of Nanoparticles

Since the beginning of the 1980s, the door has opened for research in nanostructures, especially in materials science, and further it extended in nanobiotechnology to every field. The important properties of this material are that they show unique behaviour when they shrunk to nanodimension. When the material is under a confined system in the range of 100 nm, the physical, chemical, hardness and all properties change. In this state, the nanomaterial surface creates some defects and the defect states create traps which show special properties in biological substance and affinity for device application.

The decrease in particle size from metal to semiconductor has shrinking effect and yields high chemical reactivity and good physical properties. When the confined dimension is less than de Broglie wavelength, quantum confinement occurs and shows unique optical properties that have opened the door for device application, especially in diagnostic purpose, as most of the instruments are based on the optical system. Once confinement happens, the energy levels are quantized within the valance band and conduction band mass of the electron changes and further known as effective mass [11–14]. Depending upon the confinement of nanoparticles, three categories are shown in Fig. 3.1.

Confinement in

1. one-dimension have the shape in thin films,
2. two dimensions have the shape in tube or wires and,
3. three dimensions have the shape particles called quantum dots.

Figure 3.1 shows the three different confinement systems where thin film, quantum dot as well as quantum wire concepts are developed.

The confined dimensions of nanostructure are governed by the density of states (DOS). The DOS is different for different shapes and structures and it varies from quantum film to quantum dot. DOS is a continuum for bulk material and stepwise in

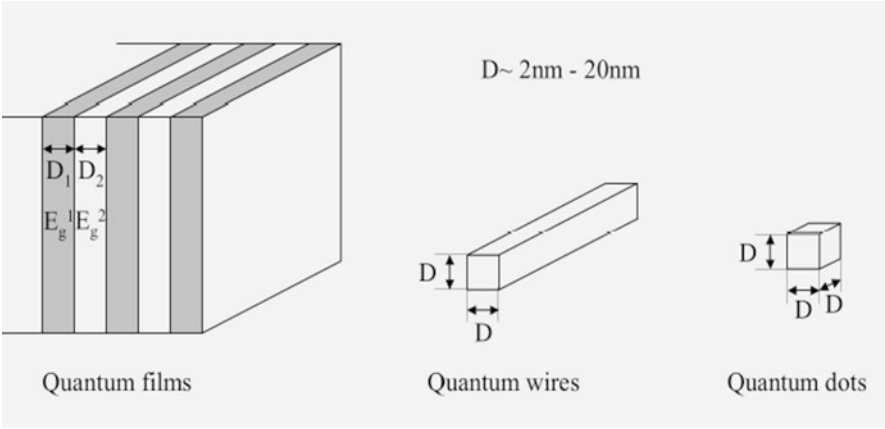

Fig. 3.1 Carrier confinement in low-dimensional systems of nanomaterial [5]

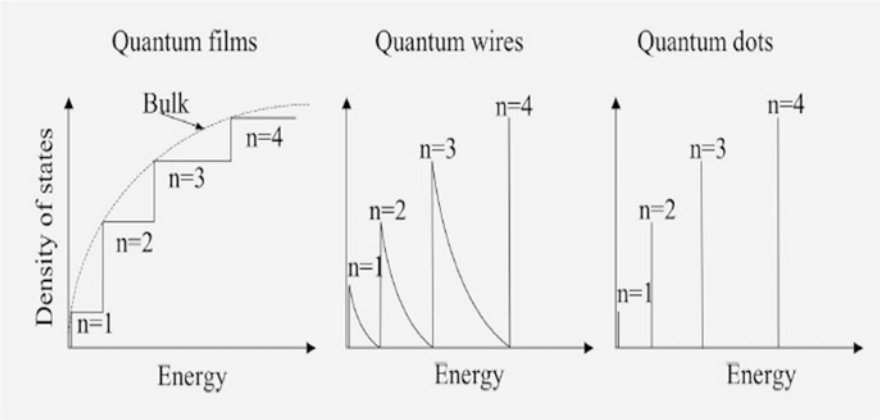

Fig. 3.2 Density of states in the reduced dimensional system of nanomaterial [5]

wire and discrete in quantum dots. The three types of DOS are shown in Fig. 3.2 [15].

Ekimov in 1980 reported the quantum size effect in semiconductor nanoparticles and later the size effect also found in all materials [15].

3.1.2 Properties of Quantum Dots

Quantum dots possess some unique properties by which they differ from bulk material. These are:

3.1.2.1 Enhancement of Band Gap

Due to the quantum confinement, the continuous DOS in conduction and valance bands are split into some discrete states compared to bulk.

3.1.2.2 Blue Shift

As the band gap of quantum dot increases, the strong absorption of the optical pulse occurs in the UV region, that is absorption edge shifts towards UV. For that reason, it is said that quantum dots possess blue shift in optical absorption spectra.

3.1.2.3 Large Surface to Volume Ratio

Most important property associated with quantum dot is the existence of large surface to volume ratio. Due to this, within the band gap some discrete electronic states are created and that process is the generation of 'Traps'.

3.1.2.4 Intense Photoluminescence

Due to the formation of traps in nanostructure, the trapping as well as detrapping rate of electrons is very fast. That is why they show intense photoluminescence spectra. Quantum dots (QDs) are also a group of NPs which are highly useful for clinical purpose. QDs have the ability to create fluorescence in different levels of spectral range and even in infrared range also [16], which makes them a suitable candidate for identifying and imaging cells with cell structures and pathogenic agents [16–18], which has made quantum dots suitable for diagnostic applications [10, 18, 19].

Half metal, super paramagnet, has lots of application in diagnostic devices like magnetic resonance tomography (MRT) and these materials are used to contrast the imaging biological tissues [20]. Again, carbon nanotube shows unique properties and it is highly used to designing biosensors [21, 22], detecting specific biomolecules [23] and identifying structural change of cells [24–26].

However, NPs have a large number of disadvantages towards the application in medicinal point of view. One such is toxicity, and it may rise due to the unique behaviour of nanostructural properties. Further advanced research is streaming to studying the causes and mechanisms of NPs to control the toxic effect.

3.1.3 Nanobiosensors

Nanobiosensor is the instrument where identification of different biological phenomena can be detected. Diagnosis practice is mainly associated with this biosensor where first biological behaviour is converted to an optical signal, and further the optical signal is converted to different forms such as voltage, current, phase shift, etc., and from these data, other measuring parameters can be correlated and captured. Nanobiosensors are an integral part of the clinical diagnosis of biological samples. A transducer is acting as an instrumental part for accessing the signal from active cell to diagnostic purpose [27–29]. The present available diagnostic technology has a limitation that it creates tumour in blood vessels and to overcome the restriction,

nanobiosensor is used which act in smart way and become functional even in the repeating process.

3.1.3.1 Bimolecular Transduction

Biomolecule transductions are classified into two classes and they are label-based and label-free detection.

3.1.3.2 Label-Based Detection

Immunoassays are the basis of most label-based detection technologies and they are based on the interaction of antigen–antibody. The resent label-based detection is based on biomolecule transduction and can perform the amount of protein existing in the blood. Till now, the immunoassay has been regarded as the standard diagnostic tool.

3.1.3.3 Label-Free Detection Methods

With the help of nanotechnology, lots of label-free detection methods have been developed and they have high efficiency in accuracy and within a short period, millions of data can be analysed. The label-free biosensors are based on the following principles.

Electrical Detection

Electrochemical and electrical detection biosensors are primarily based on the principle of change in currents and voltages with input biomolecules. The result is synthesis with specific input parameters, which is monitored with specific software.

Optical Detection

Another important procedure for biological sample analysis is optoelectronic technique. In this process, light energy is converted to electrical energy with the help of a transducer. Most of the biological samples have optical activity where energy is transformed into an electronic signal and analysis is performed with synthesis compound. During the interaction of active cells with a chemical process, some energy is released in the form of light and which is converted to desired electromagnetic signal. Some metal nanoparticles have a good response in thin film form for optical sensitivity with biomolecules [29–33]. The sensing element which has a biological response is measured with the help of a transducer and the collected signal can be recorded for data analysis. The main advantage of the nanomaterial sensor is that it can be manipulated in desirable properties [34–43].

3.2 Medical Applications of Nanostructures

Presently, the term nanodiagnostics is specially used in diagnosis purpose in the field of nanotechnology [44–48]. Though various types of diagnoses are available, yet nanodiagnostics is popular because of the early and rapid process associated with nanotechnology. Due to the unique characteristics and capability to analyse in the

desired area, it has potential application in clinical purpose [49, 50]. For the diagnostic purpose, the nanostructures are as follows.

3.2.1 Nanostructured Surfaces

Nanostructured surfaces show many interesting and unique properties. These nanostructure surfaces have shown adsorption of cells and change in cell structure [51]. The absorption of bacteria and protein absorbed by the nanostructure surface was detected by the lithography technique and shows enhanced properties. The modified adhesion was explained from the principle of expansion of the surface area to the existence of functional groups ratio [52]. With the help of Dip pen nanolithography (DPN) technique, structured surfaces can manipulate directly in modified buffer of silicon chips [53]. In this field of nanolithography, lots of work has been reported. With the help of Atomic force microscope (AFM), the surface can be enhanced up to a single molecule level which makes possible to establish the DNA pattern [54–56].

3.2.2 Nanoscale for Molecular Identification

Detection of single cells and few molecules is possible due to the development of nanotechnology. The present nanostructure-based sensor has high resolution to detect the cancer cell to the range of a single cell.

Conventional methods have some limitation that it can't establish the relation protein to DNA level. Therefore, mass spectrometry method is used in two dimensions by connecting electric field with biomolecules [57–59].

3.2.3 Gold Nanoparticles for Diagnostics

Gold nanoparticles are highly used for cancer diagnostics in the last decade. The gold nanoparticle whose diameter is less than 10 nm can be attached to small pieces of DNA. The gold nanoparticles are kept in a sensor surface which has high affinity for target element and this technique can detect different DNA multiplexing [60, 61].

3.2.4 Quantum Dot towards Application in Cancer Cell

QDs are inorganic as well as semiconducting material which have significant advantages over conventionally used materials. Due to the high sensitivity, large excitation spectra and stable fluorescence, therefore it is now the option instead of common laser application. QD-based laser has overcome the restriction and ability to heat the targeted particular effected cells [62]. Another important technique of QDs of different sizes embedded in tiny polymer porous provide the detecting of the

specimens. Different sizes of QDs have different properties which have significant application in clinical diagnosis [63, 64].

3.2.5 Nanotechnology-Based Biochips

The size of the cell is generally in the range of nanometre, and nanostructure has the same dimension with respect to cell dimension which is now regarded as 'Nanotechnology on a chip' [62]. The chips can be designed to interact with cellular constituents. This chip has the ability to detect cancer cells. Photolithographic is one of the technique through which silicon nanowire can be developed on a substrate. Using this process, the investigators can create nanotubes with diameters less than 10 nm. In this range, it is possible to trap DNA molecules with the device channels [57, 65–67]. In the nanotube, electrodes are used to accelerate the DNA molecules through the tube.

3.2.6 Infectious Diseases with Nanodiagnostics

The rapid and accurate detection of pathogenic bacteria has an utmost important factor. The conventional diagnostic methods have poor sensitivity due to instrumental limitations. QD hybridization-detection technique has been achieved by nanoprobes of single-molecule hybridization using multicolour oligonucleotide-functionalized nanoprobes.

With the help of silver nanoparticles, high degree accuracy can be achieved in spectroscopic method because spectroscopic analysis has lots of advantages with virus multiplexing. In the detection process, the principle spectroscopic method is the change of frequency with scattered infected DNA or RNA. The change in frequency can be monitored and analysed with proper parameter and can be considered as a fingerprint in the diagnosis process [68, 69].

3.2.7 Nanoparticle Hyperthermia as Clinical Cancer Therapy

Early discovered cancer has more cure rate. Therefore, early detection and timely diagnosis of cancer is essential to reduce the mortality rate of patients. Tumour imaging technology has an important role in cancer diagnosis and the choice of late clinical treatment options. The conventional methods do not thermally discriminate between target and surrounding normal tissue and heating of non-selective tissue leads to major unwanted effect. Menopausal Hormone Therapy (MHT) has attracted a lot of interest in recent years due to its proper use in clinical purpose. Magnetic nanoparticles are able to convert electromagnetic energy into heat. Therefore, the most popular application for MNPs is most likely the destruction of tumour cells by heating them to their appropriate target.

The magnetic nanostructures applied by an alternating magnetic field is presently explored as as technique for targeted cancer cells. In this process, the hysteresis loss is also an important factor, as increasing the hysteresis loss improves the heating efficiency. The conventional method like chemotherapy has lots of side effects and magnetic hyperthermia can be considered as an alternative process for better treatment [70–72].

3.3 The Next Prospects

In the coming decade, it will be possible that all diagnosis measurements related with nano-based techniques can play a vital role with better accuracy in a rapid manner. From the clinical point of view, it is less toxic than the conventional method and from the therapeutic point of view, the target element is a point-to-point heating effect which reduces the burning of other normal cells. Due to the multiple prospects, QD nano laser-based technique helps in surgery with high efficiency [73–76]. From the present issue, it is clear that nanostructure is not only applicable in diagnosis but also fruitful in therapeutic medicine as well as in surgery. The next healthcare system will be totally based on nanobiotechnology as theory and practice concern [77, 78]. The most striking area of nanobiotechnology is cancer diagnostics. From the nanostructure-based diagnosis, early detection of cancer cell possibility increases which help to cure in nanotherapeutic application rather the present available time-consuming methods.

3.4 Challenges with the Use of Nanostructures

Lots of effort has been given for synthesized nanostructures with all possible dimensions. This nanostructure has a large surface to volume ratio compared to bulk material and has the ability to attract physicochemical properties. Due to high reactivity and small size, they allowed them to cross many boundaries across cell membranes and bind to molecules such as DNA, RNA, virus and protein [16, 20, 39, 42, 51, 57, 63, 77–79].

Although lots of development has taken place, yet toxicological effects of nanoparticles and nanostructures are yet to be perfectly known. They may react with other internal parts of living animal [78].

Studies have shown that carbon nanotubes to be toxic and toxicity will act up to a long period. They can enter the lungs and create granules of laboratory animals [77]. Nanoparticles and quantum dots made of metals, insulator and semiconductor have shown the toxic effects on cells [78].

From in vitro study, it is clear that a metal nanoparticle has the ability to damage cell membranes, linking with C18-4 and stem cells. Again, magnetic nanoparticles can pass the blood-testis barrier causing aggregation in blood cells.

The above discussion indicates that nanostructures have an important impact on animal health by producing phenotypic damage to the cells [80].

To understand the nanostructures' properties relating to human health, detailed interaction and toxicity must be known. The different type of synthesis procedure created different structure, size and morphology and in terms of the clinical context, improvement should be done with quality, control and safety issues.

3.5 Conclusion

The present chapter discusses different types of nanostructures and their dimension related with biological interest. The nanobase technique has an interesting appeal towards the application in medical diagnostics. Due to the smaller size and large surface to volume ratio as well as rapid interaction with cell, it becomes a candidate for future diagnostics issue. Some important challenges have to be implemented to overcome the present situation related to toxicity. The physical characteristics of nanostructures determine the clinical approach towards the diagnosis. More study and research are necessary to overcome the present issues.

Conflict of Interest None. All the figures are original and self-made.

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Anti-diabetic Nano-formulation from Herbal Source

4

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Abstract

Diabetes is one amongst the chronic metabolic diseases affecting millions of people across the world. Apart from proper selection of drugs and doses, conventional drugs pose unwanted side effects to the diabetics. Due to the limited side effects, cost and easy accessibility, there is a rising interest in the field of research incorporating compounds from natural backgrounds. However, most of the biologically active constituents have low absorption capability despite their property of high solubility in water. Due to their low absorption capability, most of them are unable to cross the lipid bilayers of the cell owing to their large molecular sizes that produce failure in achieving bioavailability followed by the loss of efficacy. In recent years, nanotechnology-based formulations have given new lease of life to such problems with their myriad of formulations that include nanospheres, nanocapsules, liposomes, proliposomes, solid lipid nanoparticles [SLNs] and nano-emulsion. Combining herbal drugs with nanotechnology may potentiate the action of the plant extracts or active constituent by increasing their solubility, bioavailability and efficacy as well as by reducing the required dose and side effects. Therefore, the objective behind presenting this chapter is to outline works on the nanotechnology-based anti-diabetic herbal formulations reported till date.

Keywords

Diabetes · Nanotechnology · Bioavailability · Nanospheres · Nanocapsules · Liposomes · Proliposomes · Solid lipid nanoparticles [SLNs] · Nano-emulsion

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M. K. Das, Y. V Pathak (eds.), *Nano Medicine and Nano Safety*,
https://doi.org/10.1007/978-981-15-6255-6_4

4.1 Introduction

Diabetes mellitus is a group of metabolic diseases characterized by an increase in blood glucose level associated with atypical metabolism of basic foods like carbohydrates, proteins and fats due to inadequate or lack of insulin secretion by beta cells of islets of Langerhans of the pancreas and peripheral tissues like adipose, skeletal muscle and liver tissue. It is also associated with hyperaminoacidaemia and hyperlipidaemia [1, 2]. Long-term suffering from diabetes mellitus may lead to critical complications like retinopathy leading to blindness, neuropathy, including dysfunctions of endocrine organs and nephropathy, which may cause renal failure. Patients suffering from diabetes mellitus are also at high risk of vascular, cardiovascular, peripheral and cerebrovascular diseases [3].

The world prevalence rate of diabetes mellitus may increase from 8.3% (366 million) in 2011 to 9.9% (522 million) approaching the year 2030 as estimated from recent studies and surveys. The highest increase may occur in developing countries like India and China. The United States of America also has a huge number of patients suffering from diabetes mellitus. Newly developed drugs are frequently tested for the prevention and treatment of diabetes and its complications. New approaches of current therapies for the treatment of diabetes mainly include maintaining diet, exercise and use of carbohydrate digestive enzyme inhibitors, which is responsible for inhibition of glucose absorption in intestine and reduction of cellular glucose uptake. Diabetes mellitus can be treated with allopathic oral hypoglycaemic agents, which are associated with mild-to-severe side effects related to hypoglycaemia, skin reactions, gastrointestinal problems, nausea and haematological disorders [4].

Treatment and mitigation of diabetes mellitus without any adverse effect is still a challenge to the healthcare profession. In the present era, a huge number of people are using herbs/natural products for the treatment of diabetes. *Since ancient times*, plants are being used by people to recover from their disease [2]. The active principles of plants are important sources of vitamins, minerals and natural antioxidants. The therapeutic efficacy of the plant extracts is more when consumed in crude form. The major disadvantage for using crude extract is that the quantity of herbal extract required for treatment is higher due to the degradation of plant metabolites such as flavonoids, terpenes, alkaloids, amides, phenols, steroids, etc. in the gastrointestinal tract as they are very sensitive to the acidic pH of the stomach. Acidic pH promotes the destruction of plant metabolites and loss of the pharmacological activity. In recent times, several scientific studies focused on encapsulation of the herbal extracts to provide sustained release of active compounds in the beta cells of islets of Langerhans in intestine ensuring maximum absorption for treatment of diabetes mellitus. In the present time, nanotechnological approaches involving medicinal plants have contributed to cutting-edge drug delivery systems. The nanotechnological approaches provide controlled drug delivery of the active compounds to the site of action by developing into nanoparticles. Research studies have justified that nano-formulation improves the solubility, therapeutic efficacy, bioavailability, minimizes the toxicity and improves the pharmacological activity. It

is anticipated that herbal remedies being incorporated with nanocarriers can significantly enhance the efficiency of new-age drug delivery systems. Nanoformulation provides a feasible way to overcome the solubility problem of herbal medicines, facilitating their administration. Furthermore, the lack of specificity in the drug delivery to the target site often leads to undesirable side effects. Herbal Nanoformulation enhances the target selective activity of the loading drug in vivo, hence making it a promising delivery system for herbal medicine. Inclusion of nanotechnology on bioactive compounds of medicinal plants to tailoring for their exclusive benefits has been proved an imperative circumstance in the current era [5]. Extensive exploration of nanotechnological approach will give new insight into the regimen of treatment of diabetic mellitus. This could be an important approach towards building a new arena for the development of novel clinically tested drugs from place sources by using an advanced procedure and drug designing.

4.2 Anti-diabetic Drugs from Herbal Sources

Since ancient times, natural products have been an integral part of history and culture and have been the backbone of all traditional systems of healing throughout the globe. Natural sources are being utilized intensely to treat diabetes mellitus; this effort has resulted in producing more than 700 herbal formulas containing more than 400 plants used for their anti-diabetic activity [6, 7]. As per the review published by Newman and Cragg, in the last 25 years, almost 32 New Chemical Entities have been filed with FDA for treatment of both type I and II Diabetes. These drugs include a significant number of biologics based upon varying modifications of insulin produced in general by biotechnological means.

4.2.1 Anti-diabetic Herbal Sources Indigenous to India and Special Emphasis on Northeast India

In nearly all the cultures, medicinal plants have been used as essential sources of medicine [8]. Ayurveda and other literature have mentioned the usage of plants in the treatment of various human ailments. The Indian subcontinent houses more than 45,000 plant species with several thousands of them claiming to possess medicinal properties. Of late, exponential growth has been noticed in the field of herbal medicine with many people in developing and developed countries leaning more towards natural drugs because of their origin and lesser side effects [9]. Various ethnomedicinal plants have so far been studied for their beneficial role in treating different forms of diabetes and its complications. A substantial number of bioactive medicinal plants were subjected to clinical trials and were found effective. Over the past few years, a steady trend of anti-diabetic phytochemicals showing higher potential than most synthetic drugs has been observed. This has resulted in a considerable shift of scientific attention towards classification/identification of

traditional medicinal plants with antihyperglycaemic activity that may be used for daily consumption along with the food.

India happens to be one of the mega biodiversity hotspots in the world, the northeast India comprising eight states, namely Assam, Arunachal Pradesh, Meghalaya, Manipur, Mizoram, Nagaland, Sikkim and Tripura, has a total geographical area of 262,180 km^2, which comprises about 8% of the country's total area. Over 1748 (23.4% of India) plant species of known medicinal value are supported in the Eastern Himalayan hotspot. Of these, considerable representation of medicinal plants is from the North East region [10].

North East India boasts of numerous plants used to treat diabetes traditionally; these include mostly the tribal and folk medicines in practice across various ethnic groups in remote villages and tribal pockets of the region. Varying levels of scientific studies have been done on less than half of these plants. Out of these plants studied, most of them exhibited anti-diabetic and/or hypoglycaemic activities in in vitro and/or in vivo pharmacological investigations. A list of such medicinal plants with anti-diabetic effect found in North East India is given in Table 4.1.

4.2.2 Anti-diabetic Herbal Sources from Rest of the World

A study on the global distribution of anti-diabetic plants has found a wide dispersion across all six continents, and along some particular regions, viz. Mediterranean, Caribbean and across the Middle East. The worldwide distribution of anti-diabetic plants is depicted in Fig. 4.1.

The figure indicates that Asia (56%) & Africa (17%) dominate the global concentration of the majority of the anti-diabetic herbs. This can be attributed to the fact that both Asia and Africa are situated in the tropic and sub-tropic regions, and are endowed with large rain forests. In addition, these two regions own certain pre-established traditional healthcare systems that rely heavily on locally available herbal sources.

India and China are the leading countries in herbal plant research. Most of the research studies conducted here draw inspiration from the ethno-medicinal systems of Chinese Herbology and Indian *Ayurveda*. These two traditional healthcare systems form the cornerstones of the herbal plant medicinal research in the irrespective regions. Some common medicinal plants used in the treatment of diabetes mellitus are *Allium sativum, Artemisia herba-alba, Artemisia dracunculus, Azadirachta indica, Asphodelaceae, Andrographis paniculata L, Caesalpinioideae, Carthamus tinctorius, Swertia, Coccinia grandis, Bauhinia, Gymnema sylvestre, Ferula assafoetida, Sarcopoterium, Salvia officinalis, Caesalpinia bonducella, Combretum, Syzygium cumini, Mangifera indica, Momordica charantia, Ocimum tenuiflorum, Pterocarpus, Trigonellafoenum-graecum, Tinospora cordifoli, Liriope, Panax, Cinnamomum verum, Abelmoschus moschatus, Vachellia nilotica, Achyranthes, Fabaceae, Mentha, Pachira aquatic, Gongronema latifolium, Nigella sativa, Tinospora cordifolia (guduchi), Symplocos, Zingiber zerumbet, Symphytum, Cactaceae, Chrysanthemum morifolium, Perilla frutescens, Terminalia chebula* and

Table 4.1 List of anti-diabetic medicinal plants found in North East India [11–17]

Sl. No.	Botanical name and family	Part used	Local name	Preparation
1	*Acacia Concina* DC (Fabaceae)	Leaves	Khangthur	An infusion of the leaves is taken orally
2	*Aegle marmelos (L.) Correa* (Rutaceae)	Leaves	Bel	Leaves boiled with water
3	*Ajugabracteosa* (Lamiaceae)	Leaves	Neel-kantha	Leaves boiled with water
4	*Albizzia procera* Benth (Mimosaceae)	Roots, leaves, flowers	Koroi, Tantari-asing	The juice from leaves is taken orally
5	*Alocacia indica* schott (Araceae)	Rhizomes	Mankachu, Bangla-Maankachu	Dried rhizome is used
6	*Aloevera tournex.* Linn (Liliaceae)	Leaves	Ghritakumari	Leaves' paste is used
7	*Annona reticulate* L. (Annonaceae)	Leaves, fruits	Atlas	Leaves, fruit juice is used
8	*Antidesma acidum* Retz (Euphorbiaceae)	Leaves	Nikhutenga, Abu-tenga, Saruheloch	Leaves' juice is used orally
9	*Artocarpus lokoocha* Roxb (Moraceae)	Barks	Diwatenga	Barks' infusion is used
10	*Artemisia maritma* (Asteraceae)	Leaves	Chinglaibaknag	Boiled leaves' extract
11	*Boenninghausenia albiflora* (Rutaceae)	Roots	Yomri,Nukmam	Root juice is taken orally
12	*Caesalpinia crista* Linn. (Caesalpiniaceae)	Seeds	Lataguti	Crushed powder is used
13	*Cassia occidentalis Linn.* (Caesalpinaceae)	Seeds, stem	Bonoriadadol	Seed powder is used
14	*Centella asiatica* (L.)urban. (Apiaceae)	Whole plant	Manimuni	Whole plant juice is taken in empty stomach
15	*Cichorium intybus* (Asteraceae)	Seeds	Kasni	Seed powder is used
16	*Cinnamomum tamala* fr. Nus (Lauraceae)	Bark, roots	Tezpaat	Bark powder made infusion
17	*Coccinia indica cogn.* (Cucurbitaceae)	Fruits, root	Balipoka	Fruits juice is used.
18	*Coix lacrymajobi (Poaceae)*	Roots	Chaningangouba (Manipur)	Crushed extract of roots is used
19	*Costus speciosus* (Koeing). Smith (Zingiberaceae)	Rhizomes	Jamlakhati	Rhizome paste taken orally

(continued)

Table 4.1 (continued)

Sl. No.	Botanical name and family	Part used	Local name	Preparation
20	*Curcuma aromatic* Salisb (Zingiberaceae)	Rhizomes	BonoriaHalodhi	Powder Rhizome is used
21	*Cynodon dactylon Pers* (Poaceae)	Whole plant	Dubori	Crushed and boiled with water
22	*Dillenia indica Linn.* (Dilleniaceae)	Fleshy perianth	Aautenga	Juice is used.
23	*EquisetumdebileRoxb.* (Equisteraceae)	Whole plant	Lai-utang Manipur	Boiled with root of male *Caricapapaya.*
24	*EugeniajambolanaLinn* (Myrtaceae)	Fruits, Barks, Seeds.	KalaJamu	Fruit juice is used orally.
25	*Euphorbia hirta Linn*. (Euphorbiaceae)	Whole plant	Gakhirotibon	Crushed and made infusion
26	*Flacourita jangomas* Lour. (Flacourtiaceae)	Fruits	Heitroi	Raw fruits are used
27	*Garcinia padunculata* Roxb. (Clusiaceae)	Fruits	Borthekera	Fruit juice or raw fruit is used.
28	*Gloriosa superba* Linn. (Liliaceae)	Whole plant	Gloriosa	Made infusion and used
29	*Heliotropium indicum* Linn. (Heliotropiaceae)	Aerial part	Hatisur	Dried and infusion is filtered before use
30	*Holorrhena antidysenterica* Wall. (Apocyanaceae)	Barks, fruits	Kutaz	Fruit juice is used
31	*Ichnocarpus frutescent* R. Bn. (Apocyanaceae)	Roots	Syamalota	Root decoction is used
32	*Ipomoea aquatic* Forssk. (Convulaceae)	Leaves	Kalmou	Dried leaf powder mixed with *Piper nigrum* and taken orally
33	*Jatropha curcus* Linn. (Euphorbiaceae)	Leaves, twigs	Bongaliara	Leaves' juice is used
34	*Jussieua repens* (Onagraceae)	Leaves, whole plant	Ishing-kundo	Boiled extract of the plant is used
35	*Justicia adhatoda* (Acanthaceae)	Leaves	Nongmang- khaangouba	Boil the leaves with the leaf of *Clerodendrunspihonanthus*.

36	*Kyllinga triceps* Rottb	Whole plants	Chumthang	Boiled extract
37	*Leucaena glauca* (Mimosaceae)	Leaves	Cialag Manipur	Leaves' decoction is used
38	*Leucas aspera* Spreng. (Lamiaceae)	Whole plant	Doron	Stomach along with Monopteruscuchia fish
39	*Lindernia bracheata* Linn *(Scruphulariaceae)*	Whole plant	Kachidoria	Juice used.
40	Ludwigia octovalvis Jacq.	Whole plant	KaboKaji	Boiled extract is used.
41	*Melia azadirachta* Linn (Meliaceae)	Leaves	Mohaneem	Leaves' juice is used
42	*Mentha arvensis* (Lamiaceae)	Plant part	Nungshi-hidak	Mixed with honey
43	*Meyna spinosa* Roxb. *(Rubiaceae)*	Fruits	Lam-heibi	Boiled extract of fruits
44	*Mimosa pudica* Linn. (Mimosaceae)	Whole plant	Nilazibon	Whole plant boiled with water
45	*Moringa oleifera* Linn. (Moringaceae)	Barks,flowers, leaves	Sogina	Bark infusion is used
46	*Mormordica charantia* Linn. (Cucarbitaceae)	Leaves, fruits	Tita-Karela	Leaves' and fruit juice is used orally.
47	*Mucuna pruriens* DC. (Papillionaceae)	Roots	Bandarkakura	Root powder soak with water
48	*Murraya koeningii* Sprang (Rutaceae)	Leaves	Narasingha	Leaves' juice is used
49	*Musa paradisiacal* Linn (Musaceae)	Flowers, fruits	Kashkol	Fruit juice is used
50	*Nigella sativa* (Ranuculaceae)	Seeds	Keman	Seed powder is used.
51	*Ocium sanctum* L. (Lamiaceae)	Leaves	Tulsi	Leaf powder taken with honey to treat Diabetes
52	*Peristrophe fera* C.B.Clarke (Acanthaceae)	Leaves or whole plants	Ishinglangthrei	Extract of the plant is used
53	*Phologocanthus tubiflorus* Nees. (Acanthaceae)	Barks	Sang-chi	Decoction of bark with *Zingiberofficinale*
54	*Phyllanthuse emblica* Linn (Euphorbiaceae)	Fruits	Amlakhi	Fruit juice or raw fruit is taken orally.
55	*Phyllanthuse urinaria* Linn	Leaves or whole plant	Heikruman	Boiled extract is used orally

(continued)

Table 4.1 (continued)

Sl. No.	Botanical name and family	Part used	Local name	Preparation
56	*Plumeria acuminata* (Apocyanaceae)	Barks	Sun-Champa	Powder bark decoction is used
57	*Portulaca oleraceae* (Portulaceae)	Whole plant	Kulfa	Crushed Powder is used
58	*Swertia chirata* L.(Gentianaceae)	Whole plant	Chirata	Whole plant extract is consumed
59	*Saraca indica* Linn (Caesalpiniaceae)	Fruits	Ashok	Fruit juice is used.
60	*Scleria terristris* (Linn) (Cyperaceae)	Plant part	Thangjou	Boiled extract of the plant part is used.
61	*Sesamum orientale* (Pedaliaceae)	Seeds	Senum	Seeds
62	*Smilax lanceifolia* Roxb. (Smilaceae)	Roots	Kwamanbi	Boiled extract of root
63	*Spondias mangifera* wild (Anacardiaceae)	Fruits	Amara	Raw fruit is used
64	*Sterculia villosa* Roxb. (Starculiaceae)	Roots	Udal	Root infusion is used
65	*Syzigium cumini* (Linn.) (Myrtaceae)	Seeds	Jamhei	Boiled extract of seeds
66	*Terminalia chebuta* Roxb. (Combretaceae)	Fruits	Selekha	Fruit juice is used
67	*Thevetia peruviana* (pers) Merill (Apocyanaceae)	Barks	Halodhia-korobi Utonglei	Powder bark is used
68	*Vinca rosea* Linn (Apocyanaceae)	Leaves	Nayantora	Leaves chewed in morning or juice is used
69	*Zanthoxylum armatum* DC. (Rutaceae)	Leaves and roots	Muthrubi	Root and leaf decoction is used

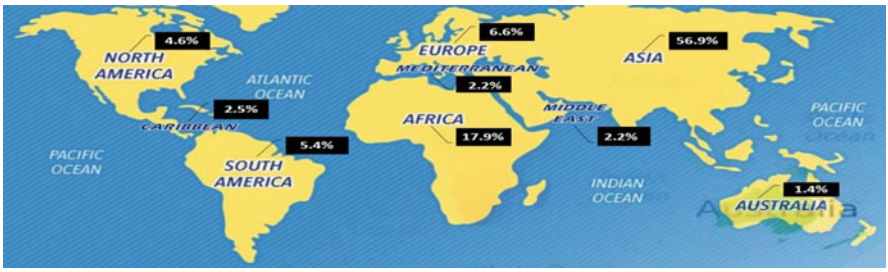

Fig. 4.1 Worldwide distribution of plants with anti-diabetic potential

Aloe vera. The anti-diabetic activity of these medicinal plants can be credited to the presence of terpenoids, polyphenols, coumarins, flavonoids and other constituents, which can lower the blood glucose levels [18, 19].

4.3 Some Prominent Isolated Compounds Extracted from Herbal Sources with Their Pharmacological Targets for Mitigating Diabetes

As the mechanism of action of drugs from herbal sources is very diverse, appropriate utilization of herbal drugs depends upon the stage of diabetes mellitus at which it should be used. Diabetes mellitus is having a myriad of pathophysiologies at different stages ranging from dysfunctional pancreatic β-cells to destroyed β-cells, peripheral insulin resistance to reduced insulin secretion, and inactivation of prolactin receptor (PRLR) and MafB in islet β-ells to diabetes mellitus-specific defective gene. Oxidative stress worsens the condition of diabetes mellitus and may produce complications and comorbidities such as diabetic nephropathy. Candidates from natural sources when taken as a therapeutic agent or as supplement reduce the injury caused by oxidative stress in diabetes mellitus. Pharmacology of some herbal drugs source at different stages of diabetes mellitus is discussed now.

4.3.1 Regulation of Insulin Secretion By Herbal Drugs

Development of T2DM is mainly due to a defect in the secretion of insulin. β-cell gets damaged due to overstimulation of pancreatic islets owing to long-term use of a conventional synthetic secretagogue, glibenclamide [20, 21]. So, the last decade has witnessed a huge interest in studying drugs from herbal sources and their bio-active components.

4.3.1.1 Dandelion (*Taraxacum officinale*)

Dandelion (*Taraxacum officinale*) is one such herbal drug that has given excellent results in mitigating diabetes mellitus both in the form of extracts and bio-active

compounds present in it [22]. Dandelion dose dependently improves the insulin secretion capacity of β-cells, thereby rejuvenating the β-cells as well as decreases plasma glucose concentration in a rat model of diabetes [23]. The most abundant component of dandelionarechicoric acid (CRA) and chlorogenic acid (CGA), which are reported to stimulate insulin secretion by acting on sulphonylurea-binding site 1 (SUR1) [24].

4.3.1.2 *Vitis vinifera* L

Grape by-products (*Vitis vinifera L*) such as stems, seed and skin of PusaNavarang and Merlot showed promising result in augmenting insulin secretion. Grape by-products, rich in polyphenolics, acted as antioxidants, which increases the secretion of insulin 2–eightfold [25].

4.3.1.3 Cuminaldehyde, Cuminol and Cuminol

Cuminum cyminum is also reported to regulate insulin secretion and at the same time having a protective effect on the β-cells. Cuminaldehyde, cuminol and cuminol isolated from *Cuminum cyminum* are reported to demonstrate an insulinotropic effect in streptozotocin-induced diabetic rat and β-cells protective as seen from comet assay [26].

4.3.1.4 Resveratrol

Resveratrol (3,5,4-trihydroxylstilbene) is a naturally occurring stilbenoid, a polyphenol mainly found in grapes and nuts. It possesses a diverse range of pharmacological activities, including analgesic, neuroprotective, anti-inflammatory, antioxidant and antiplatelet activities. It also corrects glucose metabolism. Nanoliposomes loaded with Resveratrol were prepared and tested for their antidiabetic activity in Streptozotocin-induced diabetic animals. The nanoformulation loaded with resveratrol could be a beneficial formulation for the treatment and mitigation of diabetes mellitus. Use of resveratrol and its analogue combined with nanotechnology could be a potential treatment and prevention of diabetes mellitus in future [27]. It improves the structure of pancreatic islet and decreases the insulin resistance in diabetic animals [28]. Resveratrol is reported to regulate insulin secretion as well as protect β-cells. A study reported the protective action of resveratrol in high-fat diet in C57BL/6 J mice. The report states that resveratrol ameliorated the abnormal insulin secretion by promoting SIRT1 and inhibiting uncoupling protein 2 (UCP2) in isolated islets that was caused due to HFD-induced morphological changes in the pancreas [29, 30].

4.3.1.5 Berberine

Berberine is a very important benzylisoquinoline alkaloid isolated from the plant *Coptis chinensis*. It is widely used for the treatment of inflammations, intestinal infections, congestive heart failure, hypertension, cardiac arrhythmia, cancer, hyperlipidaemia, skin diseases and diabetes. Nanoformulation of berberine had shown better bioavailability with high therapeutic efficacy. Berberine promotes insulin secretion from beta cells of the pancreas in a dose-dependent manner in

adult Wistar rats. Research studies suggested that berberine can be used as an effective insulin-sensitizing and insulinotropic agent. It improves glucose metabolism, increases secretion of insulin, stimulates glycolysis by suppressing adipogenesis, inhibits mitochondrial function, activates the 5′ adenosine monophosphate-activated protein kinase (AMPK) pathway and increases glucokinase activity [31].

4.3.1.6 Gymnemic Acid

Gymnemic acid is a triterpenoid compound isolated from the plant *Gymnema sylvestre*. It produces a strong anti-diabetic activity by increasing the insulin secretion from pancreas. Gymnemic acid possesses a wide range of pharmacological activities such as taste sensitivity suppression, inhibiting intestinal absorption of glucose and reducing the level of glucose in diabetic patients. The major drawback of this compound is its poor water solubility, which decreases its pharmacological effects. A novel approach is essential to enhance the bioavailability and solubility of gymnemic acid. The nanoformulation of this compound demonstrated better antihyperglycaemic activity and exerted hypoglycaemia. The mechanism of action of Gymnemic acid is through stimulation of insulin secretion from the pancreas. It also improves the impaired pancreatic islet cells to enhance enzyme-mediated uptake of glucose [32].

4.3.2 Antioxidant Perspectives of Herbal Drugs in Oxidative Stress

Chronic insulin resistance in Type-2 diabetes mellitus accompanied by obesity results in persistent hyperglycaemia leading to the formation of free radicals due to glucose auto-oxidation. When the generation of free radicals exceeds the scavenging ability of the endogenous antioxidant property, it results in vascular snags and high serum concentration of inflammatory markers [33]. Dietary antioxidants or supplements from natural sources aid as an antioxidant during oxidative stress in diabetes mellitus.Nrf2 (NF-E2-related factor 2), a sensor for oxidative stress, are reported to upregulate the concentration of some anti-oxidant enzymes and Keap1 (Kelch-like ECH-associated protein 1). Keap1 ensures degradation of Nrf2 through the ubiquitin–proteasome pathway under unstressed condition; however, under oxidative condition, it loses its ability to ubiquitinate Nrf2 and thus the latter exerts its power in mitigating oxidative stress [34].

4.3.2.1 Swertiamarin

Swertiamarin, from *Enicostemma littorale* blume leaves, is reported to regulate protein carbonyls, total lipid peroxides and hydroperoxides - all oxidative stress markers in high-fat diet fed streptozotocin-induced T2DM rats. Swertiamarin displayed promising antioxidant properties in the form of superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and glutathione s-transferases (GST) as well as demonstrated potent anti-diabetic properties in diabetic rats [35].

4.3.2.2 Corn Silk (*Zea mays* L.)

Corn silk (*Zea mays L.*), a waste by-product in corn cultivation, is traditionally used as anti-obesity, anti-oxidant and anti-diabetic [36]. In a recent study, ethyl acetate fraction (ECS) and *n*-butanol fraction (BCS) of corn silk demonstrated potent scavenging activity, total antioxidant potential and strong reducing power against 1-picrylhydrazyl 2,2-diphenyl (DPPH) and hydroxy radicals [37].

4.3.2.3 Silybin

Silybin is the major bioactive ingredient of silymarin. This bioactive compound possesses beneficial pharmacological effects like anti-oxidant, hepatoprotective, anti-inflammatory, and anticarcinogenic effects with less toxic effect. Silybin has been studied in obesity-induced insulin resistance model related to inflammatory biomarkers. Nanoformulation of PLGA loaded with Silybin were prepared and studied for their efficacy on streptozotocin-induced diabetic rats. The loading efficiency of silybin was found to be more than 92.11%. The results confirmed that nanoformulation of Silybin improves diabetic complications and reduced hyperglycaemia. Silybin also has regenerative impacts on beta cells and is responsible for increasing membrane permeability. This nano-formulation provides a new approach for the treatment and mitigation of diabetes mellitus [38].

4.3.3 Herbal Drugs-Assisted Alleviation of Peripheral Insulin Resistance

Insulin resistance is the impaired sensitivity of insulin to peripheral tissues such as liver, skeletal muscle and adipose tissue. It occurs when the normal energy utilization is exposed to chronic surplus of energy due to decreased peripheral glucose disposal and increase in hepatic glucose and lipoprotein [39, 40]. All these results in high serum glucose during fasting and postprandial that lead to the development of Type-2 diabetes mellitus. Insulin sensitivity may be increased in the peripheral tissue utilizing natural products and their active principles.

4.3.3.1 Botanical Mixture of American Ginseng, Fenugreek Seed, Mulberry Leaf Extracts

All these three extracts individually reported to improve glucose uptake and insulin sensitivity in human adipose tissue when examined using radiolabelled 2-deoxyglucose and decreased insulin resistance in a rat model of insulin [41]. With the increasing demand for non-drug interventions targeting insulin resistance and regulation of blood glucose level, dietary supplements from natural sources may substitute the conventional drug that are loaded with adverse effects and complications.

4.3.3.2 Emodin

Emodin is basically an anthra-quinone derivative mainly found in *Radix* et *rhizoma*. It imparts a diverse range of pharmacological activities like anti-oxidant anti-

inflammatory, anti-diabetic, anti-nociceptive and anti-cancer activities. Emodin enhanced the binding affinity in differentiated 3 T3-L1 adipocytes via induction of increased glucose uptake and increased GLUT1 and GLUT4 mRNA expression [42]. Nano-encapsulation of emodin was found to be beneficial for the treatment of diabetic neuropathy. Emodin exerts its anti-diabetic activity by suppressing the elevated glucose level, which occurs due to glucose disposal into peripheral tissues [43].

4.3.4 Inhibition of Glucose Absorption By Herbal Drugs

Glucose is absorbed from the small intestine to the systemic circulation, which aids in high serum concentration of glucose in absence of adequate supply of insulin and thus leads to the development of Type-1 and Type-2 diabetes mellitus. Interestingly, too rapid or increased glucose absorption is not a cause for hyperglycaemia in gestational diabetes mellitus as reported by a study done on human subjects [44]. Because of the low cost and significantly high safety profile, herbal drugs and their active principles have garnered a lot of attention worldwide for their diverse pharmacological effects. α-glucosidase is a class of enzyme that facilitates the absorption of intestinal glucose to the systemic circulation and thus exacerbates hyperglycaemia. GLUT2 is a glucose transporter that gets activated on sensing high glucose levels in the lumen and facilitates the uptake of glucose [45]. Conventional α-glucosidase inhibitors like voglibose, miglitol and acarbose are quite potent oral hypoglycaemic drugs, but they come with adverse effects of gastro-intestinal origin and are dose dependent [46].

4.3.4.1 *Nigella Sativa*

Nigella sativa is reported to exert potent anti-diabetic effect and its crude aqueous extracts of its seed are reported to inhibit intestinal glucose absorption by using the in vitro technique of short circuit current. *Nigella sativa* dose-dependently inhibited the sodium-dependent transport of glucose across an isolated rat jejunum, thereby carrying out an inhibition rate of more than 80% with IC_{50} of 10 pg/ml [47].

4.3.4.2 Feruloylated Arabinoxylan Mono- and Oligosaccharides (FAXmo)

Phenolic compound FAXmo is obtained from corn bran and wheat aleurone is reported to exert its anti-diabetic efficacy by inhibiting the absorption of glucose in human caco-2 cells. FAXmo significantly inhibited the maltase and sucrase function of the α-glucosidase, thereby reducing the glucose uptake in Caco-2 cells by 40% and completely inhibiting the GLUT2 activity in *Xenopus laevis* oocytes and thus demonstrated its ability as a potent α-glucosidase inhibitor [48].

4.3.4.3 Tomatoside A

Tomatoside A, an active steroidal saponin isolated from tomato seed, is reported to inhibit the glucose transport in caco-2 cells by suppressing the expression of GLUT2

transporter. 10μM of tomatoside A in Caco-2 cell for 3 h reduced the glucose transport by 46.0% by suppressing the expression of GLUT2 [49].

4.3.4.4 Stevioside

Stevioside is a glycoside-derived compound isolated from the leaves of the plant *Stevia rebaudiana*. This compound is well known for its potent anti-diabetic activity. Several research works explained that stevioside exhibits a very strong impact on renal function and glucose metabolism. It also possesses the ability to regulate the glucose level in blood by stimulating insulin utilization and secretion in diabetic rats. Though stevioside is a potent anti-diabetic agent, it has less therapeutic efficacy due to its poor intestinal absorption and poor bioavailability. Nano-bioconjugation of this compound on biodegradable copolymer Pluronic-F-68-based Polylactic acid (PLA) nanoparticles prepared by the method of nanoprecipitation (spherical, size range: 110-130 nm) was found to be beneficial to overcome the poor intestinal absorption and to improve the bioavailability. The drug loading efficiency was found as 16.32 ± 4% (w/w). The in vitro drug release study demonstrated the initial burst followed by the sustained release. The half release and complete release were obtained on 25 ± 4 h and 200 ± 10 h, respectively. This novel formulation Nanostevioside showed very high anti-diabetic efficacy in streptozotocin-induced rats [50].

4.3.4.5 Quercetin

Quercetin is a widely used flavonoid mainly found in vegetables and citrus fruits. It possesses a diverse range of pharmacological activities like anti-diabetic, anti-cancer, antioxidant and anti-inflammatory activities. Quercetin has been demonstrated to improve the metabolic abnormalities of diabetes, including lipid profile, liver enzyme levels, postprandial blood glucose and waist circumference. Nano-formulations of Quercetin possess higher bioavailability with high anti-diabetic activity [51]. Quercetin reacts with several molecular targets in small intestine, pancreas, skeletal muscle, liver and adipose tissue to control the glucose homeostasis of the entire body. The anti-diabetic mechanism of Quercetin is pleiotropic. It inhibits the intestinal glucose absorption, stimulates insulin secretion and produces insulin-sensitizing effect as well as improves glucose utilization in peripheral tissues.

4.3.4.6 Myricitrin

Myricitrin is a flavonol glycoside isolated from the medicinal plants like *Pouteriagender, Myrica rubra, Manilkara zapota* and *Eugenia uniflora*. Myricitrin possesses very potent anti-diabetic, anti-nociceptive, antioxidant, anxiolytic and anti-inflammatory activity. The metabolism and bioavailability of flavonoids are the key factors considered for the nano-formulations of myricitrin. Due to its high polar nature, it cannot cross biological membranes. Solid lipid nanoparticles (SLN) of Myricitrin demonstrated a protective effect against cytotoxicity induced by streptozotocin (STZ) in β-cells of islets of Langerhans. This research study was conducted to evaluate the anti-diabetic activity of Myricitrin-loaded solid lipid

nanoparticles (SLN) on streptozotocin-nicotinamide- (STZ-NA) induced type-2-diabetes in mouse. The plasma samples, pancreas and muscle tissues, and myotubes were taken for experimental assessments after the last treatment with Myricitrin-loaded nanoparticle. Diabetes induced increased lipid peroxidation and reduced antioxidant defence along with the hyperglycaemia, insulin resistance and pancreas apoptosis was seen in Diabetic mouse. Myricitrin-loaded solid lipid nanoparticles (SLN) improved hyperglycaemia complications in the in vivo and in vitro studies. Solid lipid nanoparticles (SLN) of Myricitrin also exhibit antioxidant, anti-diabetic and anti-apoptotic effects in mouse and myotube cells. It initiates the stimulation of glucose uptake and inhibition of α-glucosidase enzyme, which is responsible for its anti-diabetic activity [52].

4.3.5 Diverse Pharmacological Role of Herbal Drugs in Alleviating Diabetes Mellitus

Pancreatic β-cells are reported to be destroyed by $CD8^+$ T and $CD4^+$ cells that follow the infiltration of macrophages to the islets. Maturity-onset diabetes of the young (MODY) or monogenic diabetes are caused by several defective genes and may be managed by modification of personalized dietary intake depending upon the genetic makeup of an individual. Although personalized diet is the need of the hour for a diverse population of a diabetic patient, nutrigenetics is still in the nascent stage. A Thorough study of the genetic makeup and their interaction with nutrients from herbal source is needed for a successful implementation of the technology.

4.3.5.1 Compound K

Pancreatic β-cells are reported to be destroyed by $CD8^+$and $CD4^+$ T cells that follow the infiltration of macrophages to the islets. Compound K, a ginseng metabolite, reportedly reduced the rate of $CD8^+$ and $CD4^+$ cells and in spleen and lymph nodes, and thus prolonged the survival of islet allograft and inhibited inflammatory cell infiltration other prominent inflammatory markers to the islet allograft [53].

4.3.5.2 Mediterranean Diet

Most recently, gene-based dietary advice has gathered momentum where genetic makeup of an individual and their response to a particular nutrient were studied and thus evolved the term ‘nutrigenetics’ [54]. In a recent study covering 7000 Type-2 diabetes mellitus cases that reported on the gene Mediterranean diet interaction, it was found that subjects adhering to the Mediterranean diet, the risk of T2DM was low. But it was 20% higher among MC4R rs17782313 and FTO rs9939609 variant allele carriers without the Mediterranean diet [55].

4.3.5.3 Vitamin D

A study was conducted to know the variation of response to vitamin D intake in subjects suffering from diabetes mellitus. Interestingly, the study revealed that subjects with VDR Fok-I ff genotype are low responders to vitamin D supplement

in terms of circulating 25(OH)D and some inflammatory biomarkers. The study concluded with a demand of nutrigenetics approach for diabetic subjects to protect them from vitamin D deficiency [56].

4.3.5.4 Curcumin

Curcumin is a potent bioactive compound obtained from the plant *Curcuma longa*. It comes under the curcuminoid subgroup of polyphenols with a diverse range of pharmacological activities like antioxidant, antitumour, hypolipidaemic, anti-diabetic and antiulcer activities. Several research works have demonstrated that Curcumin possesses a very potent anti-diabetic activity along with hypolipidaemic effect. It also improves obesity-related metabolic dysfunctions such as hyperglycaemia, hyperlipidaemia and insulin resistance. Clinical trials conducted on Curcumin support its anti-diabetic activity as an adjuvant therapy for type 2 diabetes. The major drawback of Curcumin is poor water solubility, which can be overcome by nano-structured drug delivery system. Various methods have been incorporated and designed to overcome this problem along with formulating this bioactive compound in a nanosized structure [57]. Encapsulation of curcumin in multipolymer poly (gamma-benzyl l-glutamate)-poly (ethylene glycol)-poly (gammabenzyl l-glutamate) nano-particles (NPs) is an effective method to improve its therapeutic efficacy and water solubility. This type of nano-formulation possesses a potent activity on recovering diabetic cardiomyopathy (DCM). The underlying mechanisms of action of Curcumin are diverse and mainly involve the regulation of various molecular targets, including transcription factors, growth factors, inflammatory cytokines, protein kinases and other enzymes such as cyclooxygenase 2 and 5 lipoxygenase [58].

4.3.5.5 Capsicum Oleoresin

Capsicum oleoresin has been isolated from dried, ripe fruit of the *Capsicum* plant. It is widely used as an additive in the food industry for taste improvement and preservation of food. The crude ethanolic extract of capsicum exhibits diverse pharmacological activities, including antioxidant, anti-diabetic, anti-inflammatory and anticancer effects. It has been demonstrated that administration of a Nanoemulsion loaded with Capsicum oleoresin in obese rats decreases the glucose level and increases the metabolism of carbohydrate, which is a key factor in the treatment of type −2 diabetes. It decreases the increased body weight and reduced the level of adipose tissue mass in the obese rats. Capsicum oleoresin decreases the adipogenic gene expression and increased the expression of PPAR-α, UCP2, and CPT-1α and helps in regulating hyperglycaemia along with its complications [59].

4.3.5.6 Naringenin

Naringenin is a very effective bioactive compound mainly present in vegetables and citrus fruits like grapefruit and oranges. It belongs to the class of flavonoids called flavanone. It shows diverse pharmacological activities like antimutagenic, anti-inflammatory, anti-diabetic, antihyperglycaemic and antioxidant activities. It lowers the levels of lipids and diabetic complications. A drastic increase in the

immunological and haematological parameters of blood along with 100% survival was seen in Naringenin-treated diabetic mice. The main disadvantage of naringenin is its poor water solubility and poor absorption in the intestine after oral administration. Nanostructured delivery of this flavonoid can be considered as an effective remedy for the treatment of diabetes and related complications. Naringenin exhibits its anti-diabetic activity by inhibiting gluconeogenesis through upregulations of AMPK. It also possesses hypoglycaemic effects like metformin that mitigate inflammatory conditions and cell proliferation [60].

4.3.5.7 Baicalin

Baicalin is an essential flavonoid isolated from the plant *Scutellaria radix*. Baicalin also possesses anti-inflammatory activity by a radical scavenging effect. It has low water solubility and poor absorption after oral administration because of its glycosylic group. The nano-formulation loaded with Baicalin was established by loading with lipid nanocarriers and evaluated for its anti-diabetic effect in streptozotocin-induced diabetic rats [61]. Baicalin exerts its anti-diabetic activity by decreasing glycogen breakdown, plasma glucose levels, glycosylated haemoglobin, mRNA and protein expression levels of gluconeogenic genes like phosphoenol pyruvate carboxykinase.

4.3.5.8 Scutellarin

Scutellarin is one of the important active principles of the traditional Chinese herb *Erigeron breviscapus (Vant.) Hand. Mazz.* It comes under the flavone group of flavonoids. It has been reported for use against vascular endothelial cell dysfunction. A novel intestinal target-specific nanoformulation of Scutellarin loaded with amphiphilic chitosan derivatives increased its bioavailability and efficacy for treatment of diabetic retinopathy. Results concluded that administration of Scutellarin minimized retinopathy in diabetic rats. Treatment with the nano-formulation loaded with Scutellin was more efficient than Scutellinalone. Scutellarin promoted glucose disposal in mice and adipocytes. It selectively enhanced Akt phosphorylation [62].

4.4 Application of Nanotechnology for Anti-diabetic Herbal Formulation

In recent years, a budding interest has been generated in nanopharmaceuticals due to increased number of advancements with an aim to focus on engineering novel applications. The active phytoconstituents and standardized extracts are the main sources from which nanophytomedicines are prepared. By lowering the side effects and toxicity associated with the drugs, the herbal treatment not only helps to increase the bioavailability but also increases the therapeutic value at the same time [63]. In this regard, nanotechnology has a vital role to play in herbal medicines. Further, drug delivery systems incorporating nanotechnology is all set to spread extensively. The problems associated with synthetic drugs can be overcome by nanotechnology-based herbal drug delivery systems and subsequently enhancing the potency of

medicinal plants in the near future [64]. Due to the insufficient processing difficulties and logistic justification since long time, herbal medicines were not taken into consideration so as to develop and design novel formulations. However, these shortcomings have been resolved as scientific needs (such as pharmacological mechanistic pathway, pharmacokinetics determination, accurate dose calculation, site of action, etc.) of herbal drugs are solved by modern phytopharmaceutical research such that it can be incorporated in novel drug delivery systems such as solid dispersions, nanoparticles, matrix systems, microemulsions, solid lipid nanoparticles and liposomes. Thus, herbal drug incorporated modern dosage forms with enhanced efficacy and potency can be utilized in a way better for designing and developing novel drug delivery systems [63].

4.4.1 Material-Based Nanoformulation

4.4.1.1 Nano-carriers

Herbal drugs entrapped in nanocarriers bypass all the systemic barriers, including acidic pH of the stomach, liver metabolism that can hinder the ease of drug release at the desired site of action. Further, an optimum quantity of drug is carried to the site where desired pharmacological action is to be produced enhancing circulation of drug in the bloodstream because of their small size [65]. Some of the nanocarriers that are commonly incorporated in nanotechnology-based drug delivery systems include polymer nanoparticles, liposomes, carbon-based conjugates, polymer conjugates, lipid-based carriers, micelles, dendrimers, polymeric nanoparticles, nanotubes of carbon, gold nanocarriers. When nanocarriers are incorporated with different nanomaterials, they allow the delivery of both hydrophobic and hydrophilic drugs throughout the body [66]. Protein-based nanocarriers have emerged as a promising gene and drug delivery system that demonstrates less cytotoxicity than synthetic molecules [67].

4.4.1.2 Polymeric Nanoparticles

Polymeric nanoparticles are the therapeutic carriers obtained from biocompatible and biodegradable polymers [68]. Polymeric nanoparticles encapsulate small drug molecules, hydrophilic and/or hydrophobic molecules, nucleic acid macromolecules and protein molecules [69]. They permit controlled and slow release of drug at the target site. Also, polymeric nanoparticles have a unique property of being tailored prior to particle assembly. Natural and synthetic elements like amino acids, nucleotides and sugars are used for the preparation of another type of polymeric nanoparticles that are branched unit of macromolecules and are called dendrimers [70]. From the experimental studies, it was observed that curcumin-loaded PLGA-PVA polymeric nanoparticles were used for the treatment of diabetic cataract in STZ-induced diabetic rat models. Berberine-loaded PLGA-PEG-PLGA block copolymers were found effective in modulating PCSK-9 mRNA for treating high LDL cholesterol. Quercetin-loaded chitosan-alginate core shells and quercetin-loaded PLGA nanoparticles were used for controlling diabetes in rat models induced

with STZ and epithelial cell line HT29 of the human colon. Naringenin-loaded alginate-coated chitosan core shells were also used for controlling diabetes in STZ-diabetic rat models. Emodin-loaded PEGMA-DMAEA-MAMMAM nanomacroemulsions on the other hand were utilized against the treatment of neuropathic pain in diabetic rat models induced with STZ. Systemic hyperglycaemia of diabetic rat models induced by STZ was treated by silybin-loaded PLGA polymeric nanoparticles. Elevation in the degree of bioavailability and intestinal absorption along with better rate of drug release and absorption in the intestine were prominently found in stevioside-loaded nanoparticles compared to free stevioside. Scutellarin-loaded amphiphilic chitosan derivatives were used for the treatment of retinopathy in STZ-diabetic rat models and Caco-2 cell lines [71].

4.4.1.3 Solid Lipid Nanoparticles

Solid lipid Nanoparticles (SLNs) offer higher physicochemical stability and protection against labile drug degradation. They are colloidal particles that contain purified triglycerides and lipids stabilized by surfactants. Solid lipid nanoparticles are used in the field of pharmacy against various routes of administration, including parenteral, oral and topical [72]. Studies have shown that solid lipid nanoparticles containing berberine improved triglyceride level, body weight and insulin sensitivity in insulin-resistant animals. However, solid lipid nanoparticles containing berberine showed higher bioavailability in comparison to berberine alone [71].

4.4.1.4 Liposomes

Liposomes constituting lipid bilayers are prepared using amphiphilic molecules that have similarities with biological membranes with improved efficacy and safety. If the active compound is water soluble it is located in the aqueous space and if it is lipid soluble then it is located in lipid membrane. In recent years, stealth liposomes have been developed, which is a new generation of liposomes that have longer half-life as compared to normal liposomes [73]. Resveratrol-loaded nanoliposomes were PEGylated covalently that not only increased the half-life but also the retention time of the nanoliposome. Further, an extended release of resveratrol along with the enhancement in the expression of ROS-inactivating enzymes such as SOD and GSH-Px were observed in diabetic pancreatic β cells [71].

4.4.1.5 Microemulsion and Nanoemulsion

Microemulsion is basically a liquid solution that is thermodynamically stable, optically isotropic and is composed of oil, water and amphiphile. It acts as a perfect replacement amongst other drug delivery systems for oral delivery of compounds that have poor water solubility. They exhibit numerous advantages like ease of preparation, enhanced dissolution of lipophilic drugs, slow viscosity, thermodynamic stability and bioavailability improvement. Microemulsion can also be administered by various routes like ocular, pulmonary, parenteral and transdermal [74]. Curcumin-loaded nanoemulsions inhibited enzyme 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGR) controlling cholesterol biosynthesis in STZ-diabetic rat models [71].

4.5 Challenges in Developing Herbal-Based Nanoformulation

Different kinds of herbal medicines have come to the market worldwide as a result of globalization of trade. Herbal medications or related products are extracted from Indian herbs, Chinese herbs, Arabic herbs and Western herbs. Integrated research, including conventional 'Herbal medicines' and 'Nanotechnology', has established therapies that are attractive to the field of pharmacy enhancing the health benefits of the people. It is thereby considered that when natural products are applied along with nanocarrier the significance of existing drug delivery system is enhanced. However, there exist significant challenges in implementing clinical therapies and methods to control the interactions of biological systems with nanomaterials [75]. Some of the issues associated with herbal drugs include pharmacological, toxicological, clinical documentation, pharmacovigilance, standardization, evaluating drug interactions, constraints with clinical trial, safety and efficacy assessment. Adulteration is also an important issue associated with herbal drugs and may occur in two possible ways, namely direct adulteration, which is also known as intentional adulteration and other one being indirect adulteration, which is also known unintentional adulteration [76].

Some additional obstacles have also been encountered in the design and development of herbal-based nanotechnology-incorporated drug delivery systems. Determining the usefulness of scale-up processes that can feature creative methods and quickly bring up therapeutic techniques to the market so as to fulfil several biological and therapeutic requirements, examining the efficiency of nanoparticles towards the target, and assuring international standards of nanoparticles against their biocompatibility and toxicity are some of the new obstacles [75]. To overcome these obstacles, science-based information on herbal medicine, dosage, efficacy, potency and contraindication should be given to the consumer. However, it is the prime duty of both the herbal manufacturers and prescribers to bring a revolution in herbal drug delivery systems and reshaping herbal medicines by utilizing our own resources so as to challenge the hindrances of twenty-first century [77].

4.6 Conclusion

Natural compounds and their derivatives have numerous therapeutic applications. However, they have a significant role to play in diabetes and complications associated with such metabolic disorder. The last decade has witnessed the rise of nanotechnology in almost all fields of healthcare sector. Nowadays, the application of nano-based formulation incorporating herbal drugs that targets specific sites in the human body in an aim to enhance their bio-availability is a top priority area in the field of bio-medical sciences. Although nano-based herbal formulation has showcased excellent efficacy and bio-compatibility in the pre-clinical setup, it has come with various challenges, too. Assessment of the biocompatibility, target-based capability of the loaded nanoformulation as well as compliance with the international regulatory toxicology guidelines and phase-wise clinical trials are some of the biggest challenges that are needed to be coped up before releasing them

commercially in the market. Herbal nanoformulations may be uptaken as practical methods to enhance the functionality and bioactivity of these natural products. Utilising this area and integrating the herbal technology with nanotechnology will not only provide a new insight in the treatment of metabolic disorder like diabetes, but it will also provide a platform to the researchers for exploring this area so as to design and develop scientifically potent herbal drug delivery systems. Even though there are some additional obstacles encountered in the design and development of herbal nanoformulation, research is to be carried out such that bioavailability of the drug is enhanced at the target site utilizing various aspects of nanotechnology.

Acknowledgements The authors would like to thank Dibrugarh University for providing necessary infrastructure and technical support in proceeding with this piece of work. The authors are also grateful to All India Council for Technical Education (AICTE), Department of Biotechnology (DBT), Indian Council of Medical Research (ICMR) and University Grants Commission (UGC), New Delhi, Govt. of India, for providing financial support under AICTE-RPS to AD and research fellowship to RS,KP,UG and MPP, respectively.

Conflicts of Interest There are no conflicts of interest among the authors to report with respect to this chapter. All the tables and figures are self-made and original.

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Nanomaterials for Alternative Antibiotic Therapy

5

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Abstract

Advancement of multidrug resistance amid microorganisms has turned into a global crisis for chemotherapy of microbial diseases. The progressive broadening of resistant organisms makes people worry regarding antimicrobial resistance. The development of alternative antimicrobials using modern technology by replacing the traditional antimicrobials is times demand. Nanotechnology-driven innovations offer expectations for healthcare professionals and peoples in prevailing over the dilemma of drug resistance. The nanotechnology has delivered many nanoparticles and that created new antimicrobial options. The small-sized nanoparticles are more advantageous for hauling out antimicrobial functions. The metals like zinc, silver, copper, and iron in their nanoparticle form possess significant bactericidal and fungicidal actions. Thus, metal nanoparticles are considered as competent antibiotic agents in wound healing and other healthcare problems. These nanomaterials exhibit efficient antimicrobial activity against several pathogenic viral and bacterial species. Nanoparticles today are a promising podium for substitute instrument to control pathogenic diseases as they proffer prolonged antimicrobial activity with negligible adverse effects compared with small molecular antimicrobial agents that show short-term action with ecological toxicity. Nanoparticles could also deliver a promising solution to multidrug resistance shown by microorganisms and may also act as a carrier for antibiotics and natural antimicrobials.

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M. K. Das, Y. V Pathak (eds.), *Nano Medicine and Nano Safety*,
https://doi.org/10.1007/978-981-15-6255-6_5

Keywords

Multidrug resistance · Nanomaterials · Antibiotics · Antimicrobial resistance · Metal nanoparticles

5.1 Introduction

When we mention nanoscience, small objects strike our mind. The branch of science deals with the substances that have a size range of 1–100 nm, is spoken as nanoscience. The International Organisation for Standardisation (ISO) has outlined that materials with any external dimension within the nanoscale or having an internal structure or surface structure within the nanoscale are coined nanomaterials.

Among the principal causes of morbidity and mortality worldwide, infectious diseases are a number of prime factors. The United Nations agency has articulated sober anxiety regarding the unrelenting rise in the expansion of drug resistance by microorganisms. Hence, the incident of antibiotic resistance is one of the very significant issues in today's world healthcare system. Due to the lack of optimized recent antimicrobials, the cases of antibiotic resistance are increasing. This has generated alarm among the world community to discover fresh and supplementary practical antimicrobial compounds moreover on developing novel deliverance and marking ways. A bacterium has developed in some ways by that they become proof against antimicrobials.

Multidrug antibacterium resistance is a big challenge in public health care around the globe. The quantity of infections made by bacterial-resistant strains is increasing globally. This noninheritable resistance of pathogens indicates a key challenge for a number of antimicrobial medications. Recent progress in engineering science provides new forecasts to develop formulations supporting distinct sorts of nanoparticles (NPs) with totally different sizes, shapes, and versatile antimicrobial activities. NPs could deliver a promising answer as they will not solely combat bacterium themselves, however, they may act as carriers for antibiotics and natural antimicrobial compounds [1]. Varied materials are explored from liposomal to chemical compounds primarily based on nano-drug carriers, bronze vectors, like gold.

The inert and nontoxic nature of NPs makes them core materials in the fight against antibiotic resistance [2]. Arguably the foremost enticing facet of dosage forms incorporating nanoparticles is to introduce a good variety of medical specialty, either sure to their massive expanse or contained at intervals to the positioning of infection successfully and safely by having a controlled rate of targeted delivery [3]. By improving the pharmacokinetic and pharmacodynamic profile along with the therapeutic index of entrapped medication compared to free treatment equivalents, the dosage needed to attain clinical effects are often considerably small. This successively cut the adverse effects related to elevated common drug concentrations and repeated dosage [4]. The increasing apprehension of concerning multidrug-resistant microorganism and biofilm-associated contamination demands the event

of further germicidal resources. Therefore, concentration is particularly dedicated to the latest and intensifying nanoparticle-based materials within the area of antimicrobial remedy. The utilization of engineering science in varied sectors of medical specialty has revolutionized the sphere of medication wherever nanoparticles of dimensions traveling between 1 and 100 nm are intended and applied in nosology, medical specialty, and medical specialty tools for analysis [5]. Usually used metals in nanoscience include silver, gold, copper, nickel, zinc, aluminum, silicon, titanium, magnesium, iron, and calcium [6]. The passion of using nanoparticles is in high priority at this stage each in the knowledge domain and in industrial applications. Engineering science is being applied broadly to supply embattled medical aid, nosology, tissue regeneration, biosensors, and alternative kit within the area of life sciences. Varied engineering science podiums in regard to nanotechnology like nanotubes, nanopores, fullerenes, quantum dots, mesmeric nanopores, dendrimers, and radio-controlled nanomaterials are being made advanced [7].

Nanoparticles could also be tactically beneficial like dynamic medicine since their expanse is extremely massive virtual to mass. Nanosized particles could give high activity though solely a little dosage of the nanoparticles is incorporated. Consequently, nanomaterials may act as another to antibiotics to normalize microorganism infections [8].

5.2 Nanoparticles as Antimicrobials

5.2.1 Inorganic Nanoparticles

Metals and metal oxides are widely studied to find out antimicrobial properties [9]. Some metal nanoparticles are well recognized for their extremely powerful antibacterial result, embody iron oxide (Fe_3O_4), zinc oxide (ZnO), titanium oxide (TiO_2), copper oxide (CuO), and silver (Ag). One in each of the prime uses of NPs in the pharmaceutical sector is their remedial relevance with antibacterial action. It is already acknowledging that certain nanoparticles like silver, iron, copper, zinc oxide, etc., bear vital antimicrobial action as they unleash metal ions after generating ROS (reactive oxygen species). Presently, there are a number of NPs which show effective response against multi-drug resistant (MDR) bacteria but resistance may be developed to these NPs as a result of their recurrent exposure [10].

5.2.2 Organic Nanoparticles

In earlier years, antimicrobial medications epitomized in Organic NP frameworks have showed up as promising alternatives that have not solely exaggerated pharmacological actions and moreover reduced adverse effects of the drug [11, 12]. Currently, the liposome is one in every of the prime unremarkably utilized antimicrobial medication as a result of it which copy the microbial plasma film and just wire with the infective bug [13]. Owing to the unrestricted combination of microorganism

plasma film and liposome, cargos (drugs) just get discharged inside the microorganism cell and finally end in its fatality [11, 14].

5.2.3 Antibacterial Properties and Mechanism of Action of Nanoparticles

Now a day's Nanomaterials are used as antimicrobial agents because of their promising results. Thus it can be assumed that these nanomaterials may fulfill the present demand of newer antimicrobial agents. This includes combating multidrug-resistant mutants and biofilm [15, 16].

A variety of nanomaterials were investigated for their antibacterial properties and found to be effective. In accepting the antibacterial activity of those nanomaterials, it is necessary to acknowledge that a few metals like silver, copper, and zinc show bactericidal mechanisms in their bulk type whereas metal like iron oxide is not bactericidal in their bulk type, however, their nanoparticles might show antibacterial properties [17]. The mode of action of these nanomaterials as antibacterial agents varies from nanoparticle to nanoparticle. In some cases, it was also noticed that the NMs show delayed toxicity, thus the ratio of NMs and bacteria is very important [18]. Additionally, several environmental factors play a task and have an effect on the deadliness of NM to microorganism together with aeration, pH, and temperature. The physicochemical properties of the particles play a crucial role in their antibacterial activity. Thus, the size, shape, chemical alteration and coating, and mixing ratio of different nanoparticles and solvent used all have an effect on antibacterial activity of nanoparticles [19]. The particular area of a dose of nanoparticles will increase because the particle size decreases, providing bigger material interaction with the encircling atmosphere. Thus, the antibacterial materials like zinc and silver enhance the antibacterial property by increasing the surface to volume proportion. A nanoparticle with inherently antibacterial components might work following different mechanisms to show antibacterial activity. In general, NM act on two key fatal pathways that are associated with one another and in several cases take place concurrently: [1] disruption of membrane potential and integrity and [2] production of reactive oxygen species (ROS) [18, 20].

Nanoparticles possess distinctive physical, chemical, electronic, electrical, mechanical, magnetic, thermal, dielectric, optical, and biological properties. Metal nanoparticles are of nice interest to be used as potential antimicrobial agents, thanks to their distinctive optical, electronic, and magnetic properties. The static interaction of nanoparticles with charged microorganism surfaces attracts the particles to the microorganism and promotes their diffusion into the membrane. There are several factors that affect NP-cell membrane interactions. Amid the potential factors, physicochemical properties of the NPs like size, shape, charge, hydrophobicity/hydrophilicity, surface chemistry, and others can greatly manipulate the NP-cell membrane interactions. Powerfully positive zeta potential of a nanoparticle encourages nanoparticle interactions with cell membranes ensuing in membrane disruption, microorganism activity, and a diminution in viability. The generation of reactive oxygen species is additionally a mechanism of nanoparticle antimicrobial activity

[21]. Auxiliary mechanisms of action of nanoparticles as antimicrobial agents consist of disrupting DNA throughout the replication and biological process of microorganisms, compromising the microorganism membrane integrity via physical interactions with the microbial cell, and releasing toxic metal ions and possessing abrasive properties that induce cell lysis [22].

The adsorption followed by penetration of nanoparticles into the cell may result in membrane damage [23, 24]. Several studies confirmed that the primary mechanism of toxicity is the adsorption on the cell wall following disintegration [23–25]. Surface assimilation of NPs results in semipermeable membrane change that changes the electric charge of the cell wall to turn into more porous. Studies reported the formation of a "hole" or "pore" within the living cell membranes by NPs as an attainable mechanistic assumption [26]. A factual hole inside the bilayer membrane of the cell wall indicates a whole loss of cell [27].

Several studies justify that the electric charge of nanoparticles is vital for antimicrobial action as microorganism's cell membrane is negatively charged. Though the scientific mechanism remains underneath discussion, it has been steered that ions like silver have an effect on membrane-bound respiratory enzymes that may lead to cell death [28]. In general, once nanoparticles come in contact with the microorganism, it will begin with doable oxidation of metastasis enzymes, thus assisting the assembly of Reactive Oxygen Species and radical species that may finally have an effect on cell structure and promote deoxyribonucleic acid degradation [29].

5.2.4 Recent Studies on Nanoparticles Against Microorganism

Newly evolved multidrug-resistant (MDR) microorganisms include vancomycin-resistant *Staphylococcus aureus* and *Enterococcus* sp. like *E. faecalis* and *E. faecium* [30], penicillin-resistant *Streptococcus pneumonia*, multidrug-resistant *Mycobacterium tuberculosis*, *Salmonella enterica*, *Pseudomonas aeruginosa*, *Vibrio cholera*, *Acinetobacter baumannii*, and carbapenem-resistant *Enterobacteriaceae* [31]. The long-term stability and biocompatibility are the two characteristics of biogenic nanoparticles which make nanoparticles a better choice as antimicrobial applications [32].

Recent scientific reports have claimed that nanoparticles have special biocidal activities to cleanse *Salmonella Typhi* and might destroy cancer-promoting Cyanobacteria and algae (*Microcystis aeruginosa*) from the surroundings [33, 34]. In the era of the origination and unfolding of microbes which are multidrug defiant, the potential antimicrobial action of gold and silver nano molecules ought to be thought about as a positive sign of the usefulness of nanoparticles as antimicrobial agents [35]. Different studies experimented green metallic nanoparticles for evaluating antimicrobial applications against several morbific microbes and found a good response. For example, biogenic AgNPs obtained from *Brevibacterium frigoritolerans* DC2 [36], *Sporosarcina koreensis* DC4 [37], and *Bhargavaea indica* DC1 [38] showed antimicrobial activity against *Vibrio parahaemolyticus*, *Salmonella enterica*, *Bacillus anthracis*, *Bacillus cereus*, *Escherichia coli*, and *Candida*

albicans. Copper nanoparticles (CuNPs) obtained from *Sidaacuta* showed antimicrobial activity against *Escherichia coli*, *Proteus vulgaris*, and *Staphylococcus aureus* [39].

Metal oxide nanoparticles of zinc (ZnO), copper (CuO), and iron (Fe2O3) were screened for their antimicrobial activity against both Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*). The screening of antimicrobial activity has revealed that the ZnO nanoparticles have utmost antimicrobial activity against most types of bacteria; on the contrary, iron oxide nanoparticles have least antimicrobial action [40]. Recently, Chitra et al. 2013 reported the antibacterial and antifungal activities of ZnO nanoparticles [41]; they claimed that ZnO was highly active against food pathogens (*E. coli* and *P. aeruginosa*) (100 μL) and *Aspergillus niger* (400 μL) at mottled concentrations. Using of nanoparticles in the food industry has started back and this study also put emphasis on the usefulness of nanoparticles in the food packaging industry. Nowadays, the production or synthesis of nanoparticles from plant sources (green synthesis) has become an emerging podium in nanoscience. An antibacterial study screening ZnO against *Pseudomonas aeruginosa, S. aureus*, and *Candida albicans* showed minimum inhibitory concentration (MIC) of 1917 μg/mL, 9 μg/mL, and 39 μg/mL, respectively [42, 43]. In this study, it was noticed that ZnO showed a significant result against *S. aureus* at a lower concentration as compared with *P. aeruginosa*. Recently, another study reported that MK-AgNPs produce satisfactory activity against both Gram-positive and Gram-negative MDR bacteria. The recent increasing interests toward the synthesis of silver nanoparticles, principally for antibacterial use against human pathogens, brings new hope in regard to antimicrobial drug innovation [44].

The nanoparticles primed from *Allium species* like ginger and garlic with $AgNO_3$ showed antibacterial action against general bacterial pathogens like *E. coli*, *Proteus spp*., *Klebsiella spp*., *Staphylococcus spp*., *Eubacteria spp.*, and *Pseudomonas spp* [45–48].

One latest report confirmed that selenium and silver nanoparticles produced by microbes isolated from coal mines were discovered to possess antimicrobial action against some bacteria like *E. coli*, *Klebsiella spp*., *Pseudomonas spp*., and *S. aureus* [49, 50].

One more study that utilized a singular technique of infusing paper with nanoparticles reported the MIC of copper nanoparticles as 140–280 μg/mL for *E. coli* strain and 140 μg/mL for *S. aureus* strains [51].

In a different study, it was observed that iron oxide nanoparticles at different doses reduce cell numbers of *Staphylococcus epidermidis* suspensions while optical density readings were taken [52]. After 48 hours, iron oxide nanoparticles reduced cell populations by about 65% at a concentration of 2 mg/mL and it reflected the efficiency of iron oxide nanoparticles when compared to control groups with no nanoparticles. As the concentration of nanoparticles was increased (100 μg/mL, 1 mg/mL, and 2 mg/mL), subsequently it amplified the quantity of dead cells observed in a live/dead analysis.

Silver nanoparticles fascinated nice attention within the medicine field attribute to their remarkable and exceptional properties together with their very important antimicrobial potency and harmless character. Among the various attainable uses of AgNPs in this specific domain, spectacular interest and efforts are made in wound dressing, tissue scaffold purposes [53, 54]. AgNP-based nanoparticles were assessed as appropriate carriers of a range of remedial molecules, counting anti-inflammatory [55, 56], anti-oxidant [57, 58], antimicrobial [59, 60], and anticancer [61, 62] biomaterials.

Thus, AgNPs represent potent candidates for the nanotechnology-derived development of novel and effective biocompatible nanostructured materials for the unconventional antimicrobial application. A number of studies reported that AgNPs directly interact with the cell membrane of bacteria and infiltrate the complete cell, as a result, cell function gets interrupted as well as structural damage occurs followed by cell death [63].

Since history, silver-based materials were used for the alternative and successful management of typical contagions [64]. Nanosilver also provides a broad array of proficient biocide actions against a remarkable variety of anaerobic, aerobic, gram-negative, and gram-positive bacteria. AgNPs or silver ions applied in porous wound dressings can interrelate with and destroy the bacteria found in exudates [65].

AgNPs play an essential role in the development of novel biomedicinal approaches because of their distinctive physicochemical properties and biofunctional options like as anti-inflammatory, anti-angiogenesis, antiviral, antifungal, and bactericide activities [66]. Recently, AgNPs were also thoroughly investigated to explore its anticancer property against diverse human cancer cell lines [67, 68].

Nanotechnology offers site-specific and target-oriented delivery of drugs and thus nanoparticles has gained priority in treating persistent human diseases. However, inadequate information regarding toxicity or adverse effects of nanostructures arises big question upon using of nanoparticles. More analytical research is a times demand to enhance the effectuality with higher safety to modify the safer implementation of those nanodrugs (Table 5.1).

5.3 Conclusion

In conclusion, microorganisms are universally capable to develop resistance against antibiotics because of their diagnostics overdose and inability. The diseases caused by MDR microorganisms are a rising concern for healthcare professionals worldwide. Despite attainable limitations, nanotechnology stands for a novel approach to develop and take a look at new drug formulation-supported metallic nanoparticles with effective antimicrobial characteristics. Silver nanoparticles largely have numerous probable uses in healthcare. At present, antibiotics are the only option to eradicate microbial infections, but the cases of resistance against these antibiotics are rapidly observing. The rational application of antimicrobial agents in healthcare practices is unquestionably answerable for this crisis. Nowadays, NPs are considered

Table 5.1 Antibacterial metal nanoparticles and metal nanoparticle conjugates

Type of nanoparticle	Antimicrobial application	Mechanism of action	Ref
Silver as part of a network of fibers	*E. coli*	Bacterial growth inhibition	[69]
Silver vanadate nanowires	*S. aureus*	Bacterial growth inhibition	[70]
Naked silver	*C. albicans*, *P. fluorescens*, *E. coli*	Bacterial growth inhibition	[71]
Thioguanine-capped gold	*E. coli*, *A. fumigatus*, *P. aeruginosa*, and anticancer effect against Hep2	Bacterial growth inhibition, cellular toxicity	[72]
Naked gold	*C. pseudotuberculosis*	Vacuole formation in cell wall and agglomeration of NPs within cells	[73]
Naked gold	*S. aureus*, *K. pneumonia*, *B. subtilis*	Bacterial growth inhibition	[74]
AgNP	*Vibrio parahaemolyticus*, *Salmonella enterica*, *Bacillus anthracis*, *Bacillus cereus*, and *Candida albican*	Bacterial growth inhibition	[39]
Copper nanoparticles (CuNPs) obtained from *Sidaacuta*	*Escherichia coli*, *Proteus vulgaris*, and *Staphylococcus aureus*	Bacterial growth inhibition	[39]
ZnO nanoparticles	*E. coli* and *P. aeruginosa* (100 μL) and *Aspergillus niger*	Bacterial growth inhibition	[41]
AgNO3 nanoparticles of *Allium species*	*E. coli*, *Proteus spp*., *Klebsiella spp*., *Staphylococcus spp*., *Eubacteria spp.*, and *Pseudomonas spp*	Bacterial growth inhibition	[45–48]

as a possible substitute as antibiotics due to their biocidal and immune potentiating properties. Even though nanoparticles have a few shortcomings, they provide hope for the development of efficient antimicrobial drugs for the future.

Acknowledgments Declared None.

Conflict of Interest The authors declare no conflict of interest, financial or otherwise.

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Part II

Nano Medicine: Concept, Development, Clinical Applications and Evidences

Nanomedicines and Nanodrug Delivery Systems: Trends and Perspectives

6

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Abstract

Nanomedicine and nanodrug delivery systems have furnished a platform to upgrade drug delivery using the new idea and carrier systems that conventional formulations have failed to acquire. Drug delivery in relation to nanomedicines should be observed as a science and technology of nanocomplex systems that drive to a special mechanism related to diagnosing, treating and inhibiting multiple diseases. As nanocomplex system comprises materials that are designed at the molecular, atomic and macromolecular level, they are generally tiny particles with distinct physicochemical properties. By manipulating their distinct physicochemical properties, nanomedicines and nanodrug delivery systems can control, monitor and repair the biological systems by aligning to address diseases. These advanced technologies can offer significant advantages like higher drug loading capacity, specificity, stability and are capable of delivering both hydrophilic and lipophilic drug molecules. Recently, several nanomedicines have already been marketed by various pharmaceutical and medical device manufacturer companies in the form of polymeric micelles, nanosuspensions, nanocrystals, liposomes, SPIONs and protein-based nanoparticles that confirmed their effectiveness over a longer period. However, the benefits of nanomedicines in the healthcare sector are escorted by challenges in the regulation of such products. Enough knowledge on their efficacy, quality, safety and toxicity must be learned to support their smooth translational research towards clinical applications. The present chapter highlights the recent trends and perspectives of nanomedicines and nanodrug delivery systems with potential benefits to targeted drug delivery.

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M. K. Das, Y. V Pathak (eds.), *Nano Medicine and Nano Safety*,
https://doi.org/10.1007/978-981-15-6255-6_6

Keywords

Nanotechnology · Nanomedicine · Drug delivery · Nanotoxicity · Regulatory evaluation

6.1 Introduction

In the past few centuries, there has been tremendous development and revolution in the field of drug delivery systems to accommodate pharmaceutical agents to their target sites for the treatment and diagnosis of various diseases [1, 2]. There are a bunch of drug delivery systems that are successfully engaged in the current times; however, challenges still remain that need to be addressed and advanced technology needs to be drafted for the flourishing delivery of drug molecules to its target sites [3, 4]. Nanotechnology is a quickly advancing field that is anticipated to have a progressive impact on diverse areas, including medicine, physics, chemistry, biology and engineering [5]. By consenting nanotechnology, a fundamental transformation in healthcare sector, drug manufacture and drug delivery is expected to influence almost half of the worldwide drug manufacture, which holds relatively US Dollar 196.02 billion in revenue [6]. There is growing positivism that nanotechnology combining with medicine has directed to the interdisciplinary field of nanomedicine, will import outstanding progress in the healthcare system by understanding the complex latent pathophysiological feature of diseases and upgrade the quality of patients' life [7, 8]. Such combined technological approaches have vastly struck the field of medicine following advancements in drug delivery and upgrading the specificity or sensitivity of current strategies to develop and identify biomarkers as well as establishing advanced nanodiagnostic machinery [9, 10]. The drug delivery system based on nanotechnology aims to deliver the drug cargo to the appropriate place at the appropriate time and indicates to bridge a barrier of physical or biological sciences by utilizing nanostructures at several areas of science, especially in nanomedicine and nanodrug delivery systems [11–13]. Nanomedicines and nanodrug delivery systems are comparably new but quickly developing fields where the materials in the nanosized range, i.e. size ranges between 1 and 100 nm, are engaged to serve as diagnostics tools and drug delivery carriers [14]. Drug delivery in relation to nanomedicines should be observed as a science and technology of nanocomplex systems, comprising partially two constituents, one of which is active pharmaceutical ingredients (APIs) and even though nanoformulations of the drug are also feasible. The whole system drives to a unique function corresponding to treating, diagnosing and preventing various diseases [15]. By manipulating their exclusive physicochemical properties, nanomedicines and nanodrug delivery systems can control, monitor and repair the biological systems by aligning to respond diseases for which recently there are only available inappropriate therapeutic and diagnostic tools [16, 17]. For that reason, multidisciplinary research is being carried out with nanoformulations on diagnosis, and treatment of various diseases has entrained several attempts to merge diagnosis and therapy within a single

scaffold referred to as Nanotheranostics, which are developed to expedite various important features of drug delivery research and are examined to be remarkably beneficial for symbolizing nanomedicine-based therapeutic mediations [18].

The use of nanomedicines and nanodrug delivery systems as drug carriers has provided a powerful sword against various complex diseases, i.e. cancers, diabetes, malaria, neurological disorders, to the early recognition and diagnosis of pathologies that could allow for excluding them before the emergence of any symptoms and novel strategies for addressing unmet clinical challenges [19–21]. These nanosized particles can easily interact with the biological cells in a very competent way due to that they are present in smaller sizes than these biological materials [22, 23]. Such close interaction of nanosized particles with biological cells is defined as Bio-nano interactions and has been employed for acquiring important capabilities like proper transportation of drugs to the diseased sites and also direct identification of excessively low concentrations of crucial biomarkers that are exhibitive of pathological phenomena, which exist in complex environments, i.e. blood, saliva, urine, etc. [24–26]. The distinct properties and behaviour of nanoparticles in biological milieu also facilitate integrative advances to investigate fundamental biological processes on the cellular level like cell division, apoptosis and stem cell fate, etc. [27]. Since interest in the biological action for nanoparticles is new, there is a reasoning for the more appealing and innovative practice of nanoparticles in the area of biology and medicine [28]. The sustained development of nanomedicines has the plausibility of providing an alternative treatment approach that is more specific and targeted to a bunch of diseases [29, 30]. During the treatment of diseases, nanomedicines create a dose distinction between the diseased site and rest of the body, resulting in enhancing the therapeutic outcomes in the disease area, while lowering the adverse effects on the remaining body parts [31, 32]. The potential of hindering the outgrowth of various diseases without any indirect damage through nanoparticle-based drug delivery system has constructed outstanding appeal and nanoparticles design the field for bio-nanomaterials, which provide major steps in engineering drug delivery systems based on multi-functionalized nanoparticles [33]. Thus, such nanodrug delivery systems can be developed to have drug conjugated or absorbed onto the surface of particles and entrapped inside the core of polymer/lipid or dispersed within the mould of the nanoparticles [34–36]. As a result, drugs can be guarded from a detracting environment and adverse biopharmaceutical properties can be concealed and retrieved with the properties of nanoparticles [37].

Conventional drug delivery system associates the formulation of the drug into a relevant form, like compressed tablets or capsules, suspension for oral administration, solutions for parenteral administration, topical liquid/solution eye drops for ocular administration and ointment, cream, lotion or gels for topical application purposes [38]. These dosage forms have been spotted to have severe limitations in terms of the maximum dose required, less effectiveness, poor water solubility, abandon therapeutic drug level, poor permeability, induction of drug resistance, adverse side effects, etc. [39–42]. Moreover, conventional drug delivery systems having minor control beyond their drug release and nearly no control beyond the efficient concentration at the target disease sites may result in the immediate release

of drug, dose dumping or fluctuation, unpredictable plasma concentration and faster clearance of the drug from the bloodstream [43, 44]. Also poorly water-soluble and metabolic/enzymatic unstable drugs, when taken in conventional dosage forms, may cause bioavailability problems, leading to exhibit less therapeutic action [45]. To conquer the limitations of conventional dosage form, nanoparticle-based drug delivery systems have been designed or developed to meet the demand of healthcare systems [46].

Nanomedicine and nanodrug delivery systems have furnished a platform to upgrade the drug delivery using the new idea and carrier systems that conventional formulations have been not able to attain [47, 48]. These new technologies have progressively been investigated to enhance the therapeutic efficacy and can offer magnificent benefits over conventional dosage forms in terms of higher drug loading capacity, higher specificity, higher stability, capability for sustained release and controlled release, opportunity to use in the various routes of administration and are able to deliver both hydrophilic or lipophilic drug molecules [49–51]. As nanoparticles constructed materials are designed at the molecular, atomic and macromolecular levels, they are generally small-sized particles with distinct physicochemical properties like size, surface properties, shape and molecular weight composition [52, 53]. Being nanosized, these particles can easily penetrate the tissues or cells, facilitate more uptake of the drug, directly interact with diseased tissues or cells with improved efficiency and ensure better therapeutic action [54–56]. Currently, nanomedicine has become highly admired because nanostructures could be exploited as delivery vehicles for entrapped drugs and transporting them to target cells more precisely [57, 58]. Moreover, nanomedicines stay in the systemic circulation for an extended period of time and allowing the release of blended drugs in a controlled manner and exhibit higher bioavailability because they serve regular uptake mechanisms via absorptive endocytosis [59, 60]. The main potentiality of these nanostructures is mainly correlated with their surface characteristics. Hence, the modification of nanocarrier surface is often used to control their surface characteristics in an appropriate fashion and allow them to concurrently perform multiple functionalities, i.e. enhance bioimaging modalities, prevent aggregation and severe interaction with healthy cells [61–63]. However, the benefits of nanomedicines in the area of the healthcare sector are escorted by challenges in the regulation of these nanoproducts [64]. Enough knowledge on their quality, efficacy, safety and toxicity must be acquired as well as standardized methods must be made available to support their regulatory agreement making and allowing a refined translational development towards clinical applications [65, 66]. Thus, appropriately designed nanodrug delivery systems can be major advances to figure out the problems related to drug delivery and disease treatment. Considering the above-mentioned facts, this chapter highlights the recent trends in nanomedicines and nanodrug delivery systems with potential benefits to targeted drug delivery as well as identification of future preferences to uphold the adaptation of nanomedicines towards clinical applications.

6.2 Types of Nanoparticles with Potential Benefit to Targeted Drug Delivery

The ongoing research advances in nanotechnology have designed a wide variety of nanoparticles with diverse composition, sizes, shapes and surface functionalities to permit novel strategies in the field of medicine and biomedical research [67, 68]. Based on their distinct physicochemical properties, nanoparticles are broadly classified into various classes like polymer-based, lipid-based, metal-based, carbon-based, inorganic and hybrid nanoparticles revealing entirely promising therapeutic implications with better sensitivity, specificity, functionality and efficiency [69]. The impressive features and benefits of such nanostructures are discussed in Table 6.1 and Fig. 6.1.

6.3 Nanomedicines for Improvement of Drug Delivery

The development of nanotechnology-based drug delivery systems, specifically nanomedicines, has been growing explosively due to their exclusive properties compared with conventional drug formulations [94]. The prominence of nanomedicine on drug delivery systems can be confined into the origination of the field, encouraged by the 1908 Nobel Laureate Paul Ehrlich and his proposed idea "Magic Bullet" approach, that drugs directly go to their expected cell structural targets while persisting harmless in health or normal tissues [95]. Currently, there are many promising nanocarriers used in nanomedicines and nanodrug delivery systems like polymeric nanoparticles, lipid nanoparticles, magnetic nanoparticles, metallic nanoparticles, carbon nanotubes, quantum dots, etc., that have imported progressive changes in the field of drug delivery and total healthcare systems [96]. Although nanocarriers are pledging drug delivery systems but their poor oral bioavailability, circulation instability, incompetent tissue distribution, stability, opsonization by the reticuloendothelial system, drug efflux pumps and toxicity are few deficiencies to practical application that still unsolved [97, 98]. To overcome these hurdles and to fulfil the safety, toxicity, regulatory and ethical considerations, researchers need to design nanomedicines with improved properties, which can particularly target the disease-generating pathogens or diseased cells [99, 100]. Due to the magnificent interfacial interaction among the core and surface of the nanoparticles, surface modification has an enormous deal of attention and plays an auspicious strategy to achieve better drug targeting performance [101, 102]. As the nanoparticle surface directly touched body fluids and organs, the surface of the nanoparticles is more crucial than the core [103]. The surface of the nanomaterials is mainly modified by several functional materials like small molecules ligands, polymers, surfactants, biomolecules, etc. These functional materials guide the nanoparticles to the targeted site and release the drug molecules in an appropriate manner [104–106]. In general, nanoparticles functionalized or surface modified with positively charged ligands and hydrophilic materials displayed higher internalization into cells and enhanced systemic circulation time leading to easily evade from the recognition of macrophage

Table 6.1 Structural Characteristics and Potential Benefits of Various Nanoparticles

Sl. no.	Nanoparticles	Subtypes	Structure	Potential benefits	Ref(s)
01.	Polymer-based NPs	Nanomicelles (10–100 nm)	Nanomicelles are core/shell nanoscale structures developed by self-assembled amphiphilic block copolymers	Enhanced solubility and bioavailability, prevents drug degradation	[70, 71]
		Nanosponges (100–500 nm)	Nanosponges are hyper-crosslinked polymer-based colloidal structures with a large porous surface	High drug loading, enhanced solubility, controlled drug release	[72, 73]
		Dendrimers (1–10 nm)	Dendrimers are highly branched, three-dimensional globular shape, synthetic macromolecules	Reducing drug toxicity, facilitates the targeted and controlled release	[74, 75]
02.	Lipid-based NPs	Liposomes (50–1000 nm)	Liposomes are spherical-shaped vesicles composing of one or more phospholipid bilayer membranes	Increased potency and therapeutic index of drug, reduces toxicity	[76, 77]
		SLNs (50–1000 nm)	SLNs are sphere-shaped colloidal carriers composed of lipid matrix that are solid at physiological or body temperature	Improves bioavailability, loaded both hydrophilic and lipophilic drugs	[78, 79]
		NLCs (10-1000 nm)	NLCs are smarter drug carrier systems comprised of both solid as well as liquid lipids as a core matrix	Enhanced drug loading capacity, improved bioavailability	[80, 81]
03.	Metal-based NPs	Silver NPs (1–100 nm)	Silver NPs are framework of metal silver with tuneable physical, chemical and morphological properties	Potential cellular/ microbial cytotoxicity, photothermal therapy	[82, 83]

(continued)

Table 6.1 (continued)

Sl. no.	Nanoparticles	Subtypes	Structure	Potential benefits	Ref(s)
		Gold NPs (1–100 nm)	Gold NPs are structure of novel metal gold with unique optical electrical and surface properties	Tumour therapy, medical imaging, early detection of diseases	[84, 85]
		Magnetic NPs (1–100 nm)	Magnetic NPs are configuration of magnetic elements like iron, cobalt, nickel and their oxides that can be operated using magnetic fields	Cancer therapy, organ-specific therapeutic and diagnostic modalities, tissue engineering, MRI	[86, 87]
04.	Carbon-based NPs	Nanotubes SWCNTs (0.4–3 nm), MWCNTs (2–500 nm)	Carbon nanotubes (CNTs) are allotropes of carbon with a tubular or cylindrical nanostructure with exceptional physical, thermal, electrical and mechanical properties	Increased drug loading capacity, potential cargos for the cancer therapy, reduces toxic adverse effects of drug	[88, 89]
05.	Hybrid NPs	Lipid-polymer hybrid NPs (<1000 nm)	Lipid-polymer hybrid NPs are core shell-type nanostructure composed of polymer core and lipid shell	High loading capacity, delivery of multiple drugs, improving the therapeutic efficacy	[90, 91]
06.	Semiconductor NPs	Quantum dots (2–10 nm)	QDs are nanoscale semiconductor crystals with distinct optical and electronic properties	Improves bioavailability of drugs, bioimaging, good theranostics	[92, 93]

NPs Nanoparticles, *SLNs* Solid Lipid Nanoparticles, *NLCs* Nanostructured Lipid Carriers, *SWCNTs* Single-Walled Carbon Nanotubes, *MWCNTs* Multi-Walled Carbon Nanotubes

system, which is the major barrier and main resistance mechanism for drug targeting [97, 107–109]. Drug targeting to the diseased tissues is primarily mediated by two main mechanisms, i.e. passive targeting and active targeting as shown in Fig. 6.2. Passive targeting utilizes assets of the pathophysiological character of the diseased cells or tissues, while active targeting initially takes the advantages of passive

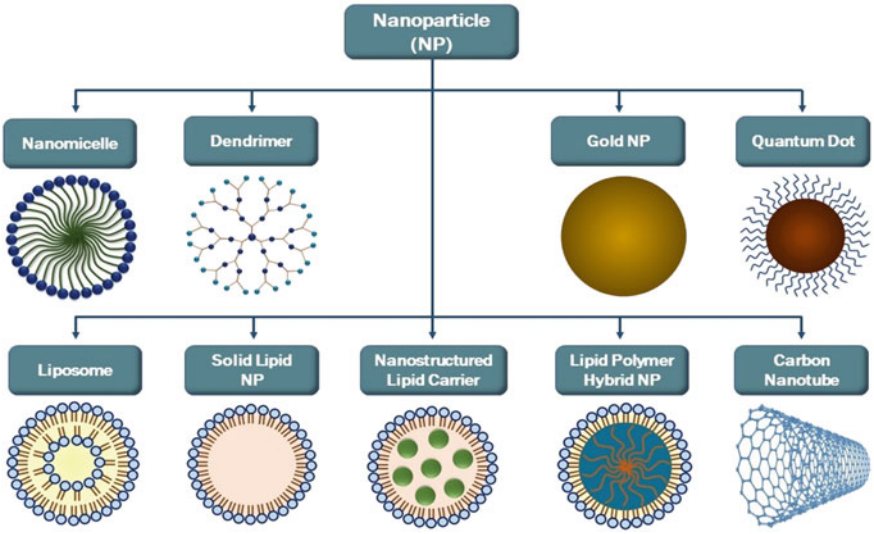

Fig. 6.1 Different types of nanoparticles

targeting to assemble into the disease sites by advantage of the enhanced permeability and retention (EPR) effect and consequently bind to the receptor overexpressed by the target diseased cells employing targeting ligand which is functionalized onto the nanoparticle surface that leads to receptor-mediated endocytosis of nanoparticles into the cells offering better cellular uptake and consecutive internalization [110–113]. Thus, achieving productive synergistic therapeutic effects and improving drug delivery as a whole, multifunctional nanoparticles for drug delivery have become enabling technology to potentiate multiple functionalities [114, 115].

6.3.1 Anticancer Nanomedicines

Cancer is one of the leading causes of death globally while lifelong speculation is still discouraging and the prevalence of cancer is continuing to increase [116]. However, the current treatment approaches mainly depend on standard cytotoxic drugs, radiotherapy and surgery that have more side effects and only limited effectivity because the likeness of cancerous and normal healthy cells are almost the same [117, 118]. Also, many anticancer drugs have poor pharmacokinetics properties that are derived from poor solubility, metabolism, stability and show various challenges including limited biodistribution, inefficacy and toxicity. Hence, it is crucial to develop an effective therapeutic strategy that can warrant the above-mentioned challenges [119, 120]. Nanomedicine has emerged as a promising substitutive technology that exhibits many benefits over conventional therapies and implements new strategies for early recognition, diagnosis and upgrades treatment strategies of cancer [121]. The nanomedicines have selectively increased the cellular uptake and drug localization within the cancerous tissues by preventing interaction with healthy

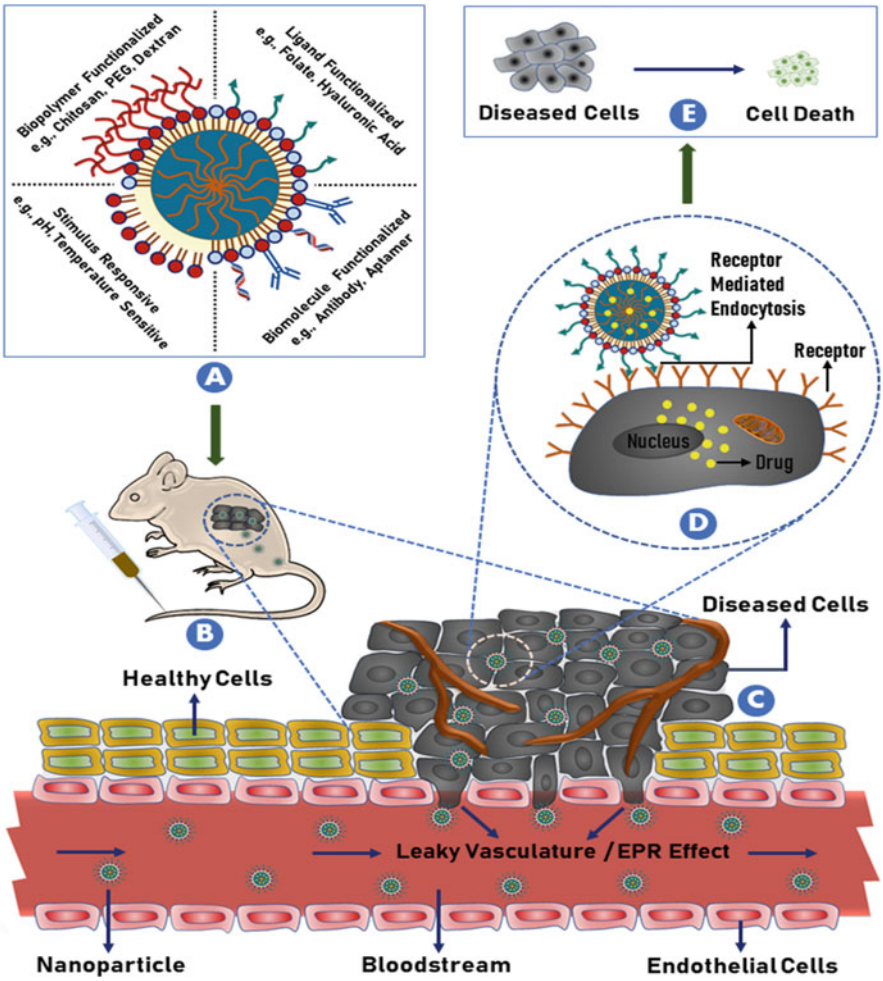

Fig. 6.2 Drug targeting strategies of nanoparticles; (A) surface functionalization of nanoparticles, (B) injecting functionalized nanoparticles to mice, (C) passive targeting via EPR effect, (D) active targeting via receptor-mediated endocytosis, (E) destruction of diseased cells resulting in better therapeutic outcomes

cells in the host body [122]. Current trends in the design of anticancer nanomedicines are mainly focusing on the construction of smart multifunctional delivery systems which ingrates the multiple collateral targeting strategies, especially passive, active and stimuli-responsive targeting [123]. The passive targeting expedites the deposition of nanocarriers within the microenvironment of cancer cells, due to their unique characteristics ingrained to the cancerous cells, not usually present in normal healthy tissues [124]. Hence, nanomedicines allow the selective accumulation or localization of consistently administered chemotherapeutic agents in the cancer cells via enhanced permeability and retention (EPR) effect due to leaky vasculature of cancer cells and compromised lymphatic drainage [125, 126]. The

EPR effect basically implies site-specific characteristics that merely occur in solid tumours and inflammatory tissues but not allied with normal healthy tissues leading to enhanced selective drug targeting [127]. Although in the active targeting, the target ligand conjugated onto the nanoparticle surface which binds to the corresponding receptors overexpressed by cancerous cell surfaces, resulting in increased cellular uptake or localization by receptor-mediated endocytosis and thus enhanced drug accumulation in cancerous cells [128, 129]. However, additional obstacles towards enhancing the potency of nanoparticles are premature drug release and poor penetration or cellular entry during circulation. Hence, the controlled stimuli-responsive nanomedicine is highly desirable due to its on request drug release by modulating the cancerous microenvironment-triggered transient properties of nanoparticles for better cancer cell penetration, leading to improved efficiency and hindering premature drug release [130, 131]. The idea of stimuli-responsive drug delivery systems arises from the evidence that the cancer cells maintain various unique features distinguished with the healthy cells [132]. Generally, stimuli-responsive nanomedicines allowing the precise release of drug in response to endogenous stimulus like pH, enzyme, redox potential, ionic microenvironment as well as exogenous stimulus like temperature, ultrasound, light, magnetic field or even a combination of more than one stimuli are considered as 'smart' nanocarriers for the delivery or transport of anticancer drugs [133, 134]. Moreover, nanomedicines enhance the therapeutic performance of anticancer drugs by wavering their pharmacokinetics and distribution to the site of action and have also been illustrated clinically [135]. Despite the range of nanoparticle-based drug delivery systems currently under the preclinical stage or in clinical trials, it is unquestionable that liposomes are superior on the market and liposomal doxorubicin (Doxil®) was the first FDA-approved anticancer nanomedicines [136–138]. The many novel nanomedicines that preclinically exhibited outstanding anticancer activity are yet to be recited clinically, as a result, the development of the marketed nanomedicines has usually been slow [139]. Although a number of nanomedicines that have reached the clinic are considered to exhibit low patient benefit due to poor conception of the biological barriers, misperception of drug delivery concepts, fabrication and scaling up [140]. Thus, future investigations will continue focusing on the development of safe and effective nanomedicines with enhanced cancer targeting or therapeutic efficacy by delivering an extensive range of therapeutic agents.

6.3.2 Antiretroviral Nanomedicines

Human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) is now a global pandemic that has become the dominating infectious murderer of adults and markedly triggered social or economic loss all over the world, especially Sub-Saharan Africa, massively affected [141, 142]. The current treatment approach for HIV/AIDS is highly active antiretroviral therapy (HAART, also known as combination antiretroviral therapy (cART), where two or three or

more antiretroviral agents are simultaneously given to patients, resulting in considerable success in upgrading the quality of patients' life [143, 144]. Despite the noticeable successes with the ongoing HAART treatment for HIV/AIDS, there are still several obstacles existing like taking medication on daily basis for a lifetime, drug-drug interaction, inadequate drug levels in the body, acute and sustainable toxicity and rebound viral replication [145–148]. The lack of complete recovery by current treatment highlights the immense need for sustained resolution in seeking novel strategies for the treatment of HIV/AIDS. Recently, nanomedicine-based anti-HIV therapeutics has also gained attention for the treatment and prevention of HIV/AIDS and jointly representing a new route for managing the amount, dosing frequency and proper delivery site of anti-HIV drugs, as well as to interrupt with particular phases of the virus life cycle by directly targeting viral enzymes or structures that are crucial for HIV revolution [149, 150]. The nanodrug delivery systems holding the most promising possibility for targeted delivery of anti-HIV drugs to CD4+ T cells and also macrophages which are the principal targets and major cellular HIV-1 reservoirs responsible for the transmission or spreading of HIV-1 to multiple sites and also delivery to the brain as well as other organs could assure that drugs reach to the latent reservoirs of HIV [151–154]. Among the two reservoirs, macrophages have been employed as cellular transporters for the distribution of nanoformulated antiretroviral therapeutics (Nano-ART) and the effectivity of antiretroviral (ARV) agents can be significantly upgraded by rewrapping them into nanoparticles because macrophage drug delivery creates antiretroviral depots [155–157]. The efficacy of Nano-ART is further enhanced due to better loading of both hydrophobic or hydrophilic drugs, enhanced solubility, intracellular uptake in nanocarriers with the increased distinct surface area that can be functionalized with the numerous functional moieties to facilitate active targeted delivery and also circumvent the blood-brain barrier (BBB) to improve CNS-assisted drug delivery for the management of neurocognitive disorders, i.e. neuroHIV/AIDS [158, 159]. Various forms of nanocarriers like polymeric nanoparticles, nanomicelles, nanosuspensions, dendrimers, liposomes and solid lipid nanoparticles are noted to improve the effective delivery of ARV drugs for the prevention of HIV. Among them, liposome has been widely used for delivering anti-HIV drugs [160, 161]. Moreover, nanomedicines have also directed the design of new targeting strategies to HIV-1 infections, including gene therapy, immunotherapy, vaccine delivery and preventive microbicides [162, 163]. Preclinical and clinical trials illustrated that DermaVir is the first and most excellent nanomedicine for the management of HIV/AIDS with promoting Phase II clinical validation, immunogenicity and safety results [164]. Other nanotechnology-based treatment strategies for HIV/AIDS include liposomes loaded with Stavudine, Zidovudine, Indinavir; dendrimer loaded with Efavirenz, Lamivudine; solid lipid nanoparticles loaded with Saquinavir and magnetic nanoparticle loaded with Tenofovir are still in preclinical stages of development that need further investigations for safety, immunogenicity and tolerability [165]. Although there are beneficial impacts of applying these systems to novel anti-HIV nanoformulations, there are some obstacles that must be conquered in the near future to efficiently translate ongoing investigations

into the clinical setting which include limited scalability, unknown toxicity. Hence, future in vivo research studies and clinical trials have to be devoted to easily producible nanoformulations with comprehensively illustrated biocompatibility and treatment proficiency.

6.3.3 Antidiabetic Nanomedicines

Diabetes is the world's fastest-growing heterogenous chronic disorder that affects millions of people and is characterized by elevated blood glucose (BG), adaptation in insulin secretion and metabolic interruptions [166, 167]. The predominance of diabetes has arrived at epidemic proportions, the latest estimate showed a global prevalence of 382 million people with diabetes in 2013 and the number is expecting to lift to around 592 million by 2035 [168]. Current synthetic drugs for the management and treatment of diabetes have been found not completely effective and may cause adverse side effects when used for prolonged periods [169, 170]. On the other hand, plant-based bioactive compounds including silymarin, rutin, glycyrrhizin, thymoquinone, naringenin, curcumin and quercetin have been found potentially effective against diabetes but possess severe problems like poor solubility, low bioavailability and required higher amount dosage to facilitate the therapeutic response. To reinforce the therapeutic potentiality and alleviate the adverse effects of these bioactive compounds, the advanced drug delivery systems are highly desirable [171–174]. In recent years, nanotechnology-based approaches in the development of antidiabetic formulations have gained significant attention due to the fact that such nanoformulations improved the therapeutic efficacy of the drugs for successful combating of diabetes in a plausible pattern [175]. Indeed, advances of nanomedicine in diabetes research have expedited the development of novel sensors which are competent for more prevalent and accurate measurements of blood glucose levels and has also enabled more powerful delivery systems for insulin that can recognize the variation in blood glucose levels and spontaneously adjust the rate of insulin release to maintain normoglycaemia, which holds the great promise to upgrade the quality of life for diabetic patients [176]. Over the past few years, a huge level of research has been conducted to develop a formulation of insulin that is active orally. Various polymeric nanoparticles loaded with insulin have exhibited lowering the levels of blood glucose in animals when administered orally, including those comprising of biodegradable or biocompatible cyanoacrylate, polyacrylic, polycaprolactone polymers and casein, and the polycationic polysaccharide and chitosan are mucoadhesive in nature and extend the residence time in the gut wall by binding or confining the nanoparticle to the mucosal surface [177, 178]. Although nanoparticles consisting of insulin and chitosan have also been spotted to stick to the surface of mucosal membranes and momentarily opens the tight junctions between gut mucosal cells and improve paracellular absorption of insulin [179, 180]. Moreover, numerous magnetic nanoparticles that have been developed as a contrast or imaging agent for β-cell imaging to early recognition of diabetes and disease progression are the important hallmarks of disease management [181]. In particular,

superparamagnetic iron oxide nanoparticles (SPIONs) are more fascinating as they are biocompatible in nature and can easily degrade into iron or oxygen and have terrific possibilities for countless biomedical applications, like quick detection of diabetes and manipulation of cell organelles. Apart from these applications, recent investigation has illustrated that surface-functionalized SPIONs have shown a magnificent role in targeted as well as site-specific drug delivery [182, 183]. Preferentially, iron oxide-based magnetic nanoparticles as a magnetic resonance imaging (MRI) contrast agent conjugated to targeting ligand (Exendin-4) resulting in better targeting of pancreatic β-cells by binding with the corresponding receptor and accumulated in pancreatic β-cells via receptor-mediated endocytosis [184]. However, the success of diabetes management is well acknowledged with the advanced control strategies using insulin pumps and repeated glucose monitors in the outpatient clinical studies, but there have been no FDA-approved nanocarriers for diabetes till date due to its safety and scalability problems. Hence, the long-term safety of nanocarriers is also under inquiry and must be properly analysed during the design of therapeutics and diagnostics for diabetes.

6.3.4 Antimalarial Nanomedicines

Malaria is one of the ancient and most widespread infectious diseases in the world that oppress humans and causes serious health problems in lower and middle-income countries like Southeast Asia and sub-Saharan Africa [185, 186]. According to the latest estimates from WHO, there were a predicted around 216 million cases of malaria, where around five million more cases than in 2015 and the number of deaths already hit 445,000 in 2016 [187]. The major contributing factor responsible for malaria proliferation has been the evolution of drug-resistant parasite clones with unrestrictedly unfolding sets of mutations that are prone to fluctuate at asexual proliferation rate [188, 189]. The current conventional therapy has several drawbacks like poor physicochemical properties and subsequently required a high dose to gain effective therapeutic outcomes that may induce toxic reactions [190]. To address these challenges, research has been executed in nanotechnology and nanomedicine, for the development of novel biocompatible schemes that are able to improve the therapeutic effect of current antimalarial drugs, controlling drug release rate, resulting in diagnosis, treatment and control of malaria by targeted delivery [191, 192]. Nanomedicine can conform to the goal of attaining the intake of total amounts adequately low to be harmless for the patient, but domestically still harmful for the parasites [193]. The most valuable feature of nanocarriers in relation to malaria is the capability to stay in the bloodstream for a longer period to upgrade the interplay with parasite membranes and infected red blood cells (RBCs) [194, 195]. Recently, targeted nanomedicines are an expeditiously developing area with clear pertinence for the treatment of infectious diseases and have been recognized as a promising tool to fight against malaria [196]. There are the two main approaches of nanoparticles for targeting antimalarial therapeutics to the infected erythrocytes and periodically the hepatocytes are active and passive

targeting [197]. These nanoparticles having the ability to carry a number of molecules, such as antimalarial drugs, proteins and fluorescent tracers, make them attractive multifunctional weapons for the targeting, destruction and eradication of both parasites and their rosette [198]. Moreover, the conjugation of particular antibodies on the surface of nanocarriers to retain outstanding antigen identification of parasitized RBCs (pRBCs) and non-parasitized RBCs has also been examined as targets for the transport of drug molecules to the infected erythrocytes resulting in complete targeting of nanoparticles to early intra-erythrocytic stages of the malaria parasite. The immunoliposomes covered with monoclonal antibodies (mAbs) exhilarated triggering across the glycophorin A (erythrocyte surface protein) are able to target 100% pRBCs and RBCs at the minimum concentration [199, 200]. Although antibody conjugated liposomes loaded with chloroquine against pRBC was assayed the first time for the treatment of *Plasmodium berghei* infections and displayed a cure of 75% to 90% in infected mice [201]. More recently, nanomedicine-based vaccination therapy, combinational therapy and small interference RNA (*si*RNA) delivery are the most promising targeting strategies to minimize or combat malarial infections. Although the fact that artemether and lumefantrine are well recognized as a combinational therapy for the treatment of simple malaria but having some limitations. Hence, nanostructured lipid carriers (NLCs) co-loaded with Artemether and Lumefantrine resulted in enhanced bioavailability of both the drugs and greater destruction of parasites in the infected mice and has proved a clear potentiality in comparison to single drug-loaded NLCs against the survival period and evolution of parasitemia [202, 203]. Additionally, urgency for a potent malaria vaccine is well accepted for the treatment and targeting of the infection process. The codon harmonized recombinant Pfs25 in *E. coli* (CHrPfs25) elicited highly effective malaria transmission inhibiting antibodies conjugated with gold nanoparticles can be designed as novel nanovaccines to improve the vaccine antigen immunogenicity by the initiation of transmission-blocking immunity and serve as an ideal vehicle to minimize the trouble of malaria [204]. Recently, the discovery of interference RNA (RNAi) and its potential adaptation against mosquitoes is now donating as an imperative weapon for interpreting the interaction of vector-parasites [205]. Hence, the efficient delivery of RNAi via nanomedicines is the massive interest in Plasmodium as it easily passes the erythrocyte membranes like parasite cytoplasm, parasitophorous vacuolar and the parasite nuclear to arrive at the Plasmodium nuclei resulting in diminished synthesis of particular proteins liable for malaria transmission [206, 207]. Nanomedicine is decisively making a competent appearance in the antimalarial field in the form of diagnostic devices, nanobiosensors, targeted drug delivery, nanoimaging and nanovaccination strategies to improve the function of the immune systems [208]. Although, it remains in the inception stage and has yet to scrutinize up to its feasibility and scale-up levels. Hence, the researchers must be kept in the mind about conditions such as production cost, socio-economic consequence and receivability within the patients.

6.3.5 Anti-Inflammatory Nanomedicines

Inflammation is the complex biological reaction of the immune systems that can be generated by a range of factors, including lethal pathogens, injured cells and toxic compounds or irradiation, that may lead to acute or chronic inflammatory responses in the kidney, liver, heart, brain, lung, pancreas, intestinal tract and reproductive system [209]. The process of inflammation is generally described by enhanced permeability of capillaries, vascular dilation, increased blood flow, leukocyte recruitment, inflammatory mediator release (e.g. histamine, serotonin, bradykinin, prostaglandins, thromboxanes) that can lead to wholesale tissue destruction [210, 211]. Currently, the most regularly prescribed classes of medication for the management of inflammation or pain is the Non-steroidal anti-inflammatory drugs (NSAIDs) [212]. However, their continuous use is affiliated with a well-identified spectrum of toxicity or side effects such as gastrointestinal damage, platelet dysfunction, acute renal failure, metabolic acidosis and an escalation cardiovascular liability to patients [213–215]. In terms of minimizing the systemic toxicity along with improving the therapeutic efficacy, a magnificent effort has been committed for the development of nanodrug delivery systems for NSAIDs [216, 217]. Nanoencapsulation of anti-inflammatory agents is one approach to achieve better bioavailability and targeting. The incorporation of NSAIDs into nanodrug delivery systems changes the in vivo biodistribution of the entrapped drug by averting its distribution to tissues that are liable to NSAID adverse effects and also diminish their touching with the mucus layer ensuing oral administration, leading to minimize their local detrimental effects on the epithelium [218]. Recent progress in nanomedicine research has supported scientists in utilizing the pathophysiological characters of inflammation, primarily leaky vasculature and overexpression of biomarkers for the treatment of the various conditions of inflammatory diseases [219]. Indeed, nanodrug delivery systems maintaining the permissible composition and particle size have been initiated to be conversely accumulate in inflammatory tissues, either by passive targeting through enhanced permeability and retention (EPR) mechanism or active targeting utilizing cell-specific targeting ligands, leading to boosting the therapeutic action of NSAIDs [220, 221]. To enhance the therapeutic action of NSAIDs, macrophage/monocyte targeted nanoparticles can be appointed as a cell-mediated drug delivery approach to pharmacologically regulate the inflammation. Such 'immunomodulatory nanoplatforms' can also be accepted for diagnosis and imaging modalities like systematically visualizing the macrophage dynamics and local inflammatory abrasion [222]. Notably, various studies suggest that gold is often utilized for treating rheumatoid arthritis. For that purpose, nanogold was engineered to investigate the antiarthritic activity in collagen-induced arthritic (CIA) rat model and results revealed that Nanogold is able to mitigate the generation of inflammatory mediators like TNF-a, COX-2, NF-κB and IL-1b, leading to displayed anti-inflammatory action [223]. Moreover, the inclusion of penetration-enhancing constituents in nanodrug delivery systems that may enhance the transdermal delivery of NSAIDs to the deeper skin layers with little systemic exposure [224]. Although to scrutinize

the transdermal transportation properties and the mechanism of penetration enhancement, triptolide loaded lipid nanoparticles was developed and results revealed that nanoparticles could penetrate the deeper layers of skin in a time-dependent aspect and exhibited an indicative anti-inflammatory response of triptolide [225]. Furthermore, a number of nanoformulations have been developed that are able to sustained/controlled release of the entrapped NSAIDs as compared to the conventional formulations and to improve the pharmacokinetics/pharmacodynamics of the incorporated drugs in preclinical models of various inflammatory diseases [226]. However, to improve the efficacy, prolong the duration of action and hinder the adverse effects of analgesic drugs, more experimental and clinical studies should be conducted to investigate the efficacy of nanotherapeutics to targeting, diagnosing and treating inflammation.

6.3.6 Antimicrobial Nanomedicines

Infectious diseases are primarily caused by microorganisms and remain a dominant cause of death, dysfunction, social and economic impairment for millions of people worldwide [227]. The efficient strategies for regulating infectious microbial diseases, antimicrobial agents also called antibiotics are the essential drugs acquired from microorganisms to hinder and prevent microbial infections [228]. However, rapid uses of antibiotics have constructed inadequacy to antibiotics and various microbial diseases are very short responding to frequently used antimicrobial drugs which have inflated multi-drug resistance and also inevitably resulted in the evolution of 'superbugs' [229–231]. The rapid evolution of 'superbugs' that resists most of the conventional antibiotics has demonstrated the urgency for the development of novel strategies or new antibiotics against multidrug-resistant or microbial infections [232]. The application of nanomedicines is quickly reverting the main driving force behind the current changes of the antimicrobial therapy and has also shown impressive defence against the multidrug-resistant infectious organisms [233]. Owing to their distinct physicochemical characteristics, nanoparticles have played a crucial role in the fast, precise and selective identification of microbial diseases. Nanoparticles for the delivery of antimicrobial drugs also offer specific advantages against drug resistance and generating limited side effects as compared to conventional antibiotics [234]. Although nanoparticles based on metallic elements that deliberate antimicrobial activity is among the widely studied. Some natural antimicrobial materials including zinc, silver, iron and copper-based nanoparticles possess higher antimicrobial properties in terms of particle size, physical structure and it can easily interact with bacteria resulting in distinct bactericidal functions [235–237]. The antimicrobial function of nanoparticles is poorly pretended, but the recently recognized mechanisms like induction of oxidative stress, the release of the metal ion and non-oxidative mechanisms are significantly inhibiting the microbial gene mutations responsible for antimicrobial resistance [238, 239]. However, the surface of the metallic nanoparticles is encircled by capping layers, which accommodate the active surface for interplay with the biological segments, expedited by

free surface-active functional groups [240]. These groups are feasible for functionalization by conjugating preferred ligands, antibodies and proteins that have the specific binding ability to target cells, thus improving their targeted drug delivery efficacy, therapeutic potency and also minimize the toxicity [241, 242]. Notably, biogenic nanoparticles are essentially applied for antimicrobial purposes owing to their biocompatibility and long-lasting stability [243]. Biogenic silver nanoparticles developed from *Sporosarcina koreensis* DC4 and *Brevibacterium frigoritolerans* DC2 exhibited antimicrobial effectivity against *Salmonella enterica*, *Vibrio parahaemolyticus, Bacillus anthracis* and *Escherichia coli*. Further, these biogenic nanoparticles enhance the antimicrobial effectivity of conventional antibiotics including rifampicin, lincomycin, vancomycin and penicillin G when adapted in combined form. Thus, combining conventional antibiotics with the biogenic metallic nanoparticles can be further beneficial for improving their antimicrobial potency [244, 245]. Recently, nanosized materials have acquired much attention as a promising delivery carrier for vaccine antigens which can simultaneously stabilize the vaccine antigens and exploit as adjuvants [246]. Adopting nanomaterials as delivery vehicles or vaccine adjuvants can stimulate more effective innate and robust immune responses across microbial infections [247]. Researchers have also engineered nanomaterial-based vaccine adjuvants to stimulate long-term immune responses. For example, gold nanoparticles coated with West Nile Virus (WNV) envelop protein which affects the in vitro and in vivo immunological responses for the generation of antibodies against WNV responsible for causing viral fever [248]. Generally, nanoparticles can conserve the antigens from the biological milieu, improve their half-life, assist the delivery of immunostimulatory or immunomodulatory substances to antigen representing cells, i.e. T cells and induce desired host immunity against infectious diseases [249, 250]. The various nanoformulations have already been marketed for the diagnosis of microbial infections, like AmBisome (liposomal amphotericin B), Fungisome (liposomal amphotericin B), Abelcet (Amphotericin B-lipid complex), etc. [251–253]. Therefore, nanomedicines have exhibited dramatic potential in overcoming nearly all forms of microbial infections by not only encountering bacteria tidily but can also exploit as carriers for antibiotics and natural antimicrobial compounds.

6.3.7 Nanomedicines for Neurodegenerative Diseases

Neurodegeneration is a characteristic of many fatiguing, incurable disorders that are continuously growing in prevalence and represent a major warning to public health. Neurodegenerative diseases are designated by liberal loss of structure or functions of neurons in diverse areas of the central nervous system (CNS), which leads to deficits in specific brain functions [254, 255]. Among the various neurodegenerative disorders, the lion's share of consideration has been inclined to Parkinson's disease (PD), Alzheimer's disease (AD), Huntington's disease (HD), multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), spinocerebellar ataxias (SA) and frontotemporal dementia (FTD) [256, 257]. Early recognition of the inception of

neurodegeneration is very crucial as it can give a chance for early treatment or diagnosis that may be beneficial to hinder further evolution of the disease [258]. The current treatment approaches of neurodegenerative disorders target only a small group of the population and barely focus on symptomatic relaxation without inhibiting disease evolution. The FDA has approved several drugs like Levodopa +Carbidopa (Sinemet), Donepezil (Aricept), Pergolide (Permax), Rivastigmine (Exelon) for palliative treatment, but they are not utilized in the long-term disease treatment and also cause numerous side effects like cardiovascular and endocrinological complications [259, 260]. The major hurdles for drug development are the existence of a restrictive blood-brain barrier (BBB), a firmly packed layer of endothelial cells that limit the entry of drug molecules into brains, resulting in irreversible neuronal damages and unwanted neuroimmune activities [261, 262]. Recent progress in nanotechnology has committed to the design of novel platforms and efficient delivering strategies to advance the treatment of neurodegenerative disorders while less irritating brain systems. The various nanocarriers including polymeric nanoparticles, dendrimers, liposomes, metallic and magnetic nanoparticles, enable the impressive delivery of drugs to the defective brain tissues. Encapsulation of drugs by the nanoparticles can arrive at more depth into targeting areas while protecting the loaded drugs from degradation [263]. With the advances of nanotechnology, nanomedicines not only serve as a vector to deliver the drug beyond the BBB, but also facilitated multiple functionalities for the detection, treatment and monitoring of brain diseases [264]. The insertion of drugs into the brain region via nanomedicines can be attained via different mechanisms including passive diffusion, receptor-mediated transport, cell-mediated transport, carrier-mediated transcytosis, adsorptive-mediated transcytosis, efflux transport, etc. [265]. However, the most plausible mechanism may be over endocytosis by endothelial cells that mark the brain capillaries. Once the nanoparticles are absorbed by the endothelial cells, they are released inside the brain tissues probably via transcytosis and also P-glycoprotein inhibition or tight junction modulation may be the other mechanisms by which nanoparticles cross the BBB [266]. Several nanoparticles have been administered to healthy animals intravenously, verifying their effectivity in crossing the BBB, basically when their surface is functionalized with surfactants or ligands that are specific to the brain tissues [267]. Apart from the ligand conjugation, nanoparticles surface functionalized with polysorbate 80 are described to cross the BBB by simulating the low-density lipoproteins (LDL), allowing them to interact with LDL receptor, resulting in nanoparticles being taken up efficiently by endothelial cells of the brain [268]. Surprisingly, nanoparticles linked with stem cell therapy are being progressively used to repair the neural circuit and hold significant promise for the diagnosis of neurodegenerative disorders. The stem cell-based therapies could offer the benefit of targeting multiple mechanisms, i.e. reducing cognitive impairment mostly occurring due to loss of synaptic function. This convergence approach commonly called theranostics provides the capability of nanoparticles to regulate the cellular response, replacement of death neural cells and enhances the survival of stem cell transplantation [269–271]. Recently, carbon nanotubes (CNTs) have emerged as a potential

nanomaterial scaffold for the regeneration of impaired nerve tissues owing to their distinct structural, mechanical, electrical properties and cell-penetrating capability [272]. Despite the promises, challenges are still being faced by CNTs in the clinical practice due to their inherent toxicity. Biofunctionalization of CNTs via biologically compatible and potent molecules is also a favourable approach to contribute excellent biocompatibility and selectivity for neural regeneration for the suppression of CNS disorders [273]. Moreover, nanoparticle-based drug delivery systems may be allowing a targeted, sustained release of old as well as new drugs, presenting a novel approach to treat neurodegenerative disorders [274]. Rivastigmine is a specific cholinesterase inhibitor with both acetylcholinesterase and butyrylcholinesterase inhibitory activity but facing severe obstacles [275]. Hence, rivastigmine loaded in chitosan nanoparticles not only improves the bioavailability and increases the uptake of rivastigmine to the brain through intranasal delivery but also providing better targeting efficiency and a promising approach for the treatment of Alzheimer's disease [276]. The application of nanomedicines exhibits an immense therapeutic effectivity in the area of neurodegenerative disease therapy, but many features are still matters of interest. Yet there is short data available from in vivo and clinical studies for the usage of nanoparticles. Thus, future research is still affirmed to verify the promising use of nanomedicines to treat neurodegenerative disorders.

6.3.8 Nanomedicines for Gene Therapy

Gene therapy is an empirical technique that describes the direct transport of genetic materials inside the tissues or cells to replace an aberrant disease-causing genes for the treatment of acquired disorders and inherited diseases [277, 278]. In gene therapy, modified strategies are utilized for the transport of genetic materials, i.e. directly inserting the genetic material into the epidermal tissues (in vivo) or indirectly tissues are excised from the host, exposed to genetic manipulation, then the transduced cells are restored into the host body (ex vivo) [279]. The therapeutic gene delivery holds the great potential of serving lifelong therapies and also cures many diseases (e.g. metabolic, neurodegenerative, immunological, haematological and diverse types of cancer) that were previously untreatable [280, 281]. The progress of gene therapy has been generally driven by advancements in non-viral and viral gene transfer vectors [282]. The efficacy of the viral vector for gene transfer is outstanding as long as their high gene expression level and innate ability to accurately infect cells. Several viruses are under observation for gene delivery including adenoviruses (AV), adeno-associated viruses (AAV), retroviruses (RV), herpes simplex viruses (HSV), alphaviruses (αV), poxviruses (PV) and Newcastle disease virus (NDV) [283]. Although recently some studies revealed that extensive use of these carriers presented severe limitations including virulent nature, toxin production, degeneration of transduced tissue and induces acute immune response [284]. Therefore, the non-viral approach was investigated with the intervention of nanomedicine. In current times, nanotechnology has gained significant advances both in terms of novel materials development with unique properties and therapeutic

delivery [285]. As such, materials are designed at the nanoscale range and their distinct physicochemical properties made them potential vector for gene delivery. These nanosized particles can easily interact with biomolecules on the cell surface or inside cells and efficiently deliver genetic materials such as DNA, RNA and siRNA into target cells or tissues [286]. The various non-viral vector including polymeric micelles, dendrimers, liposomes, solid lipid nanoparticles, nanostructured lipid carriers, carbon nanotubes, metallic nanoparticles, hybrid nanoparticles, protein and peptide-based nanoparticles having benefits over viral vector and provide versatility in design, targeting ability to specific sites in a biological system with low immune response and cytotoxicity as well as easily functionalized [287, 288]. The surface functionalization of nanosystems via various ligands or biomaterials improve their targeting ability, bioavailability, intracellular penetration and also provide a range of scope for proper delivery of genes and diagnostic agents to specific cells populations [289, 290]. Nanoparticles functionalized with dual ligands (Transferrin and Mannan) can flourishingly augment the gene expression in liver cancer cells and liver macrophages as well as magnificently improve the transfection activity of the nanocarriers in target cells [291]. Although metallic nanoparticles like gold or silver nanoparticles will continue to find use in different biomedical applications, while toxicity associated with the usage of nanoparticles, in general, and gold nanoparticles, in particular, is an interest. Hence, the surface of the gold nanoparticles modified by chitosan and Arg-Gly-Asp-Ser (RGDS) peptide not only reduced the toxic hazardous effects but also enhances the gene transfection efficiency by binding with DNA resulting to deliver genes efficiently following cellular uptake [292]. However, nanoparticles are basically carrying DNA or RNA via two systematic approaches, i.e. an encapsulating system which is a reservoir class of nanovector systems that could conserve DNA or RNA from distortion and surface binding system which holds an ionic interaction among the cationic polymers and the anionic nucleic acids [293]. Hence, the cationic lipid stationed non-viral gene delivery system is designed by utilizing the amphipathic lipids, carrying a positively charged head group that interacts with the negatively charged phosphate group that exists in nucleic acids (DNA and RNA) via electrostatic interaction to generate nanoparticles, called lipoplexes. Lipoplexes are generally entering mammalian cells by endocytosis mechanism and are able to protect their genetic cargo from degradation [294, 295]. Moreover, nanoparticle surface coating with PEG, often known as "PEGylation", is a promising strategy for upgrading the gene or drug delivery to target tissues. PEG coating provides the shield on nanoparticles surface and protecting the nanoparticles from aggregation, opsonization as well as extending the systemic circulation time [296]. In parallel, a novel cell-penetrating peptide (CPP) planted the non-viral vector that uses glycosaminoglycan (GAG)-binding enhanced transduction (GET) for extremely efficient gene delivery. GET peptides conjugate properly with DNA via electrostatic interactions to generate nanoparticles. For effective in vivo delivery, GET peptides are functionalized with PEG that protected the positively charged surface of nanoparticles, managed colloidal stability and sustained gene transfer process in human bronchial epithelial cell lines [297]. The emerging of clustered regularly

interspaced short palindromic repeats and CRISPR-associated 9 (CRISPR/Cas9) system exhibits an outstanding platform for genome editing for the diagnosis of genetic diseases [298]. However, its low transfection effectivity is a major obstacle that restricts the function of the gene editing power of CRISPR/Cas9. To overcome this hurdling, a novel PEG-phospholipid functionalized cationic lipid nanoparticle-based delivery system was constructed to encapsulate a Cas9/single-guide RNA (sgRNA) plasmid that could be used for the successful transfer of Cas9/sgRNA in A375 cells resulting in deregulation of Polo-like kinase 1 and suppression of the tumour growth [299]. Therefore, nanotechnology brings immense opportunities to generate novel and multifunctional nanocarriers that could convince to be promising carriers for gene delivery. Despite the substantial advances have been contrived, future development of novel strategies is expected for gene therapy.

6.4 Recent Patents Issued in the Area of Nanomedicine Research

Innovations at the convergence of biotechnology, medicine, engineering and information technology are triggering new pathways in research and development (R&D) sector and commercialization. The fate of nanomedicines is likely to advance in this interdisciplinary aspect [300, 301]. The grant in nanotechnology is now constantly given by governments, funding agencies, research centres and companies in both emerging markets and developed countries owing to their stability, ability to antigen recognition on particular cells in the human body, controlling drug release and enhanced bioavailability [302]. Hence upgrading therapies and nanoparticles has been the topic of research and patent application in the area of pharmaceutical technology. The significance of nanomedicine for diagnosis and treatment is distinctly mirrored in the growing number of publications and issued patents annually [303]. For the last decade, a flock of patent applications belonging to nanomedicines has been landing at the Patent and Trademark Office (PTO) in the area of cancer therapy, immunotherapy, antifungal therapy and drug delivery. The recent patents on nanoparticle systems of various compositions like polymer-based, lipid-based, metal-based, carbon-based, hybrid and semiconductor nanoparticles are outlined in Table 6.2.

6.5 Clinical Evidence of Nanomedicines (Marketed Nanoformulations)

Bringing new products to the market has always displayed a major obstacle, particularly when it comes to extremely innovational products. Nanotechnology and especially nanomedicine have been touted as the next advanced technology for medical sciences and have received an immense deal of attention in the design and development of nanoformulations for drug delivery, in vitro diagnostics and in vivo imaging [330]. Even though huge signs of progress are being spotted in the

Table 6.2 Summary of recent patented nanoparticle systems for therapeutic purpose

Sl. no.	Inventors	Title	Patent no.	Ref (s)
Polymer-based nanoparticles				
01.	Caihua Ni and co-inventor	A method for preparation of reducible degradable hyperbranched polymeric micelles	US20180360753 (A1)	[304]
02.	Zhizun Lie and co-inventor	Ceramide-rubusoside nanomicelles and their use in cancer therapy	US20170216329 (A1)	[305]
03.	Elazer R. Edelman and co-inventor	Dendrimer-drug conjugates, hydrogel compositions, and methods	US20190142953 (A1)	[306]
04.	Alice S. T. Wong and co-inventor	Amphiphilic dendrimers complexed with siRNA for treatment of cancer	US20180265872 (A1)	[307]
05.	Li X and co-inventor	Licoflavone nanosponges and its preparation process	CN108703944 (A)	[308]
Lipid-based nanoparticles				
06.	Rita Elena Serda and co-inventor	Cationic liposomes for cancer immunotherapy	US20180243216 (A1)	[309]
07.	Mahmoud Reza Jaafari and co-inventor	Peptide-conjugated liposome	US20170027868 (A1)	[310]
08.	Sun Min Park and co-inventor	Solid lipid nanoparticles including elastin-like polypeptides and use thereof	US20130197359 (A1)	[311]
09.	Indu Pal Kaur and co-inventor	Solid lipid nanoparticles entrapping hydrophilic/amphiphilic drug and a process for preparing the same	WO2013105101 (A1)	[312]
10.	Christopher B. Fox and co-inventor	Nanostructured lipid carriers and stable emulsions and uses thereof	WO2018232257 (A1)	[313]
11.	Anja Träger and co-inventor	Nanostructured active ingredient carrier system	WO2018130247 (A1)	[314]
Metal-based nanoparticles				
12.	Yunjung Choi	Anticancer and anticancer adjuvant composition containing silver nanoparticle	KR101902656 (B1)	[315]
13.	Ilaria E. Palama and co-inventor	Cancer therapy with silver nanoparticles	US20160213711 (A1)	[316]
14.	Rajesh Kotcherlakota and co-inventor	Gold nanoparticle based formulation for use in cancer therapy	US20190240186 (A1)	[317]
15.	Min Ju Kim	Composition for preventing and treating neurodegenerative diseases comprising gold nanoparticles and anthocyanins conjugates	KR101717352 (B1)	[318]
16.	Markus Barthel and co-inventor	Magnetic nanoparticles for use in the treatment of tumours	WO2019215560 (A1)	[319]

(continued)

Table 6.2 (continued)

Sl. no.	Inventors	Title	Patent no.	Ref (s)
17.	Richard Ferrans and co-inventor	Magnetic nanoparticle compositions and methods of use thereof	US20130006092 (A1)	[320]
Carbon-based nanoparticles				
18.	Hongjuan Yao and co-inventor	Drug delivery system comprising a cancer stem cell-targeted carbon nanotube, preparation and use thereof	US20170224840 (A1)	[321]
19.	Kurt W. Swogger and co-inventor	Carbon nanotube nano-therapy composites with paclitaxel	US20160095940 (A1)	[322]
20.	Vijay Krishna and co-inventor	Functionalized fullerenes as antifungal agents	US20120015045 (A1)	[323]
Hybrid nanoparticles				
21.	Say Chye Joachim Loo and co-inventor	Lipid-polymer hybrid nanoparticles	WO2019135715 (A1)	[324]
22.	Zhongyi Cheng	Polymer-lipid hybrid nanoparticles of capecitabine utilizing micromixing and capecitabine amphiphilic properties	US20190091162 (A1)	[325]
23.	Seungpyo Hong and co-inventor	Dendrimer-exosome hybrid nanoparticles as a delivery platform	US20180369410 (A1)	[326]
24.	Shanta Dhar and co-inventor	Immune-stimulating photoactive hybrid nanoparticles	US20140220143 (A1)	[327]
Semiconductor nanoparticles				
25.	Imad Naasani	5-Aminolevulinic acid conjugated quantum dot nanoparticle	US20170049891 (A1)	[328]
26.	Nathalie Gresty	Quantum dots for diagnostic imaging	US20180117184 (A1)	[329]

preclinical stages of development, still there is a lack of effective nanomedicine in the clinical setting for commercialization [331]. During the commercialization of nanomedicines, it takes a long time to acquire regulatory approval, i.e. FDA approval, passing the required clinical trials and after overcoming numerous entry barriers, finally introducing to the market [332, 333]. However, when focusing on the commercialization of this sector, the United States developed as having around half of the global merchandise for nanomedicine-based products. In fact, US industries manufacture about 45-50% of marketed nanomedicine-based products, while European industries have a 35% share [334]. The global market for nanomaterials was esteemed at US$7.3 billion in 2016 and the projection is that it will enter US$16.8 billion by 2022 [335]. Medical devices and biopharmaceutical companies are well attentive to the promising benefits of nanotechnology to the healthcare segment, as illustrated by the progressively growing collaboration among these industries and nanomedicine setup [336]. Currently, a range of nanopharmaceuticals has successfully entered the market and even more are being

examined in clinical trials for a large variety of implications. These products come from various companies all around the world and indicate the present as well as the future success of nanomaterials as therapeutic agents [337]. Some of the marketed nanopharmaceuticals are reviewed in Table 6.3 according to the type of nanoformulations like polymer-based, lipid-based, metal-based, protein-based nanomedicines and proved their safety and effectiveness over a long period.

6.6 Promises and Challenges of Nanomedicines for Drug Delivery

Nanoparticles offer numerous advantages to improve drug delivery as well as overcome many problems associated with the free drug and conventional therapy like poor aqueous solubility, requires high doses and short half-life of the drug in vivo [355]. In conventional therapeutic approaches, the drug is repeatedly administered to stimulate a therapeutic effect due to premature release of the drug prior to arriving at the targeted sites and nonspecific distribution in healthy cells or tissues [356, 357]. Nanomedicines are able to deliver the drug to a given target location in the body and has gained more popularity because it promises high precision when it comes to administering therapeutic formulations [358, 359]. Based on the preparation method, nanoparticles, nanocapsules and nanospheres can be designed to exhibit diverse properties and release behaviours for the encapsulation or delivery of the therapeutic fragments [360]. Nanoparticles possess distinct physicochemical properties owing to their tiny size, wide surface area, high reactivity, etc. [361]. Because of these unique and superior characteristics, they are suitable for enhancing the solubility of poorly aqueous soluble drugs, reduce toxicity towards normal healthy tissues and upgrade therapeutic efficacy [362, 363]. Moreover, nanodrug delivery systems exploit the nature of diseased tissues to specifically target their cargoes, either by active, passive or physical targeting [364]. Despite the benefits tendered by nanoparticles, the challenges involved should be addressed prior to developing any therapeutic nanoparticles are described below:

- Nontargeted nanoparticles could be easily opsonized by the macrophage system, present in liver, lung, kidney, bone marrow and spleen, which is a major barrier for drug targeting [365].
- If nanoparticles directly interact with the body molecules or chemical components or accumulate in the human body, they result in the induction of toxic biological responses like oxidative stress, DNA damage and inflammation. The toxic impacts of nanoparticles are fundamentally driven by their physicochemical characteristics, like their tiny size, wide surface area, exclusive surface charge, specific surface chemistry which prompt their ability to enter and settle in tissue that might be impassable to larger counterparts. Hence, toxicity issues of nanoparticles pose major challenges in assuring the safety profile of nanoparticle-based medicines [366–368].

Table 6.3 List of Marketed Nanoformulations

Sl. no.	Brand name	Drug	Nano formulation	Marketed by	Purpose	Ref (s)
01.	Genexol-PM®	Paclitaxel	Polymeric micelles	Samyang Biopharmaceuticals, South Korea	Anticancer	[338]
02.	Eligard®	Leuprolide Acetate	Polymeric Nanosuspension	Tolmer Pharmaceuticals, USA	Anticancer	[339]
03.	Diprivan®	Propofol	Polymeric Nanoemulsion	AstraZeneca, United Kingdom	Anaesthetic	[340]
04.	Rapamune®	Sirolimus or Rapamycin	Nanocrystal	Wyeth Pharmaceuticals, USA	Organ transplantation	[341]
05.	Ostim®	Hydroxyapatite	Nanocrystal	OSARTIS GmbH and Company, Germany	Orthopaedic surgery	[342]
06.	Invega Sustenna®	Paliperidone Palmitate	Nanocrystal	Janssen Pharmaceutical, Belgium	Schizophrenia	[343]
07.	Doxil®	Doxorubicin	PEGylated Liposome	Janssen Pharmaceutical, Belgium	Anticancer, Kaposi's sarcoma	[344]
08.	Lipodox®	Doxorubicin	PEGylated Liposome	Sun Pharmaceutical Industries Ltd., India	Anticancer, Kaposi's sarcoma	[345]
09.	CPX-351® or Vyxeos®	Cytarabine and Daunorubicin	Liposome	Jazz Pharmaceuticals, Ireland	Acute myeloid leukaemia	[346]
10.	AmBisome	Amphotericin B	Liposome	NeXstar Pharmaceuticals, USA	Fungal infection	[347]
11.	Marqibo®	Vincristine Sulfate	Liposome	Talon Therapeutics, Canada	Leukaemia, melanoma	[348]
12.	DepoDur®	Morphine Sulfate	Liposome	Endo Pharmaceuticals, USA	Postoperative pain control	[349]
13.	Feraheme® or Ferumoxytol®	Iron oxide	SPION	AMAG Pharmaceuticals, USA	Treatment of anaemia	[350]
14.	Resovist® or Ferucarbotran®	Iron oxide	SPION	Bayer Healthcare, Europe	MRI contrast agent	[351]

(continued)

Table 6.3 (continued)

Sl. no.	Brand name	Drug	Nano formulation	Marketed by	Purpose	Ref (s)
15.	Abraxane®	Paclitaxel	Albumin Nanoparticle	Celgene Pharmaceutical, USA	Anticancer	[352]
16.	Rebinyn®	Nonacog Beta Pegol	PEGylated glycoprotein drug conjugate	Novo Nordisk Pharmaceutical, Denmark	Treatment	[353]
17.	Adcetris®	Brentuximab Vedotin	Antibody-drug conjugate	Seattle Genetics Company, USA	Hodgkin Lymphoma	[354]

- Surface lipophilicity of nanoparticles is a key point for ameliorated absorption of blood components onto the surface of the nanoparticles and identified as foreign materials, leading to the activation of integrated pathways and depletion of nanoparticle immunogenicity [369].
- The complex essence of nanoparticle-based medicines can alter the physicochemical properties of the cargo drugs, proteins or peptides, genes and altering the solubility as well as pharmacokinetic pre-disposition upon delivery in comparison with unmodified drugs which can impact the pharmacological effect of the active agents [370].
- The uncommon size, physicochemical properties of nanoparticles exhibit challenges to insight their pharmacokinetics as diverse components will have diverse features that affect their distributions, clearance or catabolism [371, 372].
- Nanomedicines are mainly applied through a route of administration that needs sterile products that will suffer certain challenges based on their compositions since there are high risks for being damaged by sterilization especially when biological materials are involved [373, 374].
- The high intricacy of nanomedicines with their multifarious structures doesn't allow a proper characterization of physicochemical quality, posing challenges for regulatory assessment [375].
- Identifying the convenient analytical tests to entirely characterize the nanomedicines, either biological, physical or chemical may be one of the extra challenging outlooks for development of nanomedicine both from a technical, large-scale preparation and regulatory perspective [376].

6.7 Conclusion and Future Perspectives

The progress of nanotechnology in the healthcare sector is directed by the probability to design and formulation of nanomaterials which broaden the market for many drugs and projecting the source of a highly money-making compartment within the industry. The field of nanomedicine has formerly made significant success, due to the bunch of nanoformulations and imaging delivery systems are clinically approved. The advancement of nanomedicines has further expedited to develop multifunctional therapeutic nanosystems that integrate various medications to reinforce the synergistic effects or merge therapeutics for real-time tracking or diagnosis. These smart nanoplatforms concede their targeting, drug release or degradation behaviours that are controlled or guided by specific pathological alteration. Given the attention for development and application of nanomedicines, their clinical translation was still limited, hence indispensable steps are urgently needed to obtain the clinical implications such as evaluation of the formulation nature, pharmacokinetic properties, safety/toxicity, scale-up and the approval process for nanomedicines. From laboratory nanoparticles to therapeutic applications are essential suitable regulatory guidelines for assessment and monitoring of nanomedicines. Therefore, significant efforts between researchers, clinicians, pharmaceutical

industries and regulatory authorities are needed to achieve the goal of quick translation of nanomedicines and nanodrug delivery systems for the near future.

Acknowledgements The authors are thankful to Dibrugarh University for providing the research fellowship (DURF) to Mr. Sanjoy Das to conduct this work.

Conflicts of Interest The authors declare that they have no conflict of interest. All the figures and tables are self-made and original.

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Nanomedicines in Drug Delivery from Synthetic and Natural Sources to Their Clinical Applications

7

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Abstract

In recent days, nanotechnology in the form of nanopharmaceuticals and nanostructured materials has found a significant niche in different spheres of pharmaceutical science, for instance, diagnostic imaging, gene therapy, drug delivery, immunotherapy, microsurgery and dentistry. The polymer-based nanopharmaceuticals have relatively gained the interest of researchers lately, by virtue of their tuneable characteristics to achieve the intended response in targeted drug delivery. This chapter lays special prominence to the inclusion of synthetic and natural biopolymers in nanomedicines. Synthetic biopolymers have been found competent in delivering biologics besides several active pharmaceutical ingredients (API) in multiple clinical complications. This chapter additionally elucidates the employment of natural biopolymers in delivering API derived from both synthetic and natural source. The currently available FDA-approved biopolymer-based nanomedicines and those under clinical trials have been also enumerated.

Keywords

Nanopharmaceuticals · Biopolymers · Polymeric nanoparticles · Biocompatibility · Biodegradability · Biologics

7.1 Introduction

Nanotechnology has grown into an indispensable field in every walk of scientific discipline, be it electronics, robotics, physics, chemistry, molecular biology, genomics, medicine, etc. owing to its impeccable ability to downsize materials to the

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M. K. Das, Y. V Pathak (eds.), *Nano Medicine and Nano Safety*,
https://doi.org/10.1007/978-981-15-6255-6_7

molecular and atomic level, particularly to the nanoscale level. Nanotechnology is the science of extremely small structures. The prefix 'nano' is derived from a Greek word meaning 'dwarf' [1]. National nanotechnology initiatives in the USA have defined nanotechnology as 'Science, engineering, and technology conducted at the nanoscale, which is about 1–100 nanometres' [2]. 'Nanotechnology is the science and technology that measures, manipulates, and manufactures at the atomic, molecular, and supramolecular levels, aimed at creating materials, devices, and systems with fundamentally new molecular organizations, properties, and functions' [3]. Nanomaterials are substances having at least a dimension under 100 nm [4]. They have inherent characteristics, essentially magnetism, electrical conductance, optical effects, chemical reactivity, and mechanical strength, which deem them as suitable carriers of various agents for targeting diseases specifically [1, 2]. The relevance of nanotechnology in the pharmaceutical field is enormous, and it is fuelled with the booming development of various nanomaterials and nanodevices, which have been employed for the diagnosis as well as treatment of the diseases, or sometimes serving both the purposes simultaneously. This context can be effectively understood with the term 'Nanomedicine', which the National Institute of Health, USA, has defined as 'highly specific medical intervention at the molecular scale for diagnosis, prevention and treatment of disease' [2]. Nanomedicine exploits a plenitude of nanotechnological concepts and approaches, including numerous nanodevices, nanobiosensors and nanocarriers. The nanomaterials, being of size analogous to many biological macromolecules, and their significance in the domain of medicine are copious [5]. The significance of nanomedicine extends to control of diseases, understanding of pathogenesis, identifying the microscopic and decisive step in the drug delivery, and targeting process of a disease [6]. The rapid growth of nanotechnology in the pharmaceutical domain has led to the augmentation of numerous nanosystem-based pharmaceuticals, being absolutely paramount in several intended purposes, namely diagnosis, targeted drug delivery, gene therapy, immunotherapy, tissue repairing, microsurgery, dentistry, etc. The various classes of nanopharmaceuticals are presented in Fig. 7.1 [7–10].

The quest to achieve more specific, tailored, personalized treatment, and highly sensitive early state diagnosis of disease has ushered the exploitation of nanodevices. Nanodevices are devices with at least one overall dimension in the nanoscale, or comprising one or more nanoscale components required for its operation [11]. Nanodevices are nanoparticles developed such that they have the competency to interact with cells and tissues to achieve very definite functions [12]. They are extensively used as diagnostic imaging tools in ophthalmology, oncology, and are also applicable while performing microsurgery. Few examples of nanodevices include nanoporous silica chips, nanowire biosensors, nanocantilever arrays, molecularly gated single-electron transistors, nanoparticle-based biobar codes, multiplex dendrimers [13]. Diseases like cancer are associated with large degree of molecular and pathogenesis complexity, which calls for accurate sensitive diagnosis and targeted delivery of chemotherapeutics. Several nanoprecision tools are being designed and researched by the researchers throughout the years. One such example

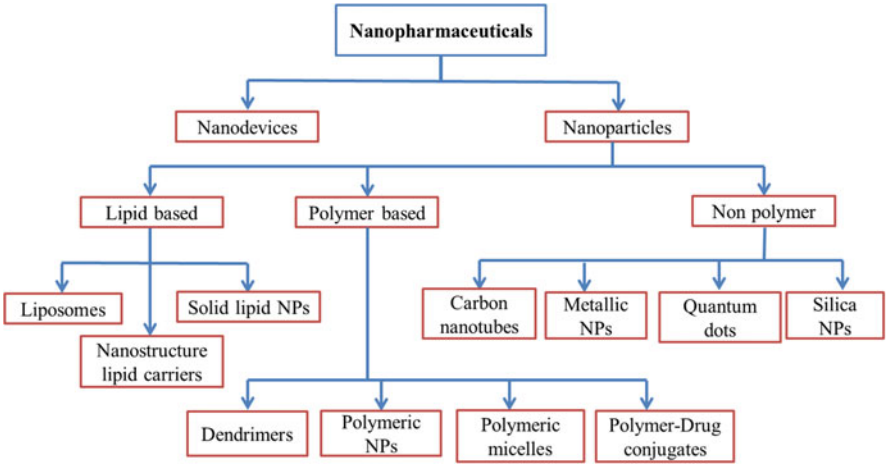

Fig. 7.1 Types of nanopharmaceuticals

is the construction of an enveloped nanodevice having multifunctional aspects developed from mesoporous silica for codelivery of therapeutic peptide and drug to tumour cells by Luo GF et al. Chemotherapeutic agent topotecan was loaded in Mesoporous silica MCM-41 nanoparticle core [14]. A few DNA nanodevices having broad-range sensor abilities have been designed in recent years. The sensitive component is generally single-stranded DNA, which promotes the annealing of complementary single-stranded biomolecules in DNA biosensors, hence achieving hybridization. These devices possess remarkable qualities to sense and react to different varieties of signals or stimuli. The DNA-based sensing devices have the ability to sense the biomolecules, to name a few proteins, peptides, ions, and nucleic acid sequences. Additionally, it can sense glucose levels, level of pH, etc. The transducer molecules can be desegregated with DNA molecule via immobilization by crosslinking, adsorption or covalent bond interaction [15].

Nanoparticles are defined as solid particles or particulate dispersions drug carrier that may or may not be necessarily biodegradable. The active agent is usually entrapped, dissolved, attached or encapsulated to a nanoparticle matrix [16]. Nanoparticles are solid particles with colloidal nature of size range from 10 nm to $<$1000 nm; however, the preferred size for nanomedical application is of that below 200 nm [17].

The methods of preparing nanoparticles are shown in Fig. 7.2 [16, 18].

Nanoparticles are evaluated for parameters like percentage yield, particle size and shape, surface charge, drug loading, drug entrapment, polydispersity index, in vitro drug release, stability and their morphological characteristics. The morphological characteristics of the nanoparticles are evaluated with the aid of techniques like Fourier-transform infrared spectroscopy, X-ray diffraction, Scanning electron microscopy, Transmission electron microscopy [16, 19].

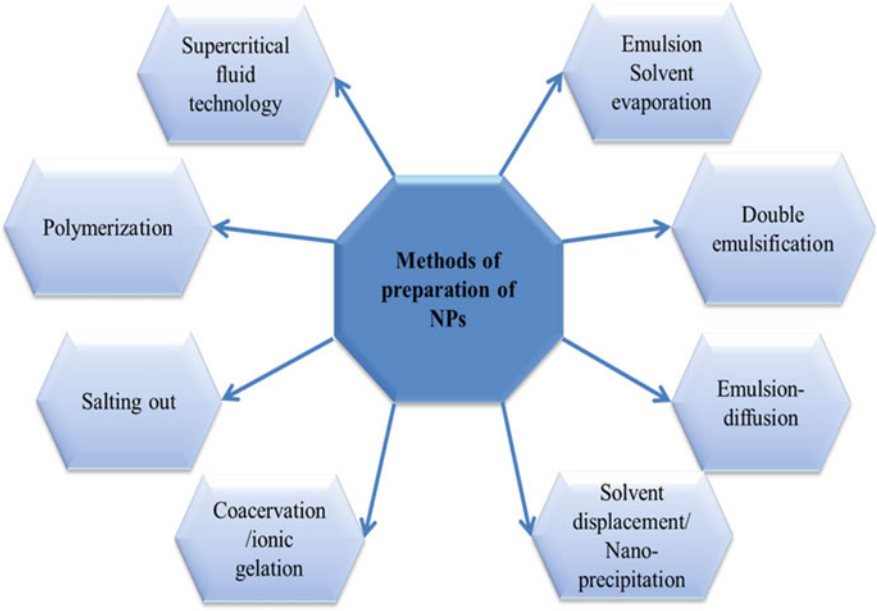

Fig. 7.2 The preparation method of nanoparticles

One of the major areas of practice of nanotechnology in pharmaceutical science arena is nanoparticle-based drug delivery system. This is adscripted to the notion that nanoparticles act as novel carriers for loading drugs or other biomolecules, by virtue of their advantages. By means of manipulating certain parameters like surface characteristics, size and material used, the nanoparticles can be formed into brilliant systems, which can deliver drug to definite organs and tissues hence providing targeted, controlled and sustained drug release and drug delivery, which improves patient's compliance by lessening the dosing frequency and drug-associated toxicity.

Few applications of several nanoformulations are listed in Table 7.1. However, major attention is given to the natural and synthetic biopolymer-based nanoparticles in this chapter.

The polymeric nanoparticles are prepared from biocompatible and biodegradable polymers where the active pharmaceutical ingredient (API) is attached, dissolved, encapsulated or entrapped to that nanosized matrix. Nanoparticles obtained can be nanospheres or nanocapsules depending on the method of preparation [36]. The polymers to be utilized for the fabrication of nanoparticles demand for certain properties among which the biocompatibility and biodegradability of a polymer are most crucial property for pharmaceutical applications. Biocompatibility is a general term referring to the properties of a material, which does not have any toxic or harmful consequences on biological systems. Non-biocompatible materials have been found to induce tissue damage, tissue necrosis, permanent tissue carnage, dystrophic calcification and fibrosis. However, it is notable that fine biocompatibility

Table 7.1 Applications of nanomedicines in drug delivery

Sl. no.	Types of nanoformulations	Characteristics	Biomedical application	Reference
1.	Polymeric NPs	Increase stability of volatile pharmaceuticals, tuneable engineered specificity, biodegradable, biocompatible	Cancer therapy, gene delivery	[8, 20, 21]
2.	Nanocrystals	Electrical and thermodynamic properties, improve dissolution of drugs, safe for intravenous administration	Photothermal Cancer therapy	[22, 23]
3.	Dendrimers	Capable of surface functionalization, stable, monodispersity of size	Anti-cancer, ocular, anti-bacterial drug delivery	[8, 24]
4.	Polymeric micelles	Biocompatible, low toxicity, high stability, core-shell arrangement	Anti-cancer, anti-influenza	[25]
5.	Carbon nanotube	Physical strength, electrical and thermal conductivity, functionalization can be done	Anti-cancer drug delivery, antibiotic delivery	[8, 26–28]
6.	Metallic NPs	Can be conjugated with antibodies, ligands, drugs	Anti-viral drug delivery, anti-bacterial, anti-microbial activity	[29–32]
7.	Liposomes	Amphiphilic, biocompatible, surface modification is possible	Delivery of peptides, DNA, anti-cancer drugs	[8, 33–35]

does not always ensure for positive biodegradability [37]. For a polymer to be used, it must be approved and accepted by the Food and Drug Administration (FDA) for therapeutic application [4].

The polymers widely used in clinical therapies are:

Synthetic Polymers Polylactides (PLA), Polyglycolides (PGA), Poly(lactide co-glycolides), Polyorthoesters, Polycyanoacrylates, Polyacrylamide, Polycaprolactone, Polyanhydrides, Polyglutamic acid, Poly(methyl methacrylate), Poly(vinyl alcohol), Polymalic acid, Poly(methacrylic acid), Poly(acrylic acid), Poly (N-vinyl pyrrolidone), Poly(ethylene glycol) [36].

Natural Polymers Starch, Hyaluronate, Human albumin, Gelatine, Alginic, Collagen, Rosin, Zein, Chitosan, Guar gum [36–38].

7.2 Synthetic Biopolymer-Based Nanomedicines and Drug Delivery

Synthetic polymers make a substantial contribution in the amelioration of pharmaceutical formulations not to mention the pharmaceutical devices for extended and controlled drug delivery and epitomize the most frequently utilized 'building blocks' for constructing diverse nanomedicines, especially nanoparticles [39]. Hence, it can be said that polymers are the essence of pharmaceutical drug delivery systems. Because of its advanced polymeric chemistry, it has exceptional diversity and conduct over the composition, configuration and purpose of the polymers, hence enabling the building of nanomaterials with mouldable properties for numerous biomedical applications [39]. Polymers have found their application in several domains of medical sciences, for instance drug-delivering system, tissue engineering, medical device implantation, dentistry, prosthesis and ophthalmology [38]. Ease in synthesis and characterization, being water soluble, non-immunogenic, biocompatible, biodegradable, inexpensive, non-toxic are the criteria of an ideal polymeric carrier [40].

Natural polymers like proteins and polysaccharides are used to a lesser degree despite being biodegradable in vivo because of the problems associated in their preparation, antigenicity, the purity of the molecule, ambiguity of the source. Similar shortcomings have been proclaimed for nanoparticles fabricated using polymerization reactions because of the generated by-products, which may be non-biocompatible and toxic as well. Therefore, synthetic-based polymers are relatively safer and preferred in pharmaceutical systems [41].

Some of the U.S. Food and drug administration (FDA)-approved biodegradable and biocompatible polymers suitable for human usage that are employed for making polymer-based nanoparticles include poly(ethylene glycol), polyacrylates, polymethacrylates, poly(DL-lactic acid), poly(lactic-coglycolic acid), polycaprolactone, cellulose derivatives, polyoxamers, poly(vinyl alcohol) [42]. The most persistently and broadly used synthetic biopolymers are discussed briefly.

Poly (Lactic-Coglycolic Acid) (PLGA) PLGA is the most competent choice of polymer used for fabricating drug delivery devices and in tissue engineering. PLGA is biodegradable and biocompatible, and exhibits a vast spectrum of erosion time and has adjustable mechanical properties [43]. It is the most investigated diblock copolymer of PLA, i.e. Polylactic acid and Poly (glycolic acid) (PGA) for drug encapsulation and has been employed to develop several available medicinal products in the market [44]. The PLGA-based nanoparticles have been found to possess appreciable mechanical stability and cramped size distribution, and additionally they prevent the drug from enzymatic degradation [4].

Poly (Lactic Acid) (PLA) Also known as Polylactide is a biodegradable, biocompatible and thermoplastic biopolymer approved by FDA for food and pharmaceutical applications [45–47]. PLA is the polymeric form of lactic acid produced from the

fermentation of sugars in sugarcane and corn by microorganisms [47]. Furthermore, different surface modification strategies (physical, chemical methods) can be applied to tune this biopolymer in order to extract the desirable nanocarriers [46]. PLA and its copolymers are an ideal choice of polymer and have been put to use in different drug delivery devices, tissue engineering by virtue of their attributes like biocompatibility, biodegradability, non-toxic degradation products and excellent mechanical properties. This biopolymer can also be spun to form fibres, thus designing nanofibres, which in turn can be used as implants and medical devices [47].

Poly (Glutamic Acid) (PGA) PGA is a polymer of glutamic acid formed due to fermentation by some Gram-positive bacteria [43, 48]. It is biocompatible with tissues and cells and provides controlled release of therapeutics. Blessed with properties like biodegradability, non-toxic, non-immunogenic, PGA is an attractive biopolymer. PGA has two isoforms poly-α-glutamic acid and poly-γ-glutamic acid [49]. The presence of end carboxylic group in poly-γ-glutamic acid allows them for conjugation with several moieties like ligands and therapeutic agents [48].

Poly (Amidoamine) (PAMAM) PAMAM was introduced as a new category of polymers by Donald A. Tomalia in 1985. It is named as 'starburst polymers'. PAMAM was the first synthesized dendrimer to be commercialized [50]**.** Its tree-like branched architecture provides excellent assistance in encapsulation of therapeutic agents, diagnostic agents, genes [50, 51]. Furthermore, the PAMAM dendrimers are water soluble, non-immunogenic and the surface functional groups present allow conjugation of diverse ligands and targeting molecules [51].

Multiple biopolymer-based nanocarriers have been researched in the last few years, namely polymeric nanoparticles, polymeric dendrimers, polymeric micelles, drug-polymer conjugates, polymeric nanofibres. Various synthetic biopolymer-based nanocarriers for delivering synthetic active pharmaceutical ingredients have been researched upon; some examples are enlisted in Table 7.2.

The application of nanoparticles is not only limited in delivering the drugs, but also has an indispensable role in delivering biologics, including genes, plasmid DNA, RNA, and antigens. The necessity to develop nano-based gene therapy arises owing to the pitfalls of both the viral and gene transfection methods, which are associated with problems such as immunogenicity, difficulties in handling, constrained gene carrying capacity, limited cell targeting and production at large scale [82]. The polymers are an ideal vector for carrying gene since they have the capability to interact with plasmid DNA and hence build complexes of nanoscale, which can cross the biological cell membrane [83]. The nanocarriers of different compositions are used in gene therapy, but in this section, the major focus has been inclined toward the importance of synthetic biopolymer-based nanoparticles in delivering biologics.

Herein, we summarize the role of few synthetic polymers and their combination-based nanocarriers, which include polymeric nanoparticles and polymeric dendrimers, in delivering the biological macromolecules in Table 7.3.

Table 7.2 Synthetic biopolymer-based nanocarriers and their clinical application in delivering synthetic active agents

Sl. no.	Nanocarriers	Polymers	API	Clinical application	References
1.	Polymeric NPs	PLGA	Camptothecin	Intracranial glioma	[52]
2.	Polymeric NPs	Eudragit FS30D, Eudragit RS100	Budesonide	Colitis therapy	[53]
3.	Polymeric NPs	PLGA	Tacrolimus	Liver targeting, reducing nephrotoxicity	[54]
4.	Polymeric NPs	PLGA	Budesonide	Ulcerative colitis	[55]
5.	Polymeric NPs	Polycaprolactone	Indomethacin	Anti-inflammatory	[56]
6.	Polymeric Nanocapsules	Eudragit RS100	Clotrimazole	Anti-fungal	[57]
7.	Polymeric NPs	PLGA	Methotrexate	Glioblastoma multiforme	[58]
8.	Polymeric NPs	PLGA	Rifampicin	Tuberculosis	[59]
9.	Polymeric NPs	Eudragit L100–55, Eudragit RS	Aspirin	Anti-inflammatory	[60]
10.	Polymeric NPs	PLGA	Bicalutamide	Prostate cancer	[61]
11.	Polymeric NPs	Polycaprolactone	Noscapine	Glioblastoma multiforme	[62]
12.	Polymeric NPs	PLGA	Gentamicin	Osteomyelitis	[63]
13.	Polymeric NPs	Polyglutamic acid	Amphotericin B	Anti-fungal	[64]
14.	Polymeric NPs	PLGA	Nelfinavir mesylate	Anti-retroviral	[65]
15.	Polymeric NPs	PLGA	Donepezil	Alzheimer's disease	[66]
16.	Polymeric NPs	PLGA, Eudragit S100	Eluxadoline	Irritable bowel syndrome	[67]
17.	Polymeric micelles	Pluronic P105 and F127 copolymers	Docetaxel	Taxol-resistant non-small-cell lung cancer	[68]
18.	Polymeric micelles	Pluronics®P85, F127 and F68	Nimodipine	Cerebrovascular disorders	[69]
19.	Polymeric micelles	Methoxy poly(ethylene glycol) poly(L-lactic acid) (mPEG-PLA)	Ursolic acid	Hepatocellular carcinoma	[70]

20.	Polymeric micelles	Copolymer hyaluronic acid-g-poly(D, L-lactide-co-glycolide)	Doxorubicin	Tumour targeting	[71]
21.	Polymeric micelles	PEG-PLA-PEG	Docetaxel	Tumour targeting	[72]
22.	Polymeric micelles	Monomethyl poly(ethylene glycol)-poly(l-lactide)-poly (trimethylene carbonate) copolymer	Zonisamide	Injury of spinal cord	[73]
23.	Polymer-drug conjugates	2-methacryloxyethyl phosphorylcholine	Temozolomide	Glioblastoma	[74]
24.	Polymer-drug conjugates	Poly(triol dicarboxylic acid)-co-poly(ethylene glycol)	Tetrodotoxin	Local anaesthesia	[75]
25.	Polymeric dendrimer	Poly(amidoamine)	Haloperidol	Psychiatric disorders	[76]
26.	Polymeric dendrimer	Poly(amidoamine)	Paclitaxel	Brain tumour	[77]
27.	Polymeric Nanofibres	Poly[(d,l)-lactide-co-glycolide]	Vancomycin	Postoperative central nervous system infection	[78]
28.	Polymeric Nanofibres	Polycaprolactone	20(S)-Protopanaxadiol	Anti-tumour	[79]
29.	Polymeric Nanofibres	Eudragit L, Eudragit S	Ketoprofen	Oral mucositis	[80]
30.	Polymeric Nanofibres	Polylactide	5-fluorouracil and Oxaliplatin	Colorectal cancer	[81]

Table 7.3 Synthetic biopolymer-based nanovehicles along with their clinical application in delivering Biologics

Sl. no.	Nanocarriers	Polymers	Biologics	Clinical application	Reference
1.	Polymeric NPs	Poly(ethylene glycol)-co-poly (β-amino ester)	DNA	Lung cancer gene therapy (small cell)	[84]
2.	Polymeric NPs	Poly(lactic-co-glycolic acid)	MicroRNAs (miRNAs)	Hepatocellular carcinoma	[85]
3.	Polymeric NPs	Calcium phosphate-embedded PLGA	Plasmid DNA (pDNA)	Gene transfection	[86]
4.	Polymeric NPs	PLGA (surface modified)	Hepatitis B surface antigen (HBsAg)	Mucosal immunization	[87]
5.	Polymeric NPs	Polyethylenimine/ polyglutamic acid (PEI/γ-PGA)	DNA/siRNA co-delivery	Cancer therapy	[88]
6.	Polymeric NPs	Conjugated Polyethylenimine	pDNA	Gene transfection	[89]
7.	Polymeric NPs	PLGA	Avian influenza antigens	Mucosal vaccine delivery	[90]
8.	Polymeric dendrimers	Poly(amidoamine) (PAMAM)	DNA	Gene transfection	[91]
9.	Polymeric dendrimers	PAMAM-PEG-PLL	siRNA	siRNA delivery and gene silencing	[92]
10.	Polymeric dendrimers	PAMAM conjugated with TAT peptide	pDNA (pIRES-H5/ GFP)	Transdermal DNA vaccine delivery	[93]

7.3 Natural Biopolymer-Based Nanomedicine and Drug Delivery

Since ages, humans have long established the practice of using medicinal products derived from plant against plethora of diseases. Medicines in the current days are principally obtained from plants based on the knowledge available from various traditional disease treatment practices. Almost 25% of the significant medicinal compounds and their derivatives accessible nowadays are retrieved from natural resources [94]. Compounds obtained from nature comprising diverse molecular backbones proposed an arena for exploration of new drugs. A current inclination in the discovery of drugs on the basis of natural products has been the curiosity for the design of synthetically liable lead molecules, which resemble their analogue chemistry [95]. Products from natural origin display miraculous features, for instance biological and chemical properties along with macromolecular specificity, extraordinary chemical diversity and low toxicity. These properties make them

favourable leads in the exploration of new drugs [96]. The phytoconstituent present and activity of innumerable natural compounds has been already studied and well established. The flavonoids, alkaloids, terpenes, tannins, saponins, steroids, phenolic compounds are the bioactive constituent found in plants. However, in maximum instances, these compounds have low absorption capacity, inadequacy to cross the lipid membranes as a consequence of their high molecular sizes, which result in decreased bioavailability and efficacy of these compounds [97]. In addition, although these molecules show extensive systemic clearance due to which a high dose of drug was administered, it makes the drug less effective for therapeutic use [98]. The advancement in formulations based on natural products can be revolutionized by implementing the scientific development of nanotechnology. The utilization of nanotechnology is able to solve the problems associated with a natural compound that restricts the application of these compounds in large-scale nanomedicine preparation [99]. Thus, the advancement in increasing the therapeutic properties can be achieved by incorporation of natural products into a nanoparticle-based drug delivery system. The biopolymers used as a carrier in nanomedicine are found from both plants and animals sources. The polymers obtained from natural sources are [100]:

1. *Plant source*: Cellulose and its derivatives, Starch, Inulin, Rosin, Pectin, Glucomannan, Agar, Guar gum, Locust bean gum, Gum Acacia, Karaya, Gum Tragacanth, Aloe Vera gel.
2. *Animal source*: Chitin, Alginates, Carageenans, Psylium, Xanthum gum, Dextran, Hyaluronic acid.

Natural polymers have garnered attention being economical, easily available and relatively low toxic materials. Furthermore, they can be chemically modified, are biocompatible and biodegradable except for few. Biopolymers are derived in vast quantities from both renewable and non-renewable resources [101]. Some of the most commonly used biopolymers in nanotechnology are discussed here [102–105].

Cellulose Cellulose is the natural polysaccharide, which is used most abundantly. It is an organic polysaccharide located within the fibre walls of plants. Cellulose can be chemically modified to produce its different derivatives (cellulosics) such as methyl cellulose, carboxymethyl cellulose, hydroxyethylcellulose, etc., which can be customized for specific industrial applications.

Chitin and Chitosan Chitin is a polysaccharide analogous to cellulose and is the second most bounteous polysaccharide. It is obtained in cell walls of some fungi and is the main component in the shell of crustaceans and also in the exoskeletons of insects. Chitosan is a deacetylase derivative of chitin. Chitosan acquires positive ionic charges, due to which it binds with negatively charged lipids, fats, cholesterol, metal ions, proteins and macromolecules. Chitosan gained an increasing commercial interest because of its admirable properties, including biodegradability,

biocompatibility, adsorption and film-forming ability. Chitosan is exploited as wound-healing remedy due to its bacteriostatic and fungistatic properties.

Starch Starch, a form of carbohydrate, is a biopolymer, which carries many properties. It can be derived from wheat, tapioca, maize and potatoes. Starch is biodegradable in nature along with unique chemical properties; therefore, it has enormous adaptability as a flexible renewable resource for applications in different fields. Although the cellulose and carbohydrate are made up of glucose, they are structurally very different. One vital characteristic is that carbohydrates are water soluble and are readily digested by human beings, whereas cellulose is not digested being water insoluble. A remarkable number of native starches show drawbacks like susceptibility to retrogradation, high viscosity, limited digestibility and limited solubility. Therefore, certain modifications are done like blending in a certain proportion with a more hydrophobic polymer like cellulose to enhance water resistance.

Inulin It is a naturally found polysaccharide, which belongs to a class of dietary fibres known as fructans. Chicory, Dandelion, Burdock, Camas, Costus Elecampane are some plants, which contain a high concentration of inulin. Chicory root is the main source for commercial production of inulin.

Guar Gum Guar gum, a natural polysaccharide, is also referred to as Guaran, Calcutta lucern, Clusterbean, Cyamopsis gum, Guarina, Gum cyamposis. Guar gum in the powder form is derived from the endosperm of the seeds of *Cyamopsis tetragonolobus* Linn (Leguminosae). This polysaccharide is chemically constituted of galactose and mannose. Guar gum is principally profitable for colon delivery due to its degradation that occurs by the specific enzymes present in this colonic environment. The gum provides protection to the drug in the stomach and small intestine environment and delivers the drug to the colon where it undergoes enzymatic degradation and release the drug.

Xanthan Gum Xanthan gum is basically a natural high-molecular-weight polysaccharide produced by the process of fermentation of *Xanthomonas campestris* in controlled condition. It is hydrophilic in nature. Xanthan gum is mainly composed of D-glucose, D-mannose, and D-glucuronic acid. It has diverse industrial application.

Alginate Alginate is a water-soluble linear polysaccharide typically extracted from brown algae (Phaeophyceae) and marine algae, namely *Ascophyllum nodosum*, *Macrocystis pyrifera* and *Laminaria hyperborea*. It is constituted of the salts of α-L-glucuronic and α-D-mannuronic acid. It is generally used in integration with other polymers like chitosan.

Carageenans Carageenans are a family of high-molecular-weight linear sulphated polysaccharides that are extracted from certain species of red seaweeds such as Gigartina, Hypnea, Chondrus and Eucheuma. Carageenans have been utilized as a

gelling agent and viscosity-enhancing agent for the controlled release of drug delivery and prolonged retention of the drug due to their strong negative charge and gelling property.

Dextran Dextran is a neutral polysaccharide produced by microorganism *Leuconostoc mesenteroides*, and consists of α (1–6)-linked D-glucose chain with varying proportions of linkages and branches. Researchers have exploited the properties of dextran such as water solubility, hydrophilicity, colloidal nature and inertness in a biological system for investigating the utilization of dextran as polymer-based carriers in novel drug delivery systems like dextran hydrogels, micelles, and also for specific targeted delivery of drugs.

Hyaluronic Acid Hyaluronic acid (HA) is an anionic natural polysaccharide composed of D-glucuronic acid and N-acetyl glucosamine repeating unit. Its unique multifunctional groups, biodegradability and biocompatibility properties help in developing novel drug delivery system. It is generally present in various connective tissues of human body matrix and hyaluronic acid-binding receptors like CD44 and CD168 are expressed abundantly on the surface of different cancer cells. HA, being a natural ligand, provides significance of hyaluronic acid-based drug delivery carrier in active targeting of tumour cells for anticancer treatment.

The biopolymers from natural sources discussed here can be used in the formulation of several polymeric nanocarriers, which are loaded with active therapeutics having desired therapeutic activity against diseases. Some of the natural biopolymer-based nanoparticles found in the literature are listed in Table 7.4.

7.4 Natural Product-Based Nanomedicine and Drug Delivery

Many natural compounds are being scrutinized and exploited for the treatment of various ailments like cancers, diabetes, cardiovascular, microbial and inflammatory diseases. Application of natural drugs in various fields is possible since they are endowed with exceptional advantages, for instance, greater therapeutic potential, low toxicity and side effects, and also low cost [159], Despite few drawbacks (low bioavailability and solubility, low oral absorption, low stability and unpredictable toxicity, issues with target-specific delivery) associated with herbal medicines limiting their use. In order to counteract such problems, nanotechnology offers a significant role in advanced medical treatment by giving targeted delivery platform and controlled drug release and delivery with extensive success [160]. Some of the phytoconstituents that have been studied till date for different ailments are shown in Fig. 7.3.

In this section, the application of the biopolymers obtained from natural sources in the delivery of therapeutics specifically obtained from natural sources is discussed. Few examples are listed in Table 7.5.

Table 7.4 Application of nanoparticles in drug delivery based on Natural polymers

Formulation	Active ingredient	Biological activity	Reference
Chitosan-nanoparticles	Mifepristone	Anti-cancer	[106]
	Alphastatin	Lung carcinoma	[107]
	Prothionamide	Anti-tubercular	[108]
	Paromomycin	Leishmaniasis	[109]
	Hexaconazole	Anti-fungal	[110]
	Doxorubicin	Breast cancer	[111]
	Risedronate	Osteoporosis	[112]
	Carvedilol	Anti-hypertensive	[113]
	Chlorpheniramine maleate	Allergic rhinitis	[114]
	Insulin	Anti-diabetic	[115]
	Paclitaxel	Anti-tumour	[116]
	Ceftriaxone sodium	Anti-bacterial	[117]
	Rivastigmine	Alzheimer's disease	[118]
Chitosan and hyaluronic acid	Ceftazidime	Anti-bacterial	[119]
	Doxorubicin/Cisplatin	Breast cancer	[120]
	Cyanine 3 (Cy3)-labelled siRNA	Lung cancer	[121]
	MiR-34a and doxorubicin	Breast cancer	[122]
	Risedronate and Teriparatide	Osteoporosis	[123]
Carboxymethyl chitosan	Carbamazepine	Anti-epilepsy	[124]
	5-fluorouracil	Breast cancer	[125]
	Metformin	Pancreatic cancer	[126]
Alginate/chitosan	Enoxaparin	Anticoagulant	[127]
	Insulin	Anti-diabetic	[128]
	5-Fluorouracil	Ocular infection	[129]
Methyl cellulose/cellulose nanocrystal	Ketorolac tromethamine	Skin infection	[130]
Methylcellulose	Docetaxel	Anti-cancer	[131]
Carboxymethyl cellulose	Cabazitaxel	Prostate cancer	[132]
	Docetaxel	Prostate Cancer	[133]
	Docetaxel	Breast cancer	[134]
	Docetaxel	Anti-tumour	[135]
Ethyl cellulose	Picroxicam	Anti-ulcer	[136]
Hydroxypropylmethyl cellulose	Doxorubicin	Anti-cancer	[137]
Cellulose nanocrystal	Hydroquinone	Hyperpigmentary disorders	[138]
κ-Carrageenan	Astaxanthin and α-tocopherol	Diabetic wound healing	[139]
Carrageenan	Melanin	Antibacterial and anti-oxidant	[140]
Starch NPs	CG-1521 (histone deacetylase inhibitors)	Breast cancer	[141]
Corn starch NPs	Ciprofloxacin	Anti-bacterial	[142]

(continued)

Table 7.4 (continued)

Formulation	Active ingredient	Biological activity	Reference
Maize starch NPs	Diclofenac sodium	Anti-inflammatory	[143]
CornStarch NPs	Ibuprofen	Anti-inflammatory	[144]
Starch NPs	Minocycline hydrochloride	Anti-microbial	[145]
Starch NPs	Neutrophil elastase inhibitor (ER143)	Psoriasis	[146]
Dextran NPs	Doxorubicin	Anti-lymphoma	[147]
Dextran Nanomicelles	Doxorubicin	Ovarian cancer	[148]
Dextran NPs	Doxorubicin	Breast cancer	[149]
Chitosan/carboxymethyl dextran NPs	hSET1 antisense	Colon cancer	[150]
Dextran NPs	Methotrexate	Rheumatoid arthritis	[151]
Guar gum/chitosan	Isoniazide/rifampicin	Tuberculosis	[152]
Guar gum/xanthan gum	5-fluorouracil	Colon cancer	[153]
Guar gum NPs	Ag85A antigen	Tuberculosis	[154]
	Isoniazide/rifampicin	Tuberculosis	[155]
	Methotrexate	Colon cancer	[156]
	Tamoxifen citrate	Breast cancer	[157]
	Tinidazole/Norfloxacin	Amoebiasis	[158]

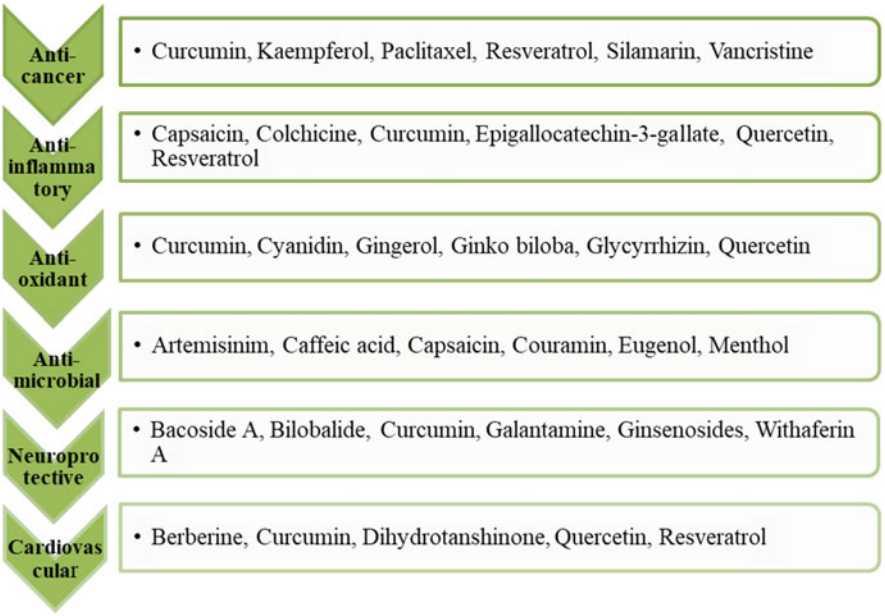

Fig. 7.3 Natural constituents obtained from plants used in nanomedicine

Table 7.5 Natural biopolymer-based nanoparticles carrying API from natural sources

Formulation	Polymers	Active ingredient	Biological activity	Reference
Polymeric NPs	Chitosan	Chlorogenic acid	Renal adenocarcinoma	[161]
Polymeric NPs	Chitosan	Epigallocatechin-3-gallate (EGCG)	Hepatic fibrosis	[162]
Polymeric NPs	Chitosan	Epigallocatechin-3-gallate	Prostate cancer	[163]
Polymeric NPs	Chitosan	Epigallocatechin-3-gallate	Psoriasis	[164]
Polymeric NPs	Chitosan	Apocynin	Anti-ulcer	[165]
Polymeric NPs	Chitosan/hyaluronic acid	Grape seed extract	Anti-oxidant	[166]
Polymeric NPs	Chitosan	Ocimum sanctum	Antibacterial	[167]
Polymeric NPs	Starch	Curcumin	Anti-inflammatory	[168]
Polymeric hydrogel	Chitosan-alginate	Curcumin	Ulcerative colitis	[169]
Polymeric NPs	Chitosan	Curcumin	Breast cancer	[170]
Polymeric NPs	Chitosan	Curcumin	Anti-malaria	[171]
Polymeric NPs	Carboxymethyl chitosan	Curcumin	Anti-cancer	[172]
Polymeric NPs	Chitosan	Curcumin	Colon cancer	[173]
Polymeric NPs	Chitosan	Curcumin/ Coumarin	Anti-cancer &anti-microbial	[174]
Polymeric NPs	Dextran	Curcumin	Liver cancer	[175]
Polymeric film	Methyl cellulose	α-Tocopherol	Anti-oxidant	[176]
Polymeric NPs	Alginate	Silk sericin	Anti-inflammatory	[177]
Polymeric NPs	Chitosan	Quercetin	Colon cancer	[178]
Polymeric NPs	Carboxymethyl chitosan	Quercetin	Pancreatic cancer	[179]
Polymeric NPs	Chitosan	Quercetin	Skin damage by UVB radiation	[180]
Polymeric NPs	Chitosan/alginate	Quercetin	Hepato-protective and anti-oxidant	[181]
Polymeric NPs	Ethylcellulose	Quercetin	Skin cancer	[182]
Polymeric NPs	Chitosan	Quercetin	Breast cancer	[183]

(continued)

Table 7.5 (continued)

Formulation	Polymers	Active ingredient	Biological activity	Reference
Polymeric NPs	Chitosan	Catechin/ Quercetin	Antioxidant and anti-bacterial	[184]
Polymeric NPs	Starch	Quercetin	Anti-oxidant	[185]
Polymeric NPs	Chitosan/alginate	Quercetin	Anti-oxidant	[186]
Polymeric NPs	Chitosan	Resveratrol	Hepatic carcinoma	[187]
Polymeric NPs	Gelatine	Resveratrol	Lung cancer	[188]
Polymeric NPs	Carboxymethyl chitosan	Resveratrol	Anti-oxidant	[189]
Polymeric NPs	Alginate	Resveratrol/ Curcumin	Prostate cancer	[190]
Polymeric NPs	Albumin	Capsaicin	Anti-oxidant	[191]
Polymeric NPs	Microcrystalline cellulose, HPMC	Capsaicin	Gastric ulcer	[192]
Polymeric NPs	Carboxymethyl chitosan/hyaluronic acid	Berberine	Anti-apoptotic	[193]
Polymeric NPs	Chitosan	Berberine hydrochloride	Nasopharyngeal carcinoma	[194]
Polymeric NPs	Chitosan	Berberine chloride	Osteoarthritis	[195]
Polymeric NPs	Chitosan	Silibinin	Prostate cancer	[196]
Polymeric NPs	Chitosan	Kaempferol	Anti-microbial	[197]
Polymeric NPs	Ethyl cellulose	*Garcinia mangostana* Linn	Anti-cancer	[198]

7.5 Clinical Application of Nanomedicines

A bulk of nano-based medicines that are validated or presently undergoing clinical trial are Nanoformulations of already-approved drugs. Currently, the Food and Drug Administration (FDA) in the USA is the decision maker in regulating the approval process for Nanomedicine in humans and is essentially identical to that of any other regulated drugs, device or biologic. The invention/discovery of new pharmaceutical material is followed by the non-clinical study, which usually involves testing on animals to manifest safety, toxicity, and efficacy and to establish appropriate dose ranges. It has become extremely significant for the nano-based medicines to have an extensive perception of the physicochemical specifications of the material, and the reproducibility and scalability of the process of manufacturing [199]. In the last

Table 7.6 FDA-approved biopolymer-based nanoparticles [22, 199–206]

Brand Name	Nature of material	Advantages offered by nanoparticles	Clinical Purpose	Year approved
Adagen®	Adenosine deaminase enzyme (PEGylated)	Enhanced circulation time and reduced immunogenicity	Severe combined immunodeficiency disease	1990
Cimzia®	PEGylated antibody fragment	Improved stability in vivo and circulation time	Crohn's disease	2008
			Rheumatoid arthritis	2009
			Psoriatic arthritis	2013
			Ankylosing spondylitis	2013
Copaxone®	Copolymers of L-alanine, L-lysine L-glutamate, L-lysine and L-tyrosine	Controlled and improved clearance characteristics	Multiple sclerosis	1996
Eligard®	Polymer (PLGH (poly (DL-Lactide coglycolide) and Leuprolide acetate	Delivery of payload in controlled manner with higher circulation time	Prostate Cancer	2002
Macugen®	Anti-VEGF (vascular endothelial growth factor) aptamers (PEGylated)	PEGylation offers greater stability of the aptamer	Age-related neovascular, macular degeneration	2004
Mircera®	Chemically synthesized erythropoiesis-stimulating agent	PEGylation-induced enhanced stability of aptamer	Chronic kidney disease associated amnesia	2007
Neulasta®	GCSF protein (PEGylated)	PEGylation-induced enhanced stability of protein	Neutropenia	2002
Pegasys®	IFN alpha-2a protein (PEGylated)	PEGylation-induced enhanced stability of protein	Hepatitis B, hepatitis C	2002
PegIntron®	IFN alpha-2b protein (PEGylated)	PEGylation-induced enhanced stability of protein	Hepatitis C	2001
Renagel®	Poly(allylamine hydrochloride)	Better circulation and therapeutic delivery	Chronic kidney disease	2000
Somavert®	HGH receptor Antagonist (PEGylated)	PEGylation-induced enhanced stability of protein	Acromegaly	2003

(continued)

Table 7.6 (continued)

Brand Name	Nature of material	Advantages offered by nanoparticles	Clinical Purpose	Year approved
Oncaspar®	PEGylated L-asparaginase	PEGylation-induced enhanced stability of protein	Acute lymphoblastic Leukaemia	1994
Krystexxa®	Polymer-protein conjugate (PEGylated)	PEGylation-induced enhanced stability of protein	Chronic gout	2010
Plegridy®	PEGylated IFN beta-1a (polymer-protein conjugate)	PEGylation-induced enhanced stability of protein	Multiple sclerosis	2014
ADYNOVATE	PEGylated factor VIII (polymer-protein conjugate)	PEGylation-induced enhanced stability of protein	Haemophilia	2015
Estrasorb™	Estradiol micelle formulation	Controlled delivery of drug	Menopause	2003
Abraxane®	Albumin-bound paclitaxel nanoparticles	Improved solubility and hence delivery to tumour site	Breast cancer, pancreatic cancer	2005 2012 2013
Zilretta®	Triamcinolone acetonide PLGA hydrogel	Extended release of therapeutics	Osteoarthritis (knee)	2017
Rebinyn®	Glycopegylated coagulation factor IX	Extended half-life	Haemophilia B	2017
Genexol®	Block copolymer poly(ethylene glycol)-poly(D, L-lactide)	EPR effect-mediated passive targeting	Metastatic breast cancer, pancreatic cancer stage IV	2007
Opaxio®	Paclitaxel polyglutamate nanoparticles	EPR effect-mediated passive targeting	Glioblastoma	2012

several years, the nanomaterial characterization issue became the core of various FDA guidance documents; therefore, it is a particularly vital aspect of research and development. The physicochemical properties, efficacy and toxicity can then be assembled into an Investigational New Drug (IND) application for FDA consideration. After approval of the IND, human trials can be inducted to actuate the safety and efficacy of the new nanomedicine [200]. Few numbers of FDA-approved biopolymer-based nanoparticles are listed in Table 7.6 to exemplify. Table 7.7 enlists those biopolymer-based nanoparticles undergoing clinical trials.

Table 7.7 Biopolymer-based nanoparticles currently under clinical trial [207–210]

Brand name	API	Nanoparticle formulation	Indication	Clinical trial Phase	Clinical trial identifier number
BIND-014	Docetaxel	PSMA targeted PEG-PLGA or PLA-PEG nanoparticle	Prostate, metastatic, non-small-cell lung, cervical, head and neck, cancers	Completed	NCT02479178 NCT02283320 NCT01812746 NCT01792479
Cynviloq IG-001	Paclitaxel	Polymeric micelle	Breast cancer	Completed	NCT02064829
Genexol-PM	Paclitaxel	Polymeric micelle	Head and neck cancer	Completed	NCT02263495
NC-6004 Nanoplatin	Cisplatin	Polyamino acid and PEG micelle	Bladder cancer	Completed	NCT03109158
NC-4016 DACH-Platin micelle	Oxaliplatin	Polyamino acid and PEG micelle	Advanced solid lymphomas	Completed	NCT01999491
NK015	Paclitaxel	Micelle	Breast cancer	Completed	NCT01644890
Docetaxel-PM DOPNP201	Docetaxel	Micelle	Head and neck cancer	Completed	NCT02274610
CriPec	Docetaxel	Micelle	Solid tumours, ovarian cancer	Completed	NCT02442531
Polymeric micelles	Amifostine	PEG, iron micelle (transferrin-mediated chelation)	Dose escalation and safety for acute radiation syndrome	Phase I	NCT02587442
ABI-011	Thiocolchicine analog (IDN 5405)	Albumin bound nanoparticle	Solid tumours or lymphomas	Phase I	NCT02582827

7.6 Conclusion and Future Perspectives

Nanotechnology is a springing discipline that opens opportunities for novel advances and innovations in health and medical precinct, especially with the advent of nanoparticles that serves as a plausible carrier for numerous therapeutic agents. The merging of biopolymers and nanotechnology has ushered the amelioration of several polymer-based nanomedicines providing unique properties, which satisfies the demands of novel targeted drug delivery system. Contribution of biocompatible and biodegradable synthetic and natural polymers has shown blistering advancement in the domain of diagnostic imaging, drugs and biologics delivery, and targeting of serious ailments like cancer, cardiovascular diseases, neurodegenerative diseases, infectious diseases, metabolic disorders. In this chapter, we have compiled few biopolymer-based nanomedicines that are FDA approved and those under different phases of a clinical trial. Taking the advantage of principally polymeric NPs, the prospect of Nanomedicine can be improved and therefore facilitate the conventional therapies to assist humans on both individual and worldwide levels. The uninterrupted research on polymeric nanoparticles in both non-clinical and clinical studies will essentially turn around and improve the diagnosis, treatment and prevention of ailments.

Acknowledgements The Authors acknowledge the library and internet facilities provided by Dibrugarh University to prepare this manuscript. S.D. acknowledges the DURF to carry out this work. T.D. acknowledges the SRF ICMR to carry out this work.

Conflict of Interest The authors declare that they have no conflict of interest. All the figures and tables are self-made and original.

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Transdermal Nanomedicines for Reduction of Dose and Site-Specific Drug Delivery

8

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Abstract

The emergence of new technologies provides unique opportunities to exploit novel approaches in drug delivery. Transdermal drug delivery systems (TDDS) are one of the imperative technologies of increasing interest with the benefits of sustained/controlled drug delivery leading to patient convenience and compliance. By definition, TDDS are topically administered medications, for example, patches or semisolids, which permeate the active ingredient through the intact skin for systemic effects in a sustained manner. Transdermal drug deliveries, therefore, are the noninvasive administration of active ingredients from the skin surface across its layers, to the systemic circulation. Nanomedicinal approaches through TDDS can be utilized for site-specific delivery of drugs which can lead to the reduction of dose, too. We have reported here TDDS providing nanomedicinal strategies to deliver drug(s) to the target tissues.

Keywords

Skin · Transdermal delivery · Nanomedicine · Dose · Site-specific delivery

8.1 Introduction

Skin, being the largest organ of our body, protects us as a physiological barrier from different infections, environmental stress, such as heat or cold, and permeates the sensation with the help of nerve endings residing beneath the skin. Certain active ingredients having the potency to cross this physiological barrier can even reach the

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M. K. Das, Y. V Pathak (eds.), *Nano Medicine and Nano Safety*,
https://doi.org/10.1007/978-981-15-6255-6_8

systemic circulation. Transdermal drug delivery systems (TDDS) are topically administered medications intended for systemic use with the benefits of sustained/controlled delivery leading to patient convenience and compliance [1, 2]. The primary goal of the transdermal route is to deliver drugs into systemic circulation at a predetermined rate across the primary barrier of drug delivery, i.e., the stratum corneum (SC) [3]. Lipophilic polymeric nanocarriers have been exploited to enhance skin penetration of larger, hydrophilic/hydrophobic drugs for various diseases.

Nanomedicine could be defined as a branch of medicine which applies the technique, knowledge, and specific tools of nanotechnology to diagnose/prevent/treat the diseases. Nanomedicine is more advantageous over conventional formulations since it enables more effective and less toxic diagnostic and therapeutic interventions. Over the last few decades, several nanosize drug/drug carrier-based approaches have been reported for the effective transport of active drug molecules through the skin. Targeted drug delivery system offers several advantages such as prolonged, localized, sustained release and site-specific drug carriers which can protect the drug from its hydrolytic/destructive/pH sensitive microenvironment and transdermal route could also be utilized for such purposes of various disease conditions using nanoformulations. Thus, the technology of nanomedicine has emerged as a novel platform for transdermal delivery of various potent drugs and other bioactive molecules. The transdermal nanocarriers have been reported to incorporate both hydrophilic and lipophilic drugs for the treatment of diabetes, cancer, viral diseases, and various dermatological disorders. A plethora of novel nano-based transdermal formulations have been developed and characterized to improve skin permeation of drugs for better pharmacodynamic profiles. In this chapter, we discuss different aspects of transdermal nanomedicines developed for targeted drug delivery with their benefits for various diseases.

8.1.1 Transdermal Drug Delivery

The concept of transdermal delivery of drugs can be traced back to the sixteenth century B.C when Ebers Papyrus suggested the use of the husk of castor oil crushed in water to be placed on an aching head and it cured headache soon [4]. Another instance of the prevalence of this type of drug delivery system was the use of medicated plasters in ancient china and two of them are available in medical practice in China [5]. One of the medicated plasters is used to stimulate circulation and the other is used in the treatment of neuralgia and soreness of bones. These include several herbal drugs and are intended for localized action in the tissue underlying the site of action. During the Second World War, munition workers working with nitroglycerine in an ammunition factory experienced less angina attacks. Later investigation showed that it happend due to nitroglycerine. The first medication delivered through the skin was dimethylsulfoxide and nitroglycerine ointment that was introduced in 1954 for the treatment of angina [6]. However, interest in transdermal drug delivery did not take place until late 1960s and early 1970s. In the 1970s, the development of female syndromes in male workers in manufacturing area for estrogen-containing pharmaceutical dosage form doubted the hypothesis of

skin's impermeable barrier. The first TDDS, Transderm-Scop, developed and approved by FDA in 1979 [7, 8], utilized scopolamine for the handling of motion sickness. Growth in the transdermal market ramped up in the mid-1980 with the introduction of anti-smoking patches designed for the cessation of mass smoking.

Some of the main advantages of TDDS are well-documented [9, 10].

- Escaping the risks and difficulties of intravenous route.
- Keeping steady and sustained drug level.
- Reduction of frequency of dosing and easy application resulting in patient compliance.
- Easy withdrawal of medication as per the need.
- Best suited for drugs with short biological half-life.
- Minimization of inter and intrapatient variability.
- Ability to bypass the hepatic first-pass effect.
- Avoidance of gastrointestinal tract discomforts during absorption caused by drug interactions with food, enzymes, etc.
- Suitability in occasions such as vomiting/diarrhea where oral route is not advantageous.
- Easy and economical manufacturing.
- Less chance of overdosing and underdosing as a result of predetermined drug delivery at a requisite therapeutic dosing interval.
- Lower total regular dose of drug by uninterrupted drug supply.
- Possibility of self-administration.
- Provides ease of quick administration of medication in emergencies for unconscious patients.

Like any other formulations, TDDS also have some limitations [11, 12].

- TDDS could be unsuitable for drugs, excipients, and permeation enhancers that cause skin irritation.
- The natural restrictions of drug access due to the skin's permeability indicate that comparatively potent drugs are appropriate only for transdermal route.
- Under various environmental conditions, adhesions of the formulations to various skin types sometimes become challenging.
- Under several environmental circumstances, drug release from transdermal patches may also change.
- The barrier function of the skin differs depending on the regions of the body of the person, from individual to individual and with age.
- Drugs with high acidic and high basic pH are difficult to deliver by TDDS.
- Drugs with large dose cannot be administered.
- Highly hydrophilic/highly lipophilic drugs are not suitable.
- High molecular weights drugs are also not suitable to formulate.

Over the past few years, commercial TDDS were developed for the treatment of hypertension, angina pectoris, pain management, osteoporosis, hormone replacement, smoking addiction, and many more. There are various types of patches with

different medications available to treat different disorders for which conventional routes are not apt for safety and patient compliance. Simplicity, effectiveness, and superior patient compliance have driven innovators toward this remarkable delivery platform in the twentyfirst century. Transdermal drug medication will modernize the perception of "dose" of drug to be delivered. Physician will prescribe a certain "rate" of drug, rather than at a certain "dose". Systems will be planned to give variable rates depending on the areas of administration [13].

8.1.2 Skin Physiology

The skin is the primary site of application for TDDS and also the main barrier of drug permeation. Hence, understanding of skin physiology is very significant for the realization of skin permeation of drugs. The skin of an average adult body measures around 2 m^2 of surface area and gets approximately one-third of the total blood circulating through the body [14]. It is one of the most vast and easily reachable organs on the human body. With a depth of only a fraction of millimeter, the skin plays several functions as mentioned underneath:

- It provides protection to the underlying blood circulation from the external environment.
- It acts as a barricade against physical, chemical, and microbial threats.
- It maintains body temperature.
- It regulates blood pressure.
- It defends the penetration of ultraviolet rays.
- It synthesizes Vitamin-D in response to sun exposure.
- It plays a role in wound healing and many more.

To understand the transportation of a drug candidate through this vast organ, one has to study in detail the anatomical features of skin that influence the absorption of the drug through its various layers. The comparative impermeability of skin is well-documented [15]. However, illumination of the factors that are responsible for skin impermeability has been investigated to use the skin as a route of drug administration.

8.1.2.1 Structure of the Skin

The skin is a multifaceted organ composed of many histological divisions but in general, it is described in terms of three major tissue layers (Fig. 8.1).

- The epidermis
- The dermis
- The hypodermis

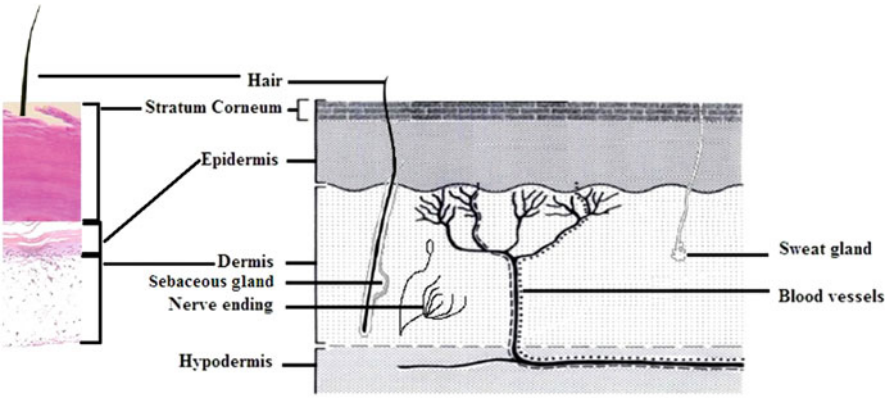

Fig. 8.1 Schematic representation of skin

8.1.2.2 Epidermis

It is the superficial layer of the skin and it comprises of five microscopic layers:

- *Stratum corneum*—This is the outermost layer of the skin and the most impervious biological membrane and the composition of this layer influences the percutaneous absorption of drugs [16]. The thickness of the stratum corneum (SC) is approximately 10–50 μ and this varies with the location in the body [17]. It consists of compacted, flattened, dehydrated and keratinized cells, protein-rich cells, and intercellular lipid layers. This layer is predominantly lipophilic barrier that is particularly impermeable in a passive sense to hydrophilic drugs or charged species [18, 19]. As the process of keratinization advances, the fatty acid in the skin decreases in phospholipid content and there is a consequent increase in triglycerides and sterol esters [20]. The intercellular lipids are rich in ceramides (50%), fatty acids (25%), and cholesterol (25%), which provides the main barrier function to the skin. Similarly, the water content of the SC is only 20% compared to 70% in the physiologically active *stratum germinativum* and hydration of this layer increases the permeability of drugs [21]. These cells are physiologically latent and are continuously covered with the continuous replacement from the underneath viable epidermal tissue.
- *Stratum lucidum*—these cells have highly acidophilic granules. The cells of this layer are non-nuclear and help in keratin formation.
- *Stratum granulosum* (*granular layer*)—Here, each keratinocyte possesses basophilic keratohyalin granules and the protein filaggrin is a foremost constituent of these granules. This protein is thought to attach to the keratin filaments to form keratin complex. The cells synthesize lipids which are believed to act as intercellular cement. Desmosomes and tonofilaments are also sometimes found in this layer.
- *Stratum spinosum/prickle cell layer*—This layer has prickly manifestation at high resolution due to fine cells which process desmosomes connecting one

polyhedral-shaped cell to another. They have numerous evenly spaced intercellular bridges called tonofilaments that are precursors of keratin.

- *Stratum germinativum/stratum basale*—It consists of a single layer of cuboidal cells connected by hemidesmosomes to a thin basement membrane, which detaches it from the underlying dermis. The cells of this layer undergo mitotic cell division to form the next layer dermis.

8.1.2.3 Dermis

Below the epidermis exists the dermis that is a tough and resilient tissue. The dermis provides nutriment to the epidermis and cutaneous appendages, namely sweat ducts, sebaceous glands, and hair follicles. The thickness of the dermis is 500–3000 μ but it does not provide a barrier to the absorption of drugs. The dermis layer is highly vascular (blood flows at a rate of 0.5–1.5 cm^3/h-cm^2) and once the drug is able to cross the SC, it easily passes through the dermal layer and is removed by the cutaneous blood vessels [22].

8.1.2.4 Hypodermis

It consists of adipose tissue and acts to connect the dermis to the underlying tissues. On average, the human skin surface has 40–70 hair follicles and 200–250 sweat ducts per cm^2 and these skin appendages have an important role in the permeation of drug at an early and steady state. Such appendages cover only 0.1% of the total human skin surface.

8.1.2.5 Reservoir Capacity of Skin

A number of researchers has reported the reservoir capacity of stratum corneum [23, 24]. Both hydrophilic and lipophilic drugs may get entrapped in the matrix structure of stratum corneum and diffuse out slowly due to low diffusivity and/or strong binding [25]. This reservoir capacity of stratum corneum should be examined before developing a transdermal therapeutic system as it affects the dynamics of transdermal delivery. Consequences of drug binding often result in an increased steady-state permeation [26].

8.1.2.6 Metabolic Activity of Skin

Epidermis is a viable, metabolizing membrane, which can be a significant metabolic barrier for drug action. This concept has long been overlooked by highlighting the barrier properties of the stratum corneum layer. However, topical bioavailability of a drug depends on both skin permeation and cutaneous metabolism [27]. Beneath the major passive barrier (i.e., stratum corneum) lies the viable epidermis and dermis and of these two layers, the viable epidermis is metabolically more active [27]. For instance, oxygen consumption was found to be 4–5 times greater in the epidermis than the dermis in mouse [28] and in humans, the activity of catechol-o-methyl transferase was 8.3 times greater in the epidermis [29]. The metabolic activity of the skin is 10% as compared to the liver [30] but its significance to the systemic uptake of some drugs cannot be ignored [31, 32]. Smith et al. [33] have demonstrated an interaction between therapeutic agents and the skin and proposed a functional role

Table 8.1 Predominant drug metabolizing enzymes present in the skin of different species

Species	Organs	Enzymes
Human	Skin	Hydroxylase
Hairless mouse	Skin	Ethoxyresrufin dealkylase
Neonatal rat	Skin and liver	Glutathione-s-transferase
Rat, neonatal rat, mouse, guinea-pig, human	Skin and liver	Arylhydrocarbon hydroxylase
Neonatal rat, hairless mouse	Skin	Ethoxycoumarin dealkylase

for CYP2S1 in the metabolism of topical drugs and in intervening the response to photochemotherapy in psoriasis. The distributions of hydrolytic enzymes which metabolize prednisone 21-acetate (PNA) to prednisolone (PN) in human skin imply that the allocation of hydrolytic activity in human skin may avert certain substances from entering the systemic circulation in their unhydrolyzed form [34]. Some enzymes involved in the metabolism of some compounds in the skin of different species are listed in [35] Table 8.1.

8.2 Mechanism of Skin Permeation

The permeation of a drug through the skin involves the following steps:

- Adsorption of drug molecules on the surface of stratum corneum
- Penetration of drug molecules through the viable epidermis
- Uptake of the drug molecule by the capillary network in the dermal layer

Routes of penetration are shown in Fig. 8.2. Diffusion through the stratum corneum is the rate-limiting step in the transdermal permeation of a drug though the viable tissue layers and the capillaries are relatively permeable [36]. The stratum corneum mainly consists of stratified flat corneocytes and the intercellular space between the corneocytes is filled by lamella of lipid bilayers [37] and the intracellular matrix is a mosaic of keratin filaments and interstitial lipids. The role of the intercellular stratum corneum lipids in the barrier properties of the skin has been demonstrated [38] and the permeability of a drug is explained in terms of diffusion and uptake in the lipid and proteinaceous phases of the skin. The presence of the lipid pathway and the proteinaceous pathway was assured by several techniques using electron microscopy and laser scanning confocal microscopy [39]. The mechanism of permeation can involve:

- *Transepidermal absorption*: Way through epidermis itself
- *Transfollicular or shunt pathway absorption*: Diffusion through shunts, mainly those offered by the relatively extensively distributed hair follicles and eccrine glands [40].

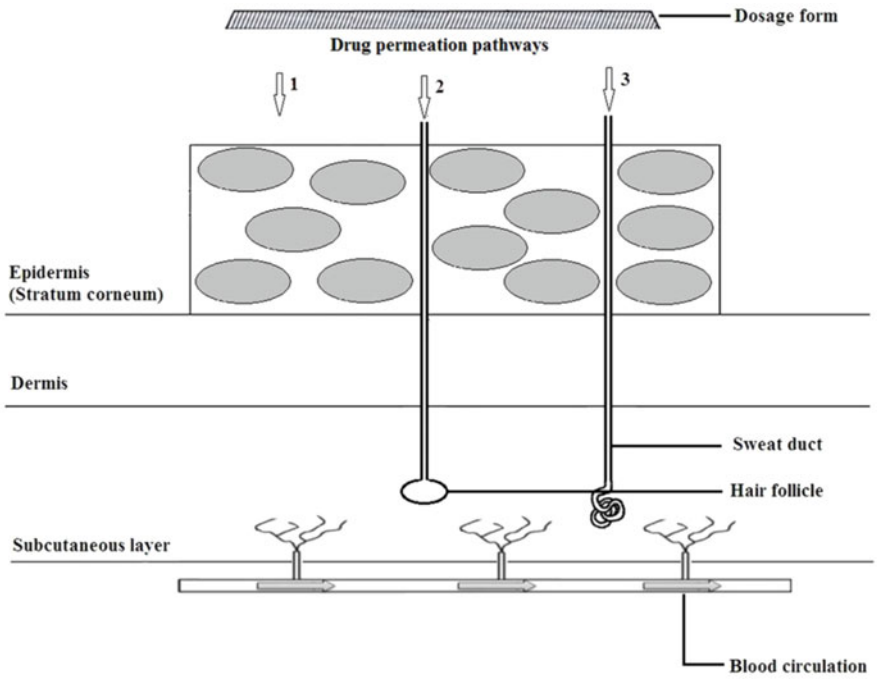

Fig. 8.2 Representation of routes of skin penetration: 1. directly across the stratum corneum; 2. via the hair follicles; 3. through the sweat ducts

In order to appreciate how the physicochemical properties of the diffusing active ingredient and vehicle affect permeation across stratum corneum and thereby maximize delivery, it is crucial to determine the major route of drug permeation within the stratum corneum. Conventionally, it was considered that hydrophilic chemicals diffuse within the aqueous portions near the outer surface of intracellular keratin filaments (intracellular or transcellular path) while lipophilic chemicals diffuse through the lipid matrix between the filaments (intercellular route) [41].

8.2.1 Skin Pharmacokinetics

The drug applied mainly permeates by passive diffusion through the stratum corneum, which is a rate-limiting step. Basically, molecular structure and molecular arrangement of lipids of stratum corneum are two important regulators of barrier function [42, 43]. Transfollicular or shunt pathway absorption also contributes to permeation. So we can say that the migration of molecules from outside the body toward the bloodstream is approximately governed by diffusion laws [41, 44]. Applying Fick's first law, the skin can be considered as complex membrane and the

quantity of drug 'J' diffusing per second per cm^2 area in the direction 'X' equals the diffusion coefficient 'D' times the concentration gradient (Eq. (8.1)) [45].

$$J = -\mathrm{D}\ \mathrm{dc}/\mathrm{dx} \tag{8.1}$$

During the diffusion of a substance in stratum corneum, the concentration gradient in the diffusion area is decreased. This distribution gradient is defined by Fick's second law (Eq. (8.2)).

$$\mathrm{dc}/\mathrm{dt} = \mathrm{D}\ \mathrm{d}^2\mathrm{c}/\mathrm{dx}^2 \tag{8.2}$$

Nevertheless, with very short distance, diffusion (and diffusion coefficient) can be considered as constant. In addition, the relationship between drug concentration in formulation and at the surface of the skin is a function of K_m, the coefficient of distribution between the vehicles and the membrane [46].

$$J = K_m\ \mathrm{DC}/d = K_p \Delta C \tag{8.3}$$

$$K_p = K_m\ D/d \tag{8.4}$$

ΔC = difference in the concentration at the top and bottom of the membrane; d = thickness of the membrane; K_p = permeability coefficient.

8.2.1.1 Mechanism of Rate-Controlled Transdermal Drug Delivery

A systematically active drug that will reach a target tissue far from the site of drug administration on the skin surface must possess some physicochemical properties that are capable of facilitating the sorption of drug by stratum corneum, the penetration of drug through the viable epidermis and also uptake by the capillary network in the dermal papillary layer [41, 47]. The rate of permeation, dQ/dt, across the skin tissues can be expressed mathematically by the following relationship [46]:

$$\mathrm{dQ}/\mathrm{dt} = P_s\ (C_d - C_r) \tag{8.5}$$

and

$$P_s = K_s\ D_{ss}/h_s \tag{8.6}$$

C_d = drug concentration on the stratum corneum, i.e., donor phase; C_r = drug concentration in the receptor phase, e.g., systemic circulation; K_s = partition coefficient between the transdermal therapeutic system and skin tissue; D_{ss} = apparent diffusivity for the steady-state diffusion of the penetrant molecule through the skin tissues; h_s = overall thickness of the skin tissues.

Thus, permeability coefficient (P_s) is a constant if K_s, D_{ss}, and h_s are constant under a given set of conditions. Therefore, to maintain a constant rate of drug delivery, the drug concentration on the surface of stratum corneum (C_d) should

always be greater than the drug concentration in the body (C_r), i.e., $C_d > C_r$, so Eq. (8.5) reduces to

$$dQ/dt = P_s\, C_s \tag{8.7}$$

In order to maintain C_d at a constant value, it is necessary to make the drug release at a rate (R_r) that is either constant or much greater than the rate of skin uptake (R_a), i.e., $R_r >> R_a$. By making $R_r >> R_a$, the drug concentration on the skin surface (C_d) is maintained at a level equal to or greater than the equilibrium solubility of drug in stratum corneum ($C^e{}_s$), i.e., $C_d > C^e{}_s$ and the maximum rate of skin permeation (dQ/dt) can be expressed as

$$(dQ/dt)_m = P_s\, C^e{}_s \tag{8.8}$$

It can be concluded that the rate of skin permeation depends on two factors primarily. One is the permeability coefficient and the other is the solubility of the drug in stratum corneum. Thus, stratum corneum is the well-known rate-limiting factor in skin permeation [48–50].

8.2.2 Dose Reduction Through TDDS

TDDS results in patient compliances through reducing unwanted harmful effects of a drug due to reduction of frequent application and dose requirement [51]. The report suggests that the dose requisite and manufacturing cost to vaccinate via skin is much lower than the conventional way [52]. TDDS has also been exploited to use opioids in decreased dose [53]. Manasadeepa et al. [54] studied the effect of pressure-sensitive polymeric patches to combat periodontal diseases locally by decreased dose compared to the conventional therapy. Damodharan and coworkers [55] showed skin permeation of rosiglitazone from patch-based formulation could be an alternative for reducing drug-related toxicities. Due to the sustained delivery of drug and avoidance of initial hepatic first-pass effect, which is common to oral delivery of drug, dose reduction is observed in TDDS [56].

8.3 Nanomedicine

Nanomedicine involves the intensive use of materials at nanoscale range; examples of such materials are metals, polymers, etc. [57]. The aim of nanomedicine mainly focuses on improving the quality of life of the patients as its (nanomedicine) less toxic diagnostic and therapeutic interventions have made it more effective. A constant emphasis has been laid on combining the diagnosis and therapy within a single platform of nanomedicine and it is referred to as ‘nanotheranostics’ [58]. It is framed to promote the crucial aspects of therapeutic drug delivery, such as evaluation of pharmacokinetics and biodistribution of the drugs and also on the targeted

accumulation of drugs to specific sites, as well as on detecting and diagnosing the disease at an early stage for optimal treatment.

8.3.1 Therapeutic Purpose of Nanomedicine

Nanomedicine for therapeutic purposes is more beneficial than the standard drugs, for several reasons such as (a) it minimizes the rapid degradation and elimination of the drugs from systemic circulation, and also prolongs its retention in the bloodstream; (b) it facilitates site-specific drug targeting to the desired organs and tissues; (c) it reduces the dose-related adverse effects to nonspecific regions of the body; and (d) it improves patient compliance by reducing the frequent administration of drugs, which ultimately leads to the reduction of dose [59, 60]. Moreover, the nanocarriers aids in overcoming the different barriers to the pathological sites for significant drug delivery.

8.4 Nanomedicine in TDDS

Nanomedicine is an application of nanotechnology which is used to prevent and treat diseases in the human body. It provides the use of sub-micrometer size particles for proper diagnosis, prevention, and treatment of diseases and helps in the betterment of human lives. In the last few years, numerous efforts and processes have been carried out in the field of nanomedicine and various formulations have been done which hold a considerable potential and enable more effective and less toxic diagnostic and therapeutic interventions.

Various interdisciplinary researches have been carried out on nanomedicine formulations which enlighten the efforts to combine diagnosis and therapy with a single nanomedicine formulation.

In the past few decades, a novel drug delivery system came into light, which is the transdermal drug delivery system. Drug delivery enhances the efficacy of drugs through controlled release, by considering several factors like carrier system, route of administration, and target of drug action. Transdermal drug delivery system is undoubtedly one of the attractive routes, but the transport of drug through the skin has been a challenge. To overcome this challenge, nanoparticulate or nanovesicular system has been adopted for better skin permeation. Vesicular system such as aspasomes, transethosomes, and nanoethosomes are used for delivering drug into the deeper layers of the skin while liposomes showed inefficiency to cross the deeper layers of skin.

8.4.1 Transethosomes

Ethosomes and transferosomes together comprise transethosomes. Since the liposomes are incapable of crossing the deeper layers of the skin, they tend to get

accumulated on the superior layer of stratum corneum. To improve skin permeation, liposomes are added with edge activators, namely Span 80, Tween 80, Span 25, and sodium cholate, which are named as transferosomes. Ethosomes are another vesicular system which is composed of phospholipid, ethanol, and water [61].

8.4.2 Nanoethosomes

As we have discussed, ethosomes are noninvasive carriers that help the bioactive agents to penetrate into the deeper layers of the skin as well to reach the bloodstream. Ethosomes are composed of phospholipid, ethanol, and water and comprise various sizes. Ethosomes of nanometer size are called nanoethosomes. A high content of ethanol when present provides a negative charge on the surface of vesicles which in turn promotes the reduction of its size [61].

8.4.3 Aspasomes

Aspasomes are multilayered vesicles formed by amphiphiles molecules, Ascorbyl palmitate in combination with cholesterol and a negatively charged lipid, dicetyl phosphate, for drug encapsulation [62, 63].

Physicochemical properties of nanocarrier systems determine the interaction with biological systems and nanocarrier internalization of the cells. The main physicochemical properties that affect the cellular uptake are size, shape, and charge in the surface and rigidity. There are various advantages and disadvantages of the transdermal nanocarriers.

1. Nanoemulsions:-

   Advantages:

- Nanoemulsions can be formulated as foams, liquids, creams, and sprays.
- They can be easily applied to the skin and are nontoxic, nonirritant.

   Disadvantages:

- Surface charge has a marked effect on the stability.
- They are susceptible to Oswald ripening.

2. Nanoparticles:-

Advantages:

- They can be made through biodegradable materials.

- They can include antibodies on their surface to reach out to the target organs.
- Both hydrophilic and hydrophobic drugs can be loaded in a nanoparticle.
- Because of their size, they are able to avoid the immune system.

Disadvantages:

- It is difficult to develop an analytical method for drug delivery.
- Not enough toxicological assessment has been done.

3. Liposomes:-

Advantages:

- Easy to manufacture and high biocompatibility.
- Liposomes increase the stability of protein.
- Controlled release based on natural lipids.

Disadvantages:

- When high-pressure homogenization is used, there is a decreased stability of high molecular weight molecules.
- They are susceptible to physical instability.

4. Dendrimers:-

Advantages:

- They increase the stability of therapeutic agents.
- They are easily prepared and show increased bioavailability of drug.
- Dendrimers act as solubility enhancers and increase the permeation of lipophilic drugs.

Disadvantages

- They have shown cellular toxicity.
- They are not good carriers for hydrophilic drugs.
- Cost of their synthesis is higher than other nanocarriers.

5. Transferosomes, Ethosomes:-

Advantages:-

- Biodegradable and low toxicity and easy to prepare.

- They can encapsulate both hydrophilic and lipophilic moieties.
- They possess the ability to target organs for drug delivery.

Disadvantages:

- Formulations may be expensive.

6. Aspasomes:

Advantages:

- High skin retention of the drug.
- Less toxic.

Disadvantages:

- Formulations may be expensive.

There are many dermal and transdermal drug delivery systems (Table 8.2) which have been licensed for the manufacture after passing through the regulatory approval and trials. Currently available medications for transdermal delivery in the market are well-documented in the literature [64].

In the field of novel drug delivery, vesicular carrier has become a highly interesting topic which provides a bright application in the field of drug delivery. It provides an ability to improve factors such as solubility, penetration, uptake as well as a better carrier facility to ensure the stability of various kinds of drugs and proteins.

8.5 Different Formulations of Transdermal Drug Delivery

Skin being the largest organ of our body, it protects us as a physiological barrier from different infections, environmental stress, like heat or cold, and permeates the sensation with the help of nerve endings residing beneath the skin. If we go in detail with the skin physiology, it may easily be speculated that just after the superficial stratum corneum and epidermis layer, the blood vessels appear with their capillaries in the dermis layer. The molecule having the potency to cross this physiological barrier can even go to the systemic circulation easily. This basic thought inclined the scientists to invent a new path of drug delivery into the system, as the conventional oral administration has significant setback with poor bioavailability of the drug molecule in the targeted tissue due to the hepatic first-pass metabolism process. Aiming this target, the TDDS was emerged to improve the therapeutic efficacy, homogeneous distribution, and reduce the amount and number of doses of drug.

Table 8.2 Various Transdermal patches with their indications

Drug	Trade name	Specific use	Type of transdermal patch
Fentanyl	Duragesic	Used in case of moderate/severe pain	Reservoir
Nitroglycerine	Deponit	Angina pectoris	Drug in adhesive
	Minitran	Prevents angina attacks (chest pain).	Drug in adhesive
	Nitrodisc	Prevents angina attacks (chest pain).	Micro reservoir
	Transderm nitro	Used to prevent chest pain in people having coronary artery disease	Reservoir
	Nitrodur	Used to prevent attacks of chest pain but will not treat an angina attack that has already begun	Matrix
	Diafusor	Used to prevent chest pain in people with coronary artery disease	Matrix
	Nitroderm TTS	Helps to prevent/reduce the frequency of angina attacks	Micro reservoir
	Transdermal-NTG	Used to prevent episodes of angina in people having coronary artery disease	Reservoir
	Nitro patch	Used to prevent episodes of angina in people having coronary artery disease	Reservoir
	NTS patch	Helps to quit smoking by replacing the nicotine	Reservoir
	Nitrocine	Prevents angina attack in people having coronary artery disease	Reservoir
Isosorbide dinitrate	Frabdol tape	Used for angina pectoris, myocardial infarction	Matrix
Testosterone	Testoderm TTS	Hypogonadism in males	Reservoir
	Androderm	Used for hormone replacement in men who are incapable of producing enough testosterone	Reservoir
Nicotine	Nicotrol	Smoking cessation	Drug in adhesive
	Prostep	Helps to quit smoking by replacing nicotines	Reservoir
	Nikofrenon	Helps to quit smoking by replacing nicotines	Matrix
	Nicotinell	Helps to quit smoking and also relieves many withdrawal symptoms	Matrix
	Habitraol	Helps to quit smoking	Drug in adhesive
Clonidine	Catapres-TTS	Used in hypertension	Membrane matrix hybrid type
Lidocaine	Lidoderm	Anesthetic	Drug in adhesive

(continued)

Table 8.2 (continued)

Drug	Trade name	Specific use	Type of transdermal patch
Scopolamine	Transderm-Scop	Used in motion sickness	Membrane matrix hybrid type
Hyoscine	Transderm-Scop	Used to prevent nausea and vomiting during motion sickness or from anesthesia given during surgery	Matrix
Acyclovir	Supravir cream	Used in case of herpes infection	Matrix
Estradiol	Vivelle	Used in case of post menstrual syndrome	Drug in adhesive
	Estraderm	Used for short-term relief of symptoms of menopause	Reservoir
	Climara	Used in treating certain symptoms of menopause	Drug in adhesive
	Esclim	Used to treat hot flashes and other symptoms of menopause	Drug in adhesive
Minoxidil 4%	Nanominox	Helps in hair growth promotion	Matrix
Ethinyl estradiol	Ortho Evra	Used in post menstrual syndrome	Drug in adhesive
Many ingredients	Cellutight EF	Used as topical cellulite	Matrix

Since the concept of TDDS emerges, the formulation to prepare the patch gets the priority for the successful delivery of respective drugs.

8.5.1 Advancement of TDDS

The first-generation delivery systems have no patch at all, but apply a liquid spray, gel, or other formulations on the skin surface. After application, it gets evaporated or absorbed which delivers the small lipophilic drugs into the stratum corneum and viable epidermis [65].

The second generation of TDDS was developed with the use of chemical enhancers in the formulation of transdermal patches. The field of second-generation TDDS has different technological approaches, like chemical enhancers, iontophoresis, and non-cavitational ultrasound. These kinds of delivery techniques have advanced small molecule delivery for localized, dermatological, cosmetic, and some systemic applications to improve in the field of clinical practice, but didn't impact optimum on the delivery of macromolecules [66–69].

The third or most advanced or modern generation of transdermal delivery system is focused and successful to target the stratum corneum more efficiently than the other generation systems to make a significant impact on TDDS. These systems have

shown their capability of stronger disruption of the stratum corneum, protecting the deeper tissues. The third-generation TDDS includes novel chemical enhancers, electroporation, cavitational ultrasound, microneedles, thermal ablation, and microdermabrasion. These modern techniques have shown to deliver macromolecules including therapeutic proteins and vaccines.

8.5.2 Passive Delivery of Protein Drugs

The average molecular weight of protein drug is 53 kDa and hydrophilic in nature, thereby antagonizing the Lipinski rules for efficient transdermal drug delivery [70]. So, the minimum diffusion of protein drugs occurs through skin. Except the chemical enhancer, all other approaches are mechanical approaches requiring force to mediate penetration of proteins by crossing the stratum corneum to the viable dermis layer of skin.

8.5.3 Iontophoresis

Iontophoresis is the medium to cross the physiological barrier, skin, especially the stratum corneum, by involving the transfer of ions using an electric current. The electric potential helps to increase the ionic movement across the skin [71]. Mechanistically, the dislodge of the total amount of drug and its transportation occurs depending on the concentration of the drug, ionic charges, intensity of applied voltage, and the surface area of the skin contacted with the device [72]. In this regard, it may be mentioned that when the current applies through the biological membrane, the system is known as transdermal iontophoresis. It consists of two opposite charge electrodes. These are applied on the skin surface that drives the drug molecule carrying a similar charge as an active electrode by repulsion across the skin. The amount of drug delivered by this method across the skin is directly proportional to the applied electric charge and duration of application [71]. Recently, the new technique of reverse electrodialysis (RED) with iontophoresis has emerged. This technique produces energy from the mixture salinity gradient of different sources of water (river or sea) through an ion exchange membrane [73, 74]. Recently, a cheaper, ecofriendly, disposable RED-iontophoretic chip has been prepared by Lee et al. [75].

8.5.4 Electroporation

In this method, short- and high-voltage pulses are used temporarily and reversibly after the outer membrane protein–lipid bilayer texture of the skin [76, 77]. This electrophoretic driving force forms electropores to increase the transdermal transport for peptides, large proteins, DNA, and even peptide vaccine through the intact skin [78].

8.5.5 Cavitational Ultrasound

The generation of cavitation in the stratum corneum layer by the application of ultrasound is known as cavitational ultrasound. The cavitational bubbles formed by the ultrasound, concentrate the energy of ultrasound and thereby enabled effects at the site of bubble activity [79, 80]. These bubbles oscillate and collapse at the skin surface, generating localized shock waves. This allows liquid microjets directed at the stratum corneum [81]. This is enough to alter the stratum corneum lipid texture to increase the permeability without damaging the deep tissue. This may last for many hours. The TDDS of lidocaine has been already approved [82]. A number of experiments has been performed regarding TDDS of insulin, heparin, and even tetanus toxoid vaccine [80].

8.5.6 Microneedles

This is another significant approach in TDDS. The very short needles called microneedles are generally used to pierce the stratum corneum selectively to increase the skin permeability of different small molecules, proteins, and medicines loaded in nanoparticles. The hollow microneedles have also been used to deliver insulin and vaccines in recent time. Few years back, an innovative approach has been evolved by inventing the water-soluble polymer that made microneedles, encapsulating various compounds within the needle matrix which gets dissolved within the skin within a few minutes/without leaving any medical waste [83]. Pretreatment with microneedles also makes faster diffusion of drug from patch into the systemic circulation [84]. Many animal studies have been done on the use of solid and hollow microneedles to deliver DNA vaccines against influenza, hepatitis B, Japanese encephalitis, and anthrax [85]. Recently, the 3D printing or solid free-form fabrication by employing a virtual computer-aided design model has shown its advancement in TDDS [86], but its use in clinical practice has not yet well been accepted and recognized.

8.5.7 Thermal Ablation

In thermal ablation technique, the portion of the skin is exposed to high heat for a fraction of a second to enhance the permeability of the stratum corneum. It selectively heats locally the skin surface to about 100 °C for micro- to milliseconds to generate the microscale perforations. In spite of the application of such heating, it does not produce any damage in skin or generate pain. Recent studies provide evidence of delivery of human growth hormone, interferon, and insulin by the thermal ablation TDD techniques [87–90].

8.5.8 Carrier Supportive Adjuvants

The use of carriers to deliver drugs transdermally has emerged as new therapeutic tools in TDDS. The nanostructural carriers are being used in these techniques to enhance the skin penetration capacity and deliver the drugs as nanomedicine. This emerges as an attractive nanomedicinal approach in TDDS.

8.5.9 Peptide Chain-Mediated Delivery

Interestingly, the use of peptides as a carrier of drug delivery has been shown to enhance the penetration properties via transdermal route. The peptides have the advantages of diversity, capability of targeting specific tissue or cells, and feasibility of conjugation with drug molecules [91]. There are two types of peptides, known as cell-penetrating peptides and skin-penetrating peptides.

8.5.9.1 Cell-Penetrating Peptides

The cell-penetrating peptides are amphiphilic in nature consisting up to 30 amino acids. Cohen-Avrahami et al. [92] have shown increased permeability about many folds of diclofenac sodium and celecoxib when conjugated with HIV-TAT (human immunodeficiency virus-trans activator of transcription) and catalase (TAT-CAT) at its nine arginine residue (9Arg-CAT) and sprayed on the skin surface. They penetrated with a potential therapeutic efficacy to the dermis layer [93]. Recently, a novel approach toward the development of transdermal peptide-based cancer vaccine has been initiated with OVA25-264 antigen with Antennapedia [94]. In nonmelanoma cancer and skin infection treatment, a cationic antibacterial protein, Melittin, was used for the abdominal stratum corneum for successful penetration into the dermis [95]. Gennari et al. [96] interestingly have shown an initial failure of coadministration of DRTTLTN (a synthesized heptapeptide) and unfractioned heparin (UFH) on human skin to enhance the skin penetration. These occur due to the high affinity of UFH for keratins. Further, when DRTTLTN was conjugated with N-3-(dimethylamino propyl)-N'-ethylcarbodiimide hydrochloride and sodium N-hydroxysulfosuccinimide, the penetration significantly increased many folds. In addition, the transdermal formulation using cyclosporine A-conjugated with octa-arginine to treat psoriasis has entered the clinical trial phase II and is a good example of cell-penetrating peptide-based delivery [97]. These observations document the capability of cell-penetrating peptides as a successful component of effective transdermal formulation in TDDS.

8.5.9.2 Skin-Penetrating Peptides

The skin-penetrating peptides have usual size range 1000–1500 Da. They are considered as safe and attractive nano-therapeutic alternatives as a carrier for both small and large drug molecules to transport across the stratum corneum layer [98]. They act as an adjuvant for enhanced drug delivery. The skin-permeating

peptides are to some extent ironic in passive transport of protein and peptide drugs [99–101].

8.5.10 Antimicrobial Peptide Magainin

Magainin is also known as pore (approx. 1 nm diameter)-forming peptide (23 amino acid-long) of lipid bilayers of the skin. It is an antimicrobial peptide isolated from the skin of *Xenopus laevis* (an African frog). It can electrostatically interact and has a net charge of +4 which helps it to bind to negatively charged phospholipid membranes [102]. Interestingly, this peptide cannot enhance skin penetration without a surfactant for providing its optimum effect [102, 103]

8.5.11 Different Formulations in Transdermal Nanomedicine

The use of transdermal route for the administration of drug is always a challenging task, for stratum corneum which acts as a natural skin barrier. Over the last few decades, several nanoformulation-based approaches have been reported for the effective transport of active drug molecules through the skin [104]. Among them, lipophilic carriers (<600 Da) such as nanoliposomes, solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC), aspasomes, and nanogel show ample opportunity to easily penetrate through the stratum corneum into the deeper skin layers [105]. These nanosize vehicles usually are capable to penetrate more easily through the stratum corneum to form active depot locally, act as a locally active depot, as a solubilization matrix or a rate-limiting membrane barrier for regulating the systemic bioavailability of drugs.

Sinico et al. [106] investigated both unilamellar and multilamellar nanoliposomes for the effective transdermal delivery of tretinoin. Accumulation of drug in various skin layers was detected. However, no evidence was noted for the intact penetration of liposomes through skin. The incorporation of ethanol in liposomal preparations (ethosomes) has been reported to act as an efficient permeation enhancer for the penetration of both hydrophilic and lipophilic compounds into the deeper epidermal and dermal layers of the skin. In this context, L´opez-Pinto et al. [107] compared the liposomal and ethosomal formulations for the dermal delivery of minoxidil. The permeation pattern from both the formulations was studied by implementing β-carotene as a probe. It was observed that the ethosomal formulations delivered the fluorescent probe into the skin much more efficiently than the conventional liposomes. The in vivo studies on the transdermal delivery of ethosomes also yielded promising results in contrast with that of the liposomal formulations [108]. The same group of authors also prepared ethosomes for the transdermal delivery of protease inhibitor, indinavir. The incorporation of ethanol into the liposomal formulation played a crucial role in reducing the particle size of the vesicles, thereby facilitating its penetration into the skin to a greater extent [109].

Solid lipid nanoparticles (SLNs) are another potential lipid-based nanocarriers for actively transporting the drug molecules through the stratum corneum into the deeper dermal layer. Kim et al. [110] developed cyclosporin A (CsA)-loaded solid lipid nanoparticles for enhanced skin permeation. The skin penetration ability of the SLN formulation was twofold greater than that of CsA-oil mixture. However, SLNs possess some drawbacks such as limited drug payload. To overcome the hindrance, nanostructured lipid carriers (NLCs) were designed for significant delivery via transdermal route [111, 112]. The potential of NLCs was also evaluated for combinatorial treatment with two drugs for the treatment of psoriasis. A hydrophilic drug (methotrexate) and a lipophilic drug (calcipotriol) were incorporated into the carrier system. The amount of permeation of methotrexate through skin was 2.4–4.4 times greater as compared to the control treatment. Moreover, very limited skin irritation was noted by administering NLCs by a topical route, thereby indicating its suitability for transdermal drug delivery [112].

Apart from the lipid-based nanodrug carriers, the other nanoformulations were also reported by various researchers suggesting the capability for successful transdermal delivery of active therapeutic compounds. The novel $CaCO_3$ nanoparticles were fabricated for the transdermal delivery of insulin. The prepared nanoparticles provided sustained drug delivery for a prolonged duration, thereby restricting frequent drug administration by painful injectable means [113]. The nanoparticulate formulations are also effective in reducing the dose-related adverse effects of several potent drugs. For instance, the topical administration of glucocorticoid drugs is accompanied by several adverse effects such as skin atrophy, cutaneous reactivity, and suppression of the hypothalamic–pituitary–adrenal axis. By incorporating the drugs within the nanocapsules, their harmful effects were reduced to a greater extent. Marchiori et al. [114] developed dexamethasone-loaded polymeric nanocapsules and incorporated them within the hydrogel system in order to improve the therapeutic efficacy of glucocorticoid in skin disorders such as psoriasis. Similarly, the nanocapsules for topical delivery are also found to be effective in improving solubility, chemical stability, and photostability of retinoids (e.g., tretinoin) [115]. The nanoparticles composed of amphiphilic block copolymers are also shown to penetrate the skin to a much greater extent than the conventional formulations. The D,L-tetrahydropalmatine (THP)-loaded amphiphilic poly{[-α-maleic anhydride-ω-methoxy-poly(ethylene glycol)]-co-(ethyl cyanoacrylate)} (PEGECA) graft copolymer-based nanoparticles were delivered via transdermal route by both appendages as well as epidermal routes [116]. The stimuli-responsive nanogels are another beneficial approach for smart delivery of drugs by transdermal route [117, 118]. The methotrexate-loaded nanogel constituted with co-polymerized N-isopropylacrylamide and butylacrylate was synthesized and characterized. An alteration in the temperature of nanogel during its penetration through skin promoted its de-swelling and expulsion of the drug in situ. Further, the addition of Na_2CO_3 enhanced the solubilization and release of the drug, thereby enhancing the concentration gradient, flux, and minimizing the production of prostaglandin PGE_2 [118].

Self-assembled aspasomes are another promising drug delivery carrier for transdermal delivery. Ghosh et al. [62] developed methotrexate aspasomes and loaded

Table 8.3 Different types of transdermal drug nanocarriers

Type of nanocarriers	Drugs used	References
Liposomes	Butyl paraben	[120, 121]
Transferosomes (ultradeformable liposomes)	Eprosartan mesylate, clindamycin, asenapine malate, diclofenac, cyclosporin A, levonorgestrel, insulin	[122–127]
Nanoparticles	HRP and β-gal with gold nanoparticles, ovalbumin antigen with silver nanoparticle	[128, 129]

HRP horseradish peroxidase

into hydrogel for the management of rheumatoid arthritis. When methotrexate-loaded aspasome hydrogel was transdermally applied for 12 days, significant reductions in rat paw diameter, SGOT, SGPT, TNFα, IL β, cartilage damage, inflammation, panus formation, and bone resorption as compared to arthritic control rats were observed. On the other hand, Sengupta et al. [119] developed nanosize particles of diclofenac diethylamine (~10 nm) in situ during the preparation of hydrogel for transdermal delivery. An improved skin permeation of drug was observed from the hydrogel (composed of PVA and carbopol 71G) in comparison to the hydrogel containing microsized drug particles (Table 8.3). Moreover, in vivo studies revealed that the systemic drug concentration in case of experimental hydrogel was much improved over the commercial hydrogel formulation.

8.6 Drug Targeting and its Importance

Targeted drug delivery is to deliver medication to a patient to provide enhanced drug concentration in the targeted parts of the body compared to the other parts [139]. Targeted drug delivery system offers several advantages such as prolonged and more localized drug action to the targeted site. The conventional drug delivery system dictates the absorption of the drug and its distribution in a nonspecific way nearly to all organs or tissues across, whereas the targeted drug release system releases the drug at a specific site. The targeted release system is distinctively advantageous as it provides in decreased frequency of the dosages taken by the patient, a more uniform effect of the drug, lessening of drug side effects, and reduced fluctuation in circulating drug levels. Nanocarriers used through TDDS have been used in different diseases (Table 8.4).

Drug targeting may be categorized into two common methods: active and passive targeting [140–142]. Active targeting dictates the delivery of drugs to a target cell type using specific interactions with some cell surface proteins or other specific molecules at the target site(s). Such types of interactions include antigen-antibody and ligand-receptor binding. On the other hand, some physical parameters such as magnetic fields, pH of the microenvironment of the tissue, and temperatures may be utilized for active targeting of drugs. Several vehicles that are useful for this methodology include antibodies, liposomes, transferrin, ferrite-containing, and thermo-responsive carriers.

Table 8.4 Transdermal nanocarriers in different diseases

Disease	Drugs	Nanocarriers	References
Psoriasis and atopic dermatitis	Methotrexate, cyclosporin A	Ethosomes	[108]
Psoriasis and acne	5-Aminolevulinic acid, psoralen, tretinoin	Ethosomes, liposomes	[130–132]
Anti-inflammatory, anti-apoptotic, antioxidant	Curcumin	Propylene glycol-containing liposomes, ethosomes, liposomes	[133, 134]
Skin cancer	Paclitaxel	Ethosomes	[135]
Rheumatoid arthritis	Meloxicam	Transferosomes, liposomes, methosomes	[136, 137]
Diabetes mellitus	Insulin	Transferosomes	[138]
Parkinson's disease	Trihexyphenidyl-HCl	Ethosomes	[137]

In the case of passive targeting, the physical and chemical properties of carrier or vehicle systems boost the target/nontarget ratio of the amount of drug delivered by adjusting these properties to the physiological and the histological characteristics of the target and nontarget tissues, organs, and cells. For this type of drug delivery technique, several vehicles are used and they include synthetic polymers, some natural polymers (such as albumin), liposome, nanoparticles, microparticles, and polymeric micelles.

8.7 Nanoformulation-Mediated Site-Specific Delivery of Drug Through Transdermal Drug Delivery

Successful skin penetration of a drug or a gene to its site of action has to face different challenges such as lack of proper drug delivery system, non-biocompatibility, immune system-mediated rejection, and unstable pharmacokinetic profile. To overcome these challenges, various physical and nonphysical techniques are used for delivering the therapeutics at the target site during transdermal drug delivery system.

8.7.1 Physical Techniques

Physical methods are mostly based on ablation of the stratum corneum layer or application of an external force to facilitate drug penetration. Some successful attempts have been made with the application of physical methods such as ultrasound, laser, electroporation, iontophoresis, microneedles, etc. [143].

8.8 Nonphysical Techniques

Currently, nanocarriers have been tried and tested to overcome the skin barrier to achieve optimum transdermal permeability and site-specific delivery of their therapeutic cargo. Nanocarriers have the potential to encapsulate different types of drug targeting for their tiny size and superior surface chemistry property. Moreover, with their tunable surface layer property, a controlled drug release pattern can be maintained. Thus, they are being investigated to enhance drug penetration across the skin to deliver drugs at the specific desired part of the body.

8.8.1 Site-Specific Delivery of Drug for Cutaneous Disorder

8.8.1.1 Melanoma

Melanoma is a kind of skin cancer that originates in melanocytes and categorized with the development of malignant cells in the skin tissue. Existing topical therapy with semi-solid formulation of 5-fluorouracil (5-Fu), diclofenac, imiquimod, and photodynamic therapy (PDT) [144] comprise poor penetration pooled with unsatisfactory drug concentration of 5-Fu at the target sites. Misak et al. [145] developed target-specific magnetic NP composed of albumin, PLGA, and 5-Fu, which showed superiority in treating skin cancer over the previous one. A recent study also demonstrates that the cationic liposomal formulation of curcumin and STAT3 siRNA with iontophoresis application significantly ($p < 0.05$) inhibited cancer cell growth and death in contrast to free drug molecules [146]. Niu et al. [147] studied the delivery of plasmid DNA (pDNA) intended for melanoma treatment by means of a peptide and cationic poly-(ethyleneimine) attached with gold NP (AuPT), which packed down pDNAs into cationic complexes [147]. These AuPT/pDNAMi221 particles were seen to be efficient carriers of pDNAs and can be utilized as a prospective novel drug delivery system to specifically deliver the therapeutics to melanoma cells overturned not only progression of melanoma but also metastasis-related advanced melanoma [147].

8.8.1.2 Psoriasis

Psoriasis is characterized by lofty itchy plaques along with silvery scales and red lesions on skin surfaces. Although the exact pathology following psoriasis is still not very clear, but the known part is that a wide range of inflammatory cells such as T-cells and dendritic cells are responsible for hyperproliferation of keratinocytes. Thus, demarcation of keratinocytes and skin barrier deformities occur in psoriasis [148]. Nanoparticles conjugated with cell targeting moieties in TDDS have been investigated comprehensively to treat psoriasis. Boakye et al. [149] recently formulated a pyrrolidinium conjugated lipid nanocarrier (CYnLIP) drug delivery system. Pyrrolidinium is used to interrupt skin barrier function, where permeation enhancing lipid nanocarriers are designed successfully to encapsulate erlotinib and IL36α siRNA to overcome its delivery constraint posed by aqueous siRNA and erlotinib. The designed nanoformulation exhibited around 40-fold superior skin

retention than the conventional delivery system [150]. This novel nanocarrier is offering a smart dual drug delivery approach for psoriatic plaques in mice for better therapeutic response compared to available conventional formulations consisting of individual agents by reducing dermal cytokine infiltration [149]. Another recent study showed that encapsulated lipophilic curcumin into PLGA NP to deliver the (Cur-NP) and had a superior penetration property than curcumin gel [151]. In vivo studies revealed that the Cur-NP delivery system reduced the symptoms of psoriasis, as well as the development of plaque psoriasis, erythema, and skin thickening [151] due to their preferential delivery of the therapeutics to its target sites.

8.8.1.3 Alopecia

Androgenic alopecia (AGA) is a general form of hair loss that happens when a certain malfunction in immune system attacks hair follicle or it may be brought on by severe stress. Potential of nanoparticles through targeting hair follicles for the therapy of alopecia was explored. Among the two types of 5α-reductase, Type 2 5α-reductase activity has been observed in the hair follicle of AGA patients. Type 2 5α-reductase inhibitor finasteride and hydrophilic compound minoxidil are responsible for the proliferation of dermal papilla cells (DPC) by facilitating a vasodilatory effect in the hair follicles for treating alopecia [152]. Research on delivery of FNS by TDDS is very much encouraged since the oral administration of FNS has a variety of side effects like impotence and erectile dysfunction, etc. A study was carried out by Roque et al. for developing FNS-loaded PLGA NP and incorporation of these NP in different types of topical formulation (shampoo, lotion, and solution) [153]. No toxic effects were observed in the *S. cerevisiae* model. In another study, Gomes and his associates formulated a nanostructured lipid carriers (NLCs) consisting of both minoxidil and FNS with a mean size of 200 nm by means of ultrasonication method [154]. A zeta potential value of approx. −30 mV was observed and a storage period over 28 days showed no significant deformation in the nanoformulation. Although the nanoparticles had desirable physical characteristics, an inadequate quantity of NP was available at the target tissue. For this reason, Hamishehkar and his associates performed another study where flutamide-loaded SLN was examined in exercised rat skin and male hamsters in vitro and in vivo, respectively [155]. It was concluded that with the SLN formulation, drug is localized more in the skin with lesser quantity into the receptor compartment in vitro as compared to the hydroalcoholic solution [155]. In vivo results after 45 days showed that the flutamide-loaded SLNs augmented the number of hair follicles in contrast with hydroalcoholic solution in hamster, demonstrating that flutamide-loaded SLN has potential for the treatment of androgenic alopecia due to its preferential delivery at the target site with a desirable concentration [155].

8.8.1.4 Wound Healing

The complex process of cutaneous wound healing is regulated by following four major phases: inflammation, cellular proliferation, hemostasis, and remodeling [156]. Deregulation to any of these phases ultimately results in chronic wounds with the consequence of delayed wound healing, which are commonly associated

with diabetes, obesity, and vascular diseases. MicroRNAs (*mi*RNAs) are noncoding RNAs accountable for post-transcriptional regulation of gene expression and have a role in wound healing [157]. In a study, Ghatak et al. [158] developed antihypoxamiR functionalized gramicidin lipid nanoparticles (AFGLN) which showed to model decrease miR-210 levels in murine and provide augmented ischemic wound healing [158]. In another recent study, Xiao et al. [159], have formulated folic acid-modified copper-based metalorganic framework nanoparticles (F-HKUST-1) and examined their cytotoxicity, in vitro cell migration and dermal wound healing rates in diabetic mice. The cytotoxicity was found to decrease due to the insertion of folic acid into the NP, which also offered the slower release of copper ion. Thus, it encouraged the rejuvenation of dermal tissue [159]. FHKUST-1 NPs showed much accelerated wound healing process by promoting epithelial tissue revival as well as renewal. Krausz et al. [160] encapsulated curcumin in the course of a sol–gel-based polymerization technique, which is a wet-chemical method providing hydrolysis and consequent polycondensation to make a gel-like substance on skin and offered superior wound closure. Thus, site-specific delivery of drug using transdermal route could also be useful in the wound healing process.

8.8.2 Treatment of Non-Cutaneous Disorders

A myriad of research works is being published mentioning of the encapsulation of drug or gene into phospholipid-based vesicles through transdermal route for the treatment of systemic diseases. The following examples are not exhaustive.

8.8.2.1 Rheumatoid Arthritis

Non-steroidal anti-inflammatory drugs (NSAID) are the most popularly prescribed drug in pain management as the first-line therapy. But their numerous side effects associated with systemic administration pushed researchers to develop formulations for transdermal delivery systems to be applied directly on the skin. Accordingly, numerous approaches are being investigated on NSAID skin delivery, such as transfersomes (TFS), liposomes, and menthosomes (MTS). These different approaches were studied and compared by Duangjit and his coworkers as carriers for meloxicam (MX) [136]. The study suggested that ultra-deformable and deformable liposomes (MTS and TFS) had a potentiality as transdermal drug delivery carriers for MX. Glycerosomes, glycerol-containing liposomes, were published in the literature as a competent delivery system for dermal and transdermal delivery of diclofenac [161].

8.8.2.2 Parkinson

Trihexyphenidyl HCl (THP)-loaded ethosomes were formulated as a novel approach for the treatment of Parkinson's disease [137]. THP poses a short half-life and therefore, it requires a frequent dosing that causes severe side effects. THP is an ionizable molecule with partial skin permeation. In this circumstance, Dayan and Touitou tested ethosomal formulation of THP and showed a percutaneous flux

87 and it was 4.5 times higher than those of liposomes and hydroethanolic solution, respectively. Skin retention of THP upon 18 h exposure also increased compared to hydroethanolic solution [137].

8.8.2.3 Diabetes Mellitus

Insulin-loaded transfersomes were shown to deliver the therapeutic protein through the intact skin with a reproducible drug outcome which was very much similar to that of subcutaneous insulin [138]. Thus, transfersomes stand for promising noninvasive systems for insulin delivery improving patient compliances. A glucose-mediated insulin delivery system was found to be extremely suitable for diabetes diagnosis dependent on the concentration of blood glucose in the body. Zhang et al. [162] formulated a novel microneedle (MN) delivery device incorporated with insulin and H_2O_2-responsive mesoporous silica nanoparticles (MSNs) to attain fast and painless administration through skin.

A number of transdermal nanoformulations are commercially available in the market or are under clinical development. Moreover, various patents are also available in this field. The different transdermal nanomedicine in this regard is tabulated in Table 8.5.

8.8.3 Advanced Cell Targeting by CPPs (Cell Penetrating Peptides)

The discovery of CPPs was studied with TAT, a trans-activating factor belonging to HIV virus and such peptides have been insighted for transdermal delivery. Assorted types of CPPs have been attached to different types of (oligo) nucleotide or gene carriers to boost their competence to internalize into the cells. Although CPPs have been studied widely, molecular action pathway is not fully understood yet. Boisguérin et al. [173] discussed the application of CPPs in gene delivery in detail. CPPs have been applied also in amalgamation with the newly revealed gene delivery systems such as Transcription activator-like effectors nucleases (TALENs) [174] and CRISPR/Cas9 system for the idea of genome editing [175]. It has been demonstrated that liposomes conjugated to an arginine-rich CPP have improved permeability and afterward proved drug delivery superiority in a transdermal drug delivery experiment [173]. This type of CPP can even boost the skin permeation of naked drugs. In a recent study, a novel type of CPPs (IMT-P8) was attached to KLA (a pro-apoptotic peptide). The KLA peptide conjugated with CPP had appreciably higher skin permeation [176]. CPPs can raise the transdermal permeation rate of oligonucleotides. For example, TAT was conjugated to gold/PEI particles to increase the transdermal gene delivery for a topical application [147]. Nowadays, another group of peptides (SPACE peptides) has been investigated through phage display, which is getting enormous attention due to its skin penetration competence. SPACE peptides have effectively been applied for transdermal drug delivery along with the transdermal oligonucleotide delivery [177, 178].

Table 8.5 Various patented products/products in clinical trials/commercial products on transdermal nanomedicine

Drug/active ingredients	Delivery system	Status (patents/ clinical trials / commercially available)	Outcome/ indication	References
Estrasorb^{R}; (Novavax, Inc., Malvern, Pennsylvania; Esprit Pharma, East Brunswick, New Jersey; Graceway Pharmaceuticals, Bristol, Tennessee)	Micellar nanoparticle estradiol emulsion (MNPEE)	FDA approved	An alternative to current estradiol transdermal formulations with an improved profile of local unwanted effects (i.e., skin irritation, dryness).	[163]
Insulin delivery	Using the MN system was conducted and completed in 2013	A phase III clinical trial	The primary endpoint was determination of Tmax of insulin	[164]
ADAM zolmitriptan	Microneedles	A phase III Clinical trial	Migraine	[165]
Triamcinolone acetonide	Suprachoroidally administered microneedles	A phase III Clinical trial	Macular edema associated with non-infectious uveitis.	[166]
Chinese medicine	Ethosome gel patch	CN103536700 A (patent)	Strong analgesic action	[167]
Nano repair Q10 cream and nano repair Q10 serum (Dr. Kurt Richter Laboratorien GmbH, Berlin, Germany)	Lipid nanoparticles	Commercially available cosmetics	Antiageing	[168]
Photoactive drug component	Targeted delivery of nanoparticles to skin surface	WO 2015031189 A1, 2015 (patent)	Improved drug transport through skin in atopic dermatitis	[169]
Antioxidants and anti-inflammatory agents	Chitosan nanoparticle for skin targeted drug delivery	WO 2015072846 A1, 2015 (Patent)	Treating atopic dermatitis	[170]
Paclitaxel	Ethosome gel	CN102579323, 2012 A (Patent)	Improved percutaneous permeation	[171]
Acyclovir	Ethosome	CN102133183, 2011 A (Patent)	High stability and narrow particle size distribution	[172]

8.9 Conclusion and Future Perspectives

The technology of nanomedicine has emerged as a novel platform which can be explored for the transdermal delivery of various potent drugs and other bioactive molecules. The advancement in the development of drug nanocarriers with more effective therapeutic potential and less side effects, and significant implication of nanomedicine in drug delivery can be utilized for transdermal delivery as it could help in overcoming the skin barrier and penetration of drug carrier into the deeper layers of skin and facilitate the drug into the systemic circulation for targeted and prolonged action. It is well known that the transdermal route is not suitable for administration of all class of drugs, yet the current research in nanomedicine promises an incredible hope and future of therapeutics via transdermal route. The transdermal nanocarriers have been reported to incorporate hydrophilic as well as lipophilic drugs for the treatment of many chronic diseases such as diabetes, cancer, neurological diseases, various dermatological disorders, and many more. For diseases located in the deeper tissues such as the brain, or in case of carcinoma, a specific targeting moiety coupled with the nanocarriers needs to be explored. The noninvasive transdermal nanoformulations are more advantageous to conventional means since they provide better patient-compliance and also reduce the frequent dosing of drugs. Currently, a number of these carriers is under extensive exploration for elucidating their transport path and mechanism underlying their fate in the human body. In the near future, much more investigations are warranted for the development of more efficacious transdermal nanoformulations for clinical use. Moreover, the safety profile of transdermal nanomedicine needs to be carefully investigated before their transition from laboratory scale to patients.

Acknowledgement The authors are grateful to the Department of Pharmaceutical Technology, Jadavpur University, Kolkata, for providing laboratory space to conduct such theoretical research work.

Conflict of Interest Authors declare that there is no conflict of interest. All figures and tables are original and self-made.

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Multifunctional Mesoporous Silica Nanoparticles for Biomedical Applications

9

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Abstract

Mesoporous silica nanoparticles (MSNs) are outstanding nanocarriers for drug delivery, imaging, and other biomedical applications. MSN features a well-defined mesoporous structure with tunable pore size, pore volume, high surface area, and high drug loading capacity and offers external and interior surfaces for functionalization over conventional materials. Functionalized MSNs respond upon stimuli, such as pH, redox, light, ultrasound, magnetic, enzyme, or their combinations, which have transformed their applications in biomedical engineering. Their unique mesoporous structure is capable of delivering an assortment of therapeutic regimens to alleviate the progress of diseases including cancer and inflammatory responses. In this chapter, we highlight recent advances in biomedical applications of multifunctional MSN including (1) MSN-based therapeutic delivery; (2) MSN-based bioimaging applications; (3) MSN-based materials for tissue regeneration; and (4) MSN-based antimicrobial against infections.

Keywords

Mesoporous silica nanoparticles (MSNs) · Functionalized MSNs · Bioimaging · Therapeutic delivery

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M. K. Das, Y. V Pathak (eds.), *Nano Medicine and Nano Safety*,
https://doi.org/10.1007/978-981-15-6255-6_9

9.1 Introduction

Mesoporous silica nanoparticles (MSNs) have been established as innovative inorganic nanoplatforms with substantial attention in biomedical applications [1–3]. Advances in the control of architecture and surface functionalization of inorganic mesoporous silica-based nanomaterials have opened innovative potential to biotechnological and biomedical applications. Although a plethora of nanomaterials offer optimum physio-chemical properties, MSNs stand out owing to their exemplary physio-chemical properties that include controllable size, morphology, composition, tailorable pore size, pore structure, large surface area with dual functional surfaces (internal cylindrical pore surface and exterior particle surface), dispersibility, surface chemistry, etc. [4, 5]. In addition to physio-chemical properties, their applicability either in drug delivery or in diagnostic application largely depends on the porosity that accounts for the delivery of cargo without leakage to the target biological site than other nanoparticles such as polymeric or liposomal nanoparticles. The tunable pore size can even accommodate large molecules, DNA, or proteins. Their attention to biomedical application relies on properties including excellent in vitro and in vivo biocompatibility, controllable degradability under biological surroundings, and rapid clearance and excretion [6–8]. MSNs are versatile with respect to self-transformation to core shell MSNs when other nanomaterials, such as gold, silver, semiconductor quantum dots (QDs), upconversion nanoparticles (UCNPs), and magnetic nanoparticles (MNPs), are sheltered and upgrade themselves to hybrid MSNs offering multifunctional properties (Fig. 9.1).

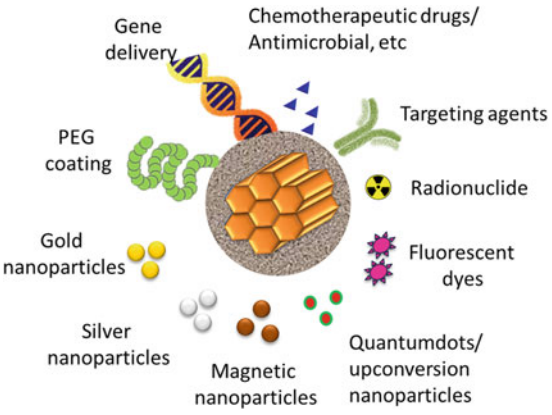

Fig. 9.1 Multifunctional MSNs

9.2 Potential Biomedical Application of MSNs

9.2.1 Multifunctional MSN for Delivery of Therapeutic Agents

MSNs present several advantages to function as perfect nano-based drug carriers owing to their exceptional properties including tunable pore size, superior loading capacity, chemical stability, large surface area, surface functionality for specific cell targeting, biocompatibility, and controlled release of cargo with or without stimuli. The large surface area and controllable porosity of MSNs have been exploited extensively to encapsulate wide-ranging bioactive molecules including chemotherapeutic drugs, peptides, enzymes, siRNA, DNA, and growth factor for different biomedical applications. MSNs offer researchers the flexibility to design them for drug encapsulation/release/delivery applications with the prospect to functionalize the surface with targeting moieties for recognition of the target site where the encapsulated drug exerts its action without being released elsewhere, capping ligands to avoid the early diffusion of loaded drugs and with stimuli-responsive groups that can be used to trigger to control the release of loaded bioactive components.

The cargo (therapeutics) loading approaches in MSN depend on the pore size, surface area, pore structure (engineering framework of MSNs), and response to signals either internal (pH, redox, and enzymatic stimuli) or external (light, heat, magnetic, and ultrasound stimuli). MSNs can accommodate drugs (hydrophobic or hydrophilic drugs) through noncovalent bonding and without the involvement of pore capping [9, 10]. However, leakage of drug limited their application and approaches to overcome drug leakage strategies were adopted. The porous outer surface was engineered for controlled release of cargo via functionalization with molecular or supramolecular nanovalves/nanomachines, nanoparticles, or coating with protein, polymer, or lipid. The reported nanomachines that blocked the diffusion of cargo include rotaxane, pseudorotaxane, molecular nanovalves, and cleavable molecular bridges and triggered the release of cargo upon either external or internal stimuli [11–14]. Nanoparticles (gold nanoparticles, QDs, and MNPs) with the diameter ranging from 2 to 10 nm were grafted via chemical bonds onto porous MSNs and controlled cargo release from MSNs [15–17]. The exterior surface of mesoporous MSNs was functionalized with proteins/polymers or lipid coatings, and the release was based on stimuli. Bovine serum albumin was coated over MSNs, and the change in pH destabilized the electrostatic interaction that triggered the release of cargo [18]. Similarly, when insulin was used as coating onto MSNs, glucose concentration determined the release of cargo from MSNs [19]. Polymer-coated MSNs performed the release of cargo by the change in confirmation of structure or degradation of the polymer upon trigger (heat, light, pH, etc.) [20–23]. Lipid-coated MSNs released cargo upon endocytosis that supported the disruption of lipid coating from MSN [24].

pH-responsive controlled drug delivery systems have been widely studied as human body reveals differences in pH. In tumor cells owing to the high glycolysis rate, the pH is acidic than normal tissue. The pH value of cellular compartments in

Table 9.1 Stimuli responsive for drug delivery using MSNs

Type	Mechanism	Reference
pH responsive	Acidic pH in the endosome and lysosome	[25, 26]
	Acid cleavable linker/bond (acetal bond and acetal linker)	[27–34]
	Polymer gatekeepers	[35–37]
	pH responsive self-destructive polymers	[38]
Redox responsive	Cleavage of disulfide bond	[39–46]
Enzyme responsive	Peptide sequence cleavage Enzyme degradable polymer	[47, 48] [49–52]
Magnetic responsive	Temperature increase upon alternating magnetic field	[53, 54]
Light	UV-vis (photoresponsive polymer gatekeeper and photoresponsive linkers) NIR absorbing materials, and NIR to thermal conversion	[55–58] [59–66]
Ultrasound responsive	Ultrasound-sensitive material and cavitation	[67–69]

cancer cells is further lower in endosomes ranging from 5.5 to 6 and in lysosomes from pH −4.5 to 5. The pH variation is beneficial for the development of pH-responsive MSN-based nanosystems [25, 26]. In most of the studies, pH-sensitive linkers such as acetal, hydrazine, ester, or boronate ester bonds are cleaved under low pH for developing pH-responsive MSNs [27–34]. pH-sensitive polymer shells are also studied, which undergo conformational transformation with the variation of external pH owing to the change in charge, solubility, etc. [35–38] (Table 9.1).

Redox potential existing between cellular microenvironments (extracellular and intracellular) and between healthy and tumor tissues has been recognized as internal stimuli for drug release. Glutathione (GSH) protects cells from the damage of reactive oxygen species (ROS), and the intracellular concentration of GSH is 10^3 times than the extracellular matrix. However, in cancer cells, GSH concentration is nearly four times than healthy cells and, therefore, in cancer cells, GSH serves as an internal trigger for drug release. The disulfide bond-based surface modifications with bulk gatekeepers are generally used in redox-responsive drug release. As disulfide bonds are sensitive to GSH, design of nanoconjugates with disulfide linkages can display redox sensitive drug release. GSH-sensitive linkers are often used to attach nanomaterials or other molecules (QDs, Au, magnetic nanoparticles, peptides, cyclodextrin, cytochrome c, etc.) onto the surface of MSN [16, 39–46] (Table 9.1). At the target site, the higher concentration of GSH results in the cleavage of disulfide bonds and opens the pores, resulting in the release of drugs. Redox-responsive MSNs for delivery of chemotherapeutic drug are a promising strategy in cancer therapy for the distribution of local concentration of drugs without leakage.

The progression of cancer is often associated with overexpression and dysregulation of many enzymes, including esterases, matrix metalloproteinases (MMPs), and others [2]. Also, several enzymes are being expressed at a high level in tumor tissues than in normal cells. Therefore, endogenous enzymes expressed in

tumor cells are being exploited as an internal trigger for drug release. Owing to their selectivity and specificity, enzyme-responsive MSNs have been designed. Coating of MSNs with gatekeepers comprising protease-sensitive sequences or enzyme-sensitive linkers is often employed to achieve enzyme-responsive release [47–52] (Table 9.1). In most studies, the pores of MSNs are caped with peptides or lipids that are removed in the presence of enzymes. MMP2-sensitive linkers are used as triggers for drug release owing to the enzymatic hydrolysis efficiency [50].

Extrinsic stimuli (magnetic field, light, or ultrasound) are employed to activate the discharge of drugs upon demand from MSNs. The major advantage of external-triggered drug release systems is the potential to turn on and off based on demand and simultaneous diagnostic applications facilitating multifunctional properties to MSN-based nanocarriers. Magnetic-responsive MSNs are extensively studied owing to their intrinsic magnetic behavior to support magnetic resonance imaging (MRI) and magnetic hyperthermia (heat generation) in the presence of alternating magnetic fields (AMFs) for the controlled release of loaded drugs. The magnetic particles have the potential to transform their magnetic energy into thermal energy owing to Neel and Brownian effects. The heat generated by MNPs in the presence of AMF also serves as a trigger for on-demand drug release. Among the magnetic nanoparticles, superparamagnetic iron oxide nanoparticles (SPIONs) are widely studied magnetic nanoparticles. In most of the studies, superparamagnetic Fe_3O_4@SiO_2 functions as a core that is surrounded by mesoporous silica shell, which in turn loaded with drugs [53, 54]. Other possible combinations of MSN and MNPs are the hollow structure comprising MNPs as core and thin mesoporous silica layer as shell, which exhibits a higher saturation magnetization value than with the intact middle silica layer; MNPs embedded in mesoporous silica nanospheres; and MNP-capped mesoporous silica through either chemical linkers or polymers [70–74]. The accumulation of magnetic MSNs and release of drug from MSNs in the target site (tumor) enhance upon alternative magnetic field. The parameters, including intensity of magnetic field, concentration of MNPs, and distance of magnetic field from the cells, can affect the buildup of nanoparticles and release of chemotherapeutic drugs that were loaded in a controlled manner without being affected by the normal healthy cells. Apart from SPIONs, MSNs combined with zinc-doped iron oxide nanoparticles, manganese ferrite nanoparticles, have also been studied as alternative magnetic-responsive drug delivery nanosystems [75–77].

Light as a trigger for the release of drug cargo has been widely studied owing to its simplicity of operation, minimum invasiveness, and remote spatiotemporal control. Light-triggered on-demand drug release can be achieved by choosing a definite wavelength from ultraviolet, visible, or near-infrared (NIR) regions. UV-vis and NIR are commonly employed for drug release from MSNs. UV has been employed for releasing cargo owing to its potential to break bonds and for triggering chemical modifications in the drug molecules or polymers [78–81]. Although UV trigger is widely studied, the low penetration property and damage to the tissues associated with UV limit the in vivo application. A possible alternate is the visible light that can release the cargo owing to its safety and high tissue penetration than UV light [55]. Photosensitizers, such as porphyrin-capped MSNs and chlorin e6-doped

MSN nanorods, have been studied for the release of drugs and singlet oxygen from MSNs upon light activation [56–58] (Table 9.1). NIR is the ideal wavelength for drug release and for deep tissue imaging owing to its minimum autofluorescence, tissue scattering, and deep penetration. NIR-absorbing nanomaterials, such as single-walled carbon nanotubes (SWNTs), gold nanoparticles, QDs, and UCNPs, convert adsorbed light energy into thermal energy for release of therapeutic payload from NIR-triggered drug delivery nanosystems [59–66] (Table 9.1).

Ultrasound (US) is yet another potential external trigger for stimuli-responsive drug release owing to its advantageous properties including noninvasiveness, non-ionizing profile, safety, portability, spatiotemporal control, real-time monitoring, deep tissue penetration, and cost-effectiveness. Tissue penetration depth can be controlled by effectively tuning the parameters such as cycles, frequency, exposure time, etc. High intensity focused ultrasound (HIFU) can deeply penetrate into the body that permits local therapy in the affected site, thus eluding side effects to healthy normal tissues. Upon US trigger, physical effects, such as cavitation, heat, pressure difference, and fluid streaming, may occur [82]. These thermal, mechanical, and chemical effects from the US have been studied as a trigger for designing various types of US responsive nanocarriers. Microbubble (MB)-encapsulated MSNs were studied for delivering drug-loaded MBs upon US image monitoring [67]. US-trigger damage of MBs permits the drug to accumulate at the target site through the vascular endothelial barrier by cavitation process, thus augmenting the drug delivery efficiency [68, 69].

The major challenges in nanotechnology-based cancer therapy are the high specificity, effective cellular uptake, and intracellular release of chemotherapeutics and the differentiation of healthy normal and diseased cancer cells. MSNs have been widely emphasized in nanomedicine owing to their large surface area and rich multifunctional surface chemistry with mesoporous channels for loading chemotherapeutics. The MSN nanostructure offers the platform for efficiently targeting them with targeting ligands such as antibodies, peptides, proteins, polysaccharides, aptamers, and small molecules [83]. MSNs functioned as an excellent nanoplatform for the loading and controlled release of various chemotherapeutics including doxorubicin [84–90], methotrexate [91, 92], 5-Fu [93–95], camptothecin [13, 96, 97], and other anticancer therapeutics RNA molecules [98–102].

However, in cancer therapy, another major challenge is the capability of tumor cells to acquire resistance. Novel therapeutic options are being explored with MSNs to overcome drug resistance by developing MSN capacity for combination therapy—a therapeutic cocktail of chemotherapeutics such as multiple drugs or with chemotherapeutics and siRNA. Combination therapy involves the application of two or more bioactive chemotherapeutic drugs with different solubility, hydrophobicity, and pharmacological actions that can synergistically reduce cell viability in cancer cells. Co-delivery of multiple drugs generating synergistic therapeutic effects was also performed by various research groups. However, parameters such as the ratio of drugs in combination and delivery kinetics can generate synergistic outcome

(resulting in augmented cytotoxicity) or an antagonistic effect (reduced cytotoxicity) [103].

The approach with drug/siRNA cocktail recipe depends on the destruction of cancerous cells and silencing the over-expression of drug efflux transporters for multidrug resistant cancer cells [104, 105]. MSNS are designed by loading anticancer drugs inside the mesopores and siRNA on the external surface. Nel and Zink demonstrated the knockdown of P-glycoprotein (Pgp) gene involved in multiple drug resistance protein 1 (MDR-1). MSNs were modified with polyethylenimine (PEI), and siRNAs were electrostatically bound to PEI polymers. The co-delivery of siRNA and doxorubicin resulted in the downregulation of Pgp and reduced IC50 by a factor of 2.5 compared to free DOX or Dox-loaded MSN [105].

9.2.2 Biomedical Imaging with Multifunctional MSNs

MSNs have garnered substantial attention in biomedical research owing to their distinctive features including tunable mesoporous structure, large surface area, tunable pore size, and large pore volume. These exclusive properties make MSNs to be designed as theranostic agents for simultaneous diagnosis and therapy by encapsulating and loading therapeutic and diagnostic agents, deliver them to the preferred site, enable the release of therapeutics in a controlled manner, and monitor them. Owing to the exceptional characteristics of MSNs, imaging moiety integrated with MSNs functions as a reliable diagnostic system for bioimaging with superior stability and supporting several imaging modalities. MSNs can support single-mode imaging such as optical imaging, positron emission tomography (PET), computed tomography (CT), magnetic resonance imaging (MRI), ultrasound imaging, and multimodal imaging for prompt diagnosis of various disorders including cancer.

9.2.2.1 Optical Imaging with MSNs

Optical imaging is one of the effective imaging modalities among several other imaging techniques owing to the high resolution and sensitivity of fluorescence compared to other imaging modalities. Optical imaging spans the spectrum ranging from visible to NIR utilizing both fluorescent organic dyes and fluorescent inorganic nanoparticles. Fluorescent dyes (including conventional fluorescent and NIR dye) and nanomaterials (QDs, UCNPs, etc.) have been developed for labeling cells [106–110].

Although fluorescent dyes are excellent imaging agents, their photobleaching, limited tissue penetration, and autofluorescence are a few major disadvantages that hinder their applications as diagnostic agents [111, 112]. In the case of fluorescent nanoparticles, most of them are designed to exhibit high quantum yield and resistance to photobleaching; however, their inherent toxicity arising from synthetic parameters owing to the use of hydrophobic organic solvents and ligands and their poor solubility limit their in vivo application. MSNs function as excellent hosts for these fluorescent dyes and nanoparticles owing to their tunable porous structure and overcome the potential limitations associated with them.

Physical adsorption and covalent conjugation are the most commonly studied strategy for the conjugation of dyes to MSN. Squaraine dye was adsorbed into the mesopores and graphene was used as wraps to protect dyes, which prevented the leaching of dye and attack of nucleophile on the dye [113]. Covalent conjugation, on the other hand, is the facile and robust approach for conjugation of dyes to MSNs. Owing to the versatility of MSNs, dyes can be conjugated either on the silica matrix or on the surface of MSNs [8, 114–120]. As mentioned previously, nanomaterials offer excellent brightness, high absorption coefficients, quantum yield, photostability, and resistance to photobleaching. Encapsulation of them in mesoporous nanostructures decreases their inherent toxicity, which enables them to be used for imaging applications. MSN-embedded hydrophobic inorganic nanoparticles were demonstrated by Kim and coworkers [121]. Ligand-stabilized QDs were transferred to the aqueous phase via the microemulsion technique, and stabilized nanoparticles were subjected to sol-gel reaction for coating MSNs to develop core/shell MSNs [122]. Dispersity in aqueous medium of core/shell MSNs was further enhanced by PEGylation [123]. A similar approach was adopted for the synthesis of core/shell-type UCNP/MSNs [124–128]. Dual-model imaging using NIR and MRI with $NaYF_4$:Tm/Yb/Gd UCNPs as core and MSN as shell ($NaYF_4$:Tm/Yb/Gd@MSNs) was reported by Liu and coworkers [126]. Administration of $NaYF_4$:Tm/Yb/Gd@MSNs at the tumor site in mice demonstrated a significant upconversion luminescence signal.

9.2.2.2 Positron Emission Tomography (PET)

PET bioimaging offers superior sensitivity with molecular-level details of a living system with positron-emitting radioisotopes [129]. Owing to noninvasive nature and reduced background interference, superior sensitivity PET is utilized for assessment biodistribution and pharmacokinetics in tumors. Frequently used radioisotopes for PET imaging include ^{11}C, ^{18}F, ^{15}O, ^{64}Cu, ^{68}Ga, ^{99}mTc, and ^{111}In and are often labeled with chemotherapeutics, targeting ligands, and functional components. Despite their advantages with regard to sensitivity and enhanced tissue penetration, their long-term stability raises concern for their in vivo applications. The general strategy for the integration of PET isotopes into nanocarriers is to attach the isotope through metal chelators. Most commonly employed chelators for PET are 1,4,7,10-tetraazacyclododecane tetraacetic acid (DOTA) and 1,4,7-triazacyclononane-N,N′, N″-triacetic acid (NOTA). ^{18}F (^{18}F-labeled fluorodeoxyglucose) is one of the widely used clinical PET-active isotopes to track high glucose-consuming brain cells, kidney cells, and cancer cells. However, its shortened half-life (109.8 min) and the necessity of extended circulation time for their target cell or organ uptake limit in vivo PET study [129, 130].

Kim and coworkers developed ^{18}F-labeled MSN based on strain-promoted alkyne azide cycloaddition (SPAAC) conjugation of aza-dibenzocyclooctyne (DBCO) and demonstrated pre-targeted PET imaging by bioorthogonal covalent ^{18}F-labeling [131]. The researchers synthesized DBCO-based PEGylated MSNs (DBCO-PEG-MSNs) and conjugated these with ^{18}F fluoropentaethylene glycolic azide to yield ^{18}F-labeled azadibenzocyclooctatriazolic PEG-MSNs (^{18}FDBCOT-

PEG-MSNs). DBCO-PEG-MSN was intravenously injected to tumor-bearing mice, and after 24 h, radiotracer was administrated and PET images were acquired. An alternative group of mice was administered with the radiotracer, and after 2 h, their PET images were acquired. The pre-targeted murine model displayed superior uptake in tumor than nonpre-targeted mice owing to the generation of radiolabeled conjugate through SPAAC conjugation reaction. This approach can be possibly tailored for PET isotopes with short half-life along with other nanomaterials that need a long circulation time in bloodstream.

Several research groups have studied the integration of radionuclides with long half-life such as ^{64}Cu or ^{89}Zr in MSNs [132–135]. In vivo vascular imaging with MSNs integrated with multimodal imaging properties of ^{64} Cu ($t_{1/2}$ = 12.7 h) and 800CW (an NIRF dye) was demonstrated by Chen et al. [134]. TRC105 (a human/murine chimeric IgG1 monoclonal antibody) was attached to MSN along with PET/NIRF imaging moieties for tumor angiogenesis. PET imaging demonstrated ~two-fold enhancement of tumor accumulation with antibody labeled nanoconjugate in 4 T1 tumor, while the uptake of nontargeted nanoparticles was less, indicating the efficiency of targeting ligand (TRC105 (Fab)) for enhanced tumor accumulation. Miller and coworkers developed ^{89}Zr isotope (half-life −78.4 h)-integrated MSNs with large pore size via covalent conjugation of p-isothiocyanatobenzyl-desferrioxamine (DFO-NCS). There was no evidence of leakage of radionuclides from the mesoporous nanoprobe, signifying the safety profile for bioimaging applications [135]. Biodistribution in mouse with prostate cancer demonstrated strong PET signal in liver, spleen, and lungs. Although no obvious buildup of ^{89}Zr-DFO-MSNs in tumor is observed, MSNs could be further optimized for enhanced tumor uptake by improved surface functionalization.

9.2.2.3 Magnetic Resonance Imaging (MRI)

Owing to excellent spatial resolution, good penetration, and strong soft tissue contrast, MRI is extensively employed as noninvasive diagnostic modality. The progress in MRI applications integrating nanotechnology resulted in improvement with nanoparticle-centered MRI imaging [136, 137]. Upon applying magnetic field and radio frequencies, MRI signals are generated from proton relaxation of components including water, lipid, and protein to produce pictures with high resolution and contrast. Based on relaxation pathways, MR images are categorized as T_1-weighted images (longitudinal relaxation time and positive contrast with bright signal) or T_2-weighted images (transverse relaxation time and negative contrast with dark signal) [138]. Owing to the developments in nanotechnology, nanomaterials have been studied as contrast agents that can enhance further the sensitivity. T_1-weighted images are often created by gadolinium (Gd)- and manganese (Mn)-based contrast agents for investigation of structural details, and T_2-weighted images are generally generated by iron-based contrast agents and mostly studied for acquisition details during inflammatory conditions and also during edema [139–141]. Among different types of nanoparticles, MSN shell over contrast agent functions as a model platform for the advancement of MR-based nanomaterials.

MSNs integrated with superparamagnetic iron oxide nanoparticles (SPIONs) and ferrite-based nanoparticles have been used for generating T_2 contrast and also as drug delivery vectors [72, 142–147]. Loaded therapeutics is released in the acidic environment of tumor and magnetic centers in MSNs are accessible for H_2O molecules, thus enhancing MR signal. The MNPs are either conjugated on the outer surface of MSN or designed as core component, which are in turn coated with MSNs. Lee and coworkers synthesized MSNs immobilized with several magnetite (Fe_3O_4) nanocrystals on the surface of MSNs, resulting in the formation of raspberry-like with enhanced T_2 MR signal [148]. Kim and coworkers synthesized distinct core shell-magnetic core/MSN shell-based nanoparticle with multifunctional properties. Incorporation of fluorescent dye in the MSN framework permitted optical imaging, MRI, and simultaneous drug delivery function [142].

Gd-based nanoparticles are studied for MRI owing to large magnetic moment as a result of unpaired electrons (seven) and prolonged electron spin-relaxation time (10^{-9} s) under magnetic field [139]. Gd chelator-Gd-diethylenetriaminepentaacetic acid (Gd-DTPA) is commonly used to lessen the toxicity associated with free Gd ions. Gd chelates grafted on MSN surface are often used for developing MR contrast agents owing to their capacity to cart huge cargo of Gd centers and better water availability of the Gd chelates [149–152]. A core-shell multifunctional theranostic MSN functionalized with photosensitizer Chlorin e6 (Ce6), carbon dots (CDs), and Gd (III) ions for simultaneous MRI and CT has been developed by Yang and coworkers. Thermoresponsive poly[(N-isopropylacrylamide)-co-(methacrylic acid)] (P(NIPAm-co-MAA)) polymer encapsulated the pores of core-shell MSNs [141]. Dox was loaded as chemotherapeutic drug. Theranostic nanoconjugate presented enhancement in the contrast, which was concentration dependent and was assigned to Gd^{3+} concentration in the MSN shell. T_1-weighted MRI at the tumor site in mice with tumor confirmed superior MR contrast.

Though Gd-based contrast agents are widely studied, health threat associated with nephrogenic systemic fibrosis induced by Gd confines its in vivo use. A safer reported substitute to Gd-based contrast agents is Mn^{2+}-based contrast agents owing to their low cytotoxicity and prolonged electronic relaxation time associated with five unpaired electrons [153, 154]. Mn-based core−shell mesoporous silica spheres were reported for simultaneous T_1- and T_2-weighted MRI [154]. MR imaging studies of Mn-based MSN exhibited better contrast effects of both T_1- and T_2-weighted MR imaging. The large r_1 value of Mn-based MSN was attributed to reduced $KMnO_4$ concentration for oxidizing CTAB molecules, formation of excess Mn^{2+} ions that might have probably enhanced T_1-weighted MR imaging, and mesoporous configuration of nanocarrier that improved the diffusion of H_2O molecules, thereby augmenting r_1 relaxivity. The high r_2 value and superior T_2-weighted contrast might have originated from MnO_2 nanoclusters (<2 nm) in MSN. Mn-based MSNs may possibly be an exceptional dual-modal MR contrast for diagnosis of cancer in the future.

9.2.3 Tissue Regeneration and Wound Healing

Another prospective application of MSN-based nanocarriers is in tissue regeneration and wound healing. Topical application of nanoformulation offers comparable efficacy and less toxicity and overcomes the limitations of conventional routes of drug administration including systemic side effects and pain from injections [155, 156]. The physicochemical parameters of nanoparticles including size, shape, zeta potential, surface charge, etc. largely influence the interaction with skin and MSN with size less than 25 nm have demonstrated penetration and not permeation in the skin. The larger nanoparticles (55 ± 6 nm) did not cross the normal or disrupted murine skin upon topical application for 5 days [157]. Therapeutics including antibiotics, antifungal, antiviral, antiseptics, anticancer, antiinflammatory, and antioxidants corticosteroids have been delivered through MSNs [95, 158–166] (Table 9.2).

Recently, novel and versatile ROS-scavenging tissue adhesive nanocomposite was developed by immobilization of ultrasmall ceria nanocrystals on the surface of MSN. The ceria nanocrystal-decorated MSN (Ceria-MSN) exhibited excellent tissue adhesion strength and ROS-scavenging effect by decreasing oxidative stress in wound microenvironment. The nanocomposite accelerated wound healing process and promoted tissue regeneration with limited scar formation. The nanocomposite exhibited a "nano-bridging" effect that promoted quick closure of wound owing to interactions between surface of nanocomposite and the tissue matrix. The in vivo studies in mice also confirmed the therapeutic effect by Ceria-MSN in wound healing [158]. The "nanobridging effect" on wound closure and healing was also

Table 9.2 Therapeutic-loaded MSN for wound healing and infection control

Cargo/modification	Features	Application	Reference
Ceria nanocrystal-decorated MSNs	Tissue adhesion property and in vivo ROS-scavenging potential	Biomedical	[158]
Quercetin	Loading of flavonoid derivatives, antioxidant, and active ingredients of cosmetic interest	Biomedical and cosmetics	[159, 160]
Octyl methoxycinnamates	Sunscreen UV filters	Cosmetic	[161]
Doxorubicin hydrochloride and indocyanine green	Treatment of superficial tumors by chemotherapy and photothermal therapy	Cancer	[162]
5-Aminolevulinic acid	Photodynamic therapy in skin cancer	Cancer	[163]
5-fluorouracil	Transdermal delivery of chemotherapeutic drugs	Cancer	[95]
Ginsenoside compound K and Rh2	Anti-cancer and anti-inflammatory efficacy	Cancer	[164]
Bismuth titanate ($Bi_xTi_yO_z$) NPs	Sunscreen UV filters	Cosmetic	[165]
Polymyxin B	Therapeutic properties with antibacterial activity	Antibacterial	[166]

investigated by Lu and coworkers with nanosilver-decorated mesoporous silica nanoparticles (Ag-MSNs) [167]. The researchers demonstrated properties including tissue adhesion, biodegradation, and enhanced biocompatibility of Ag-MSNs and antibacterial effect in animal model without any infections or side effects.

Colloidal mesoporous silica (CMS) and its adhesion property with polydimethylacrylamide (PDMA) hydrogel were evaluated for in vivo wound closure and healing in mouse and compared with conventional suture and treatment with nonporous silica nanoparticles [168]. Owing to pores and rough surface than in nonporous silica nanoparticles, the total outer surface area determined the adhesion energy in CMS. CMS nanoparticles demonstrated better wound healing compared to the conventional suturing and degradation rate, enhancing their application in the future regenerative tissue engineering.

9.2.4 Antimicrobial Applications

The acquired resistance to antibiotics and the formation of biofilms have decreased the sensitivity to antibiotics and effectiveness of treatment on microbial infections. Dose increase and frequency of antibiotics for treatment further favor the antibiotic resistance in microbes. Hence, alternate approaches for the delivery of antibiotics against microbial infections have been explored. The use of bacteriophages, toxins such as bacteriocins, probiotics, and prebiotics, has demonstrated antimicrobial effect, however, not effective as antibiotics [169–171]. Implementation of nanotechnology-based nanocarriers for antibacterial treatments is a promising approach to overcome existing challenges with bacterial infections including recurrent treatment failures associated with multiantimicrobial resistance and the insistent biofilms.

Nanomaterials have proven to be an attractive alternative approach for delivering antimicrobial agents for the treatment of infection [172, 173]. Among different nanomaterials, MSNs are the most common nanocarriers owing to their high surface area, tunable pore size, high drug loading capacity, and slow and sustained release of cargo. MSN-based antibacterial nanomaterials can be designed either by incorporating antimicrobial nanomaterials or by loading antimicrobial compounds. The MSN matrix can be tuned or tailored to incorporate ultrasmall antimicrobial nanoparticles or metal ions or encapsulate antimicrobial metal or metal oxide nanoparticles to mesoporous silica shell. Metal, metal ions, or metal oxide offers their antimicrobial action by inducing oxidative stress, release of metal ions, or nonoxidative mechanisms. In addition, the increased surface area of porous silica shell offers room for loading antimicrobial therapeutics that offer the synergetic effects in the treatment of infections. MSNs hosting nanomaterials, such as silver, copper, zinc, and nickel, have demonstrated superior antimicrobial properties [174, 175]. Although ultrasmall silver nanoclusters (Ag NCs) are excellent antimicrobial agents, the oxidation and aggregation of silver in the biological environment limit their application. Ag NC-decorated MSNs were developed for the long-term release of Ag^+ ions, and the nanoparticles demonstrated excellent antibacterial

activity against both Gram-positive and Gram-negative pathogens and minimum toxicity on mammalian cells. The uniform distribution of Ag NCs in the mesoporous MSN matrix was critical for the controlled release of Ag^+ ions, which leads to the broad-spectrum antimicrobial activity [176].

The tunable MSN surface permits the adsorption, loading drugs into the pores of MSNs, incorporating onto the matrix, or conjugation of drugs to the surface of MSNs [177, 178]. In this setting, the properties of MSNs, including large surface areas, tunable pore size, and volume, affect surface functionalization, drug release, and penetration capability through biological barriers, which make them superior to drug-delivery systems. The versatility of MSNs for surface modification allows the incorporation of different antibiotics and improves the effectiveness of the same. For example, Polymyxin B, although a potential antibiotic against Gram-negative bacteria, the inherent toxicity of the same limits its application in mammalian cells. The antibiotic was incorporated into MSNs, and researchers demonstrated enhanced antibacterial effect and biocompatibility in mammalian cells [166]. The physicochemical properties facilitate the MSNs to be tailored for loading therapeutics. In a study, lysozyme-coated MSNs improved the interaction with *Escherichia coli* and enhanced the concentration of lysozyme in the bacteria, whereas in another study, lysozyme was loaded inside the large pores of MSNs and demonstrated the hydrolysis of peptidoglycan in the bacterial cell wall [179, 180].

Another challenge in microbial infection is poly-microbial infections. The treatment of poly-microbial infections involves mainly the combinatorial therapy with antimicrobial drugs with different functions and work in synergistic mode. However, the dose of drug, pharmacokinetic behavior, and possible side effects are factors to be refined. Encapsulations of multiple drugs in nanoparticles can tremendously improve the efficacy, resulting in reduced side effects. Gounani et al. demonstrated the loading of dual antibiotics, polymyxin B, and vancomycin in surface-modified MSNs and studied the effectiveness against Gram-positive and Gram-negative pathogenic bacteria [166]. The effectiveness of nanocarrier with both drugs was superior to the activity of free antibiotics. The enhanced antibacterial efficiency was ascribed to the improved local concentration of antibiotics in particles on the bacterial surface, supporting the delivery of dual drugs to their target. The study proves that the effect of combination therapy with MSN-based drug delivery can improve the safety profile of existing antibiotics.

Eradication of bacterial biofilms that are commonly adhered on the surface of implanted medical devices or human tissue is yet another challenge in the clinical field as pathogens can survive in high dose of antibiotics, developing multiantibiotic resistance and low patient compliance [181, 182]. Antimicrobial (antibiotics, antimicrobial peptides, and proteins)-loaded nanoparticles have demonstrated the enhancement of the efficacy of treatment and superior biocompatibility. However, for the treatment of biofilms, the nanosystems are being designed to enable the penetration of therapeutic drugs into biofilms. Xu et al. recently developed novel rod-shaped hollow MSNs with large cone-shaped pores for the delivery of lysozyme into biofilms [183]. The cone-shaped pores and inner hollow cavity facilitated high loading capacity of lysozyme and sustained release of the same. The authors also

demonstrated superior antimicrobial activity toward the *E. coli* biofilm with MSN-based nanodelivery system.

Nanoparticle-based theranostic system is being developed for the simultaneous diagnosis and treatment of bacterial infections [184]. The ability of MSNs for accommodating and transporting multiple therapeutic and diagnostic components can be employed for the prevention and tracking of biofilms, which is one of the major challenges associated the treatment of polymicrobial biofilms. Multiple antibiotics with distinct mode of action are necessary for the treatment and destruction of biofilms, and multiple antibiotics and along with other antimicrobial component-loaded MSNs would be an efficient candidate for destroying biofilms.

9.3 Conclusion and Future Prospects

A plethora of engineered MSN-based nanomaterials have been developed in the past few years, which are promising nanomaterials for biomedical applications. The unique mesoporous matrix of MSNs bestows the nanoparticle capability of loading and retaining drugs and contrast agents for the development of multifunctional MSNs. Although there has been progress in the synthesis parameters and rendering biocompatibility, the MSN nanoplatform demands substantial perfection to be employed for clinical application. A comprehensive investigation of the interaction of nanomaterials in in vivo setting with other cellular components has to be explored and would deliver details for future, refining the biocompatibility of MSNs. A complete assessment of acute and chronic toxicity, including immuno-toxicity and genotoxicity of these nanomaterials, has to be extensively addressed. The pharmacokinetics and biodistribution are yet another significant area to be evaluated. The advancement in the treatment of cancer with multiple therapeutic modalities with MSN can be aimed to target challenges including the multidrug resistances (MDRs) associated with cancer. Therefore, much investigation is further required on multifunctional MSNs for their effective clinical translation for a profound effect on human health.

Acknowledgments ARG thanks the University of South Australia for providing support. VP would like to thank ARG for her immense support extended during the period of this study. Also, VP would like to thank BNERC, Toyo University, for their continuous support.

Conflict of Interest The authors declare no conflict of interest.

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Advances in Pulmonary Nanomedicine for Therapeutic Management of Respiratory Diseases

10

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Abstract

Respiratory diseases such as obstructive pulmonary diseases, acute respiratory distress syndrome, idiopathic pulmonary fibrosis, pulmonary tuberculosis, acute lung injury, lung cancer, etc. are nowadays continuously increasing worldwide in all age group population, and their treatment has been proved to be a challenging task. Nanotechnology-based drug delivery systems overcome to some extent the deficits of the conventional treatment regimen by modifying the pharmacokinetic properties, reducing drug toxicity, and enhancing the half-life of the drugs. We focus on nanomedicines like Nanoparticles, Dendrimers, Liposomes, Lipid-based nanoparticles, Lipid–polymer hybrid nanoparticles, and Micelles used for respiratory diseases. This chapter discusses the advancement of pulmonary nanomedicines for respiratory diseases and highlights the recent clinically approved nanomedicines as well as their limitations.

Keywords

Respiratory diseases · Pulmonary nanomedicine · Nanoparticle · Liposomes · Dendrimers · Drug delivery system

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M. K. Das, Y. V Pathak (eds.), *Nano Medicine and Nano Safety*,
https://doi.org/10.1007/978-981-15-6255-6_10

10.1 Introduction to Nanomedicine

This is the era of nanotechnology, and medicine has immensely benefited from advances in nanometric drug delivery systems. Nanomedicine is the contemporary term used to encompass a vast variety of systems and has become possible due to involvement and contribution from diverse scientific fields including medicine, nanoscience, and pharmaceutical technology. The major focus of researchers and industry in the field of nanomedicine is in cancer chemotherapy and theragnostics, followed by inflammation or pain relief or safety enhancement of drugs, infection control, and ophthalmic and topical therapy of diseases. Primary rationale for such systems is to optimize the pharmacokinetic profile of the drugs (often due to better targeting) and improve their safety factor or therapeutic index. Regulatory approval for nanomedicines was phenomenal in the first half of the last decade, thereafter declined presumably due to the worldwide economic recession of 2008. Nanomedicines are also being investigated as carriers for contrasting agents and theragnostics [1].

Nowadays, various nanomedicines have witnessed increasing attention for diagnosis and therapies through using the three interrelated themes—nanodiagnostics and molecular imaging, targeted drug delivery and controlled release and regenerative medicine. Nanomedicines

1. provide efficient transport capacity by fine capillary blood vessels and lymphatic endothelium and controlled release of API in the harsh environment of diseased tissue,
2. enhance the circulation period and plasma concentration,
3. increase the binding capacity to biomolecules and accumulation in the target tissue, and
4. decrease the inflammatory or immune response and oxidative stress in tissues.

compared to conventional medicines depending on physicochemical properties such as particle size, surface, and chemical composition of the nanoformulations [2, 3].

Vast varieties of nanomedicines, such as Nanoparticles, Dendrimers, Microemulsion, Liposomes, Polymeric nanoparticles, Lipid-based nanoparticles, Lipid–polymer hybrid nanoparticles, Nanostructured lipid carriers, Nanospheres, etc., were studied to concentrate drugs in selected target tissues for minimizing systemic side effects and toxicity. But still, safety assessment is a very challenging task [4–6]. Primarily, clinically available nanomedicines are administered via IV route followed by oral or sometimes transdermal pathways.

Pulmonary nanomedicines have attracted attention only lately, and we come across only a handful of clinically available or pipeline products such as Arikayce® (Insmed Inc.), Curosurf® (Chiesi Farmaceutici) [1, 7], Lipoquin and Pulmaquin (Aradigm Inc.), and Nanosilver Inhalation (nAG) [8]. In spite of several potential advantages of the pulmonary route, successful products are hard to come by.

Literature documents that nanomedicines can be given through pulmonary route for treating various respiratory diseases and are found to be very effective with

improved pharmacologic and therapeutic potency [4–6, 9–28]. It has been found that the extent of pulmonary nanomedicine uptake depends not only on the physical and chemical features of nanomedicines themselves but also the health position of the organism. Within this context, the present chapter discusses the advancement of pulmonary nanomedicines for respiratory diseases and highlights the recent clinically approved nanomedicines and their limitations.

10.2 Respiratory Diseases and Infections

Respiratory disease is termed as disorders related to different parts of the pulmonary system such as upper respiratory tract, trachea, bronchi, bronchioles, alveoli, pleura, pleural cavity, and the nerves and muscles required for breathing. Common respiratory diseases include common cold, chronic obstructive pulmonary diseases (COPDs), chronic bronchitis, emphysema, fibrosis, lung cancer, and restrictive lung diseases. Infections or inflammation in the upper or lower respiratory tract is chiefly caused by the virus such as rhinovirus, parainfluenza virus, etc. or bacteria such as *Streptococcal pharyngitis, Haemophilus influenza, and Streptococcus pneumonia* infections [10, 24]. Due to high prevalence of such diseases among all age groups and also because they are on the rise as a result of increasing air pollution, clinical management beyond the conventional systemic or inhalation therapies needs urgent attention. This is where the pulmonary nanomedicine may play a pivotal role in providing safe and effective newer therapy options to the clinicians.

10.3 Pulmonary Delivery Systems

The lungs as a route of drug delivery have been in vogue for thousands of years from China to Egypt (Ebers papyrus) to Assyria. From Hippocrates (Greece), Rhazes (Arabi) to Maimonides (Spanish physician to King Saladin of Arabia), all have left in their treatise, various forms of pulmonary medication practices. The story began as long back as 2600 BC. Starting with the practices of inhaling fume from burning medicinal herbs to the individual attempts to design inhalation devices to the systematic industrial design of various kinds of liquid and powder inhalers and nebulizers, the pulmonary drug delivery system has evolved into a complex technology intensive system with many potential [29]. What began as smoking medicinal plants has now ushered into delivery platforms for biotechnological products involving complicated formulation development exercises even at the nanometric scales. With the advent of nanotechnology, the promising potential of this route of drug delivery is taking new shapes to be enlightened in the future.

The major advantages of pulmonary delivery systems are effective targeted drug delivery with maximum local drug action using very low doses to treat various respiratory diseases particularly for chronic lung diseases through direct delivery of active ingredients to the diseased organs and cells as outlined in Fig. 10.1 [13, 30].

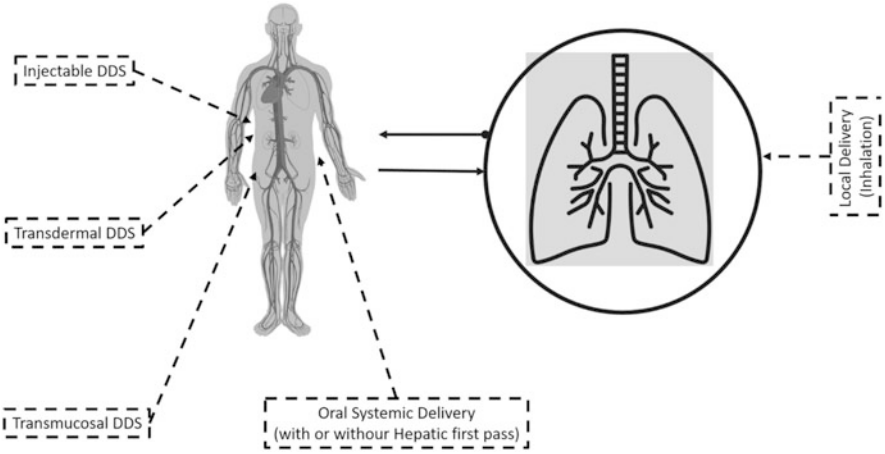

Fig. 10.1 Potential advantage of local inhalation delivery from a pharmacokinetic point of view. All other routes deliver the drug to the systemic circulation first, while local inhalation delivery delivers drug to the pulmonary region first

As lungs provide a large surface area accessible for drug absorption and a thin and permeable epithelial barrier, pulmonary drug delivery offers several advantages compared to other administration routes such as the rapid onset of action, avoidance of first-pass metabolism, decreasing the required dose with improved bioavailability of the delivered agent(s), and reducing adverse effects. In spite of these advantages, some factors such as lung toxicity of drugs, drug-induced lung disease, occupational exposure, lung defense mechanism, and drug stability can potentially limit the practical application of pulmonary delivery system in the clinic [13, 30].

In order to overcome the drawbacks of the conventional pulmonary drug delivery system, nanostructured formulations have found increasing attention for diagnosis, treatment, and prevention of respiratory diseases as they provide sustained drug release kinetics, shortening of the treatment course, reduction of required therapeutic dose, prevention of side effects, improved patient compliance, good drug encapsulation efficiencies, and protection of the active ingredient against decomposition and can be transferred into an aerosol without being adversely affected by the process of nebulization [10, 13, 24, 30].

10.3.1 Inhalation Therapy

10.3.1.1 Macro- and Microstructure of Lungs and Mechanism of Deposition from Inhalation

The anatomy and physiology of lungs play a critical role in the dynamics of drug delivery through inhalation. The macrostructure of lungs comprises two distinct zones of airways—the conducting airways and the respiratory airways.

The upper portions of the airways starting from nose or mouth followed by trachea, bronchi, bronchioles, and terminal bronchioles comprise the conducting airways. In this region, no exchange of gases takes place; rather, it transports the gas into the respiratory zones. The conducting airways bifurcate around 17 times. The surface area of conducting airways is approximately 2–3 m^2. The thickness of the cell wall is approximately 60 μm [31]. The presence of epithelial cell, goblet cell, and secretory glands keeps the airway humidified to ensure proper function of the airways as well as mucociliary clearance [32].

The respiratory airways consist of respiratory bronchioles, alveolar duct, and alveolar sacs. The presence of a thin squamous cell (Type I pneumocytes) and a large cuboidal cell (Type II pneumocytes) on the surface of the alveoli is responsible for gaseous exchange and production and secretion of surfactant in the alveoli, respectively. The alveolar surface is lined with a lipoprotein complex consisting of 10% protein and 90% phospholipids, termed as lung surfactant. It helps in reducing the surface tension of pulmonary fluids and contributes to the elastic properties of the lungs. The macrophages, near the pneumocytes, are responsible for removing particles or microorganisms from the respiratory surface by the process of Phagocytosis. The presence of lymphatic circulation in this part is responsible for fluid homeostasis and host defense mechanism in addition to gaseous exchange [33]. This area is suitable for gas exchange because of its inherent physical characteristics. The surface area is approximately 50 times greater, and the thickness of the cell layer progressively reduced from 60 μm to 0.1–0.5 μm compared to the conducting airways. Therefore, the fluid layer at the cell surface decreases substantially with the decrease in cell thickness [34]. Gaseous exchange is further promoted in this region due to the pressure gradient existing between lower partial pressure of oxygen within the alveoli compared to CO_2 rich blood in pulmonary circulation.

Therefore, the mechanism and rate of absorption of the inhaled drug can be either a paracellular or transcellular transport process [35].

The paracellular process occurs in the distal bronchioles and is suitable for hydrophilic small molecules. An electric resistance exists between apical and basal transepithelial cells, which gradually decreases from the tracheal region to the distal airways and again increases in the alveolar region [36]. The rate-limiting step is the permeation through the tight junction of adjacent epithelial cell.

Transcellular transport involves primary and secondary active transport in conjunction with passive transport. The two main influx transporters present in the lungs are a solute carrier (SLC and SLCO) and ATP binding cassette (ABC) transporters. The efflux transporters present in the lungs are P-glycoprotein (P-gp), Breast cancer resistance protein (BCRP), and Multidrug resistance protein (MRP1) [37].

10.3.1.2 Mechanism of Particle Deposition

The aerodynamic property of the particle estimates deposition and optimizes targeting in all regions of the respiratory tract. The tidal air throughout the respiratory system carries the inhaled Particles. Particle deposition occurs through the mechanisms of impaction, sedimentation, interception, and diffusion. The deposition of particles is influenced by their size, shape, and velocity [38]. Breathing

Table 10.1 Parameters influencing particle deposition in various pulmonary regions

Mechanism of deposition of particles	Particle size	Area of deposition	Expressed with
Inertial impaction	2–5 μm	Oropharyngeal and tracheobronchial region	Stokes number Deposition probability of impaction
Sedimentation	>0.5 μm 3–5 μm <3 μm	Tracheobronchial region Alveolar region	Deposition probability of sedimentation Terminal velocity of sedimentation
Diffusion	<0.5 μm	Acinar region of the lungs	Deposition probability of diffusion

pattern, lungs volume, viscosity of the fluid in the pathway, branching angle of the trachea, and the patients' health condition determine the travel mechanisms of the inhaled particles. Spherical particles are prone to deposit by the process of impaction, sedimentation, and diffusion depending on their diameter, whereas acicular-shaped particles are deposited on the wall of the respiratory tract in the direction of the air stream by the process of interception. Particles of diameter >10 μm deposit in the upper respiratory airways and are mostly removed by the mucociliary escalator through nose and mouth. For a pharmaceutical aerosol system, the particle size can range from 0.01 to 100 μm [39], but a monodisperse system is always preferred for optimal deposition and specific targeting in the region of lungs [40].

Diffusional transport of particle increases with decrease in particle size and respiratory rate. Targeting a particular region in lungs requires particle engineering, evaluation of breathing pattern, mode of inhalation, and composition of the inhaled medication (Table 10.1).

10.3.1.3 Pulmonary Clearance

The inhaled particles undergo various events prior to target interaction. Once the particles are deposited, they are taken up by mucociliary transport followed by biotransformation, receptor interaction, and nonspecific retention prior to systemic absorption. Therefore, the possible route of elimination of inhaled drugs can be by mucociliary clearance, mechanical clearance, absorption into the capillary network, enzymatic degradation, and alveolar clearance via macrophage phagocytosis. The entire mechanism acts in parallel (Fig. 10.2) and is responsible for rapid onset of action, localized high concentration of the drug, and targeted delivery of the drugs with poor oral bioavailability [41].

- *Mucociliary clearance*: The submucosal glands and the goblet cells of epithelium produce mucous. It flows in the proximal direction with the rapid stokes of cilia [42]. A healthy human produces mucous 10–20 ml/day, and in chronic bronchitis, its production increases 10 times. A majority of insoluble particles of more than 6 μm diameter are eliminated by mucociliary clearance. The large particles either penetrate the mucous or dissolve, then flow in the direction of mucous, and

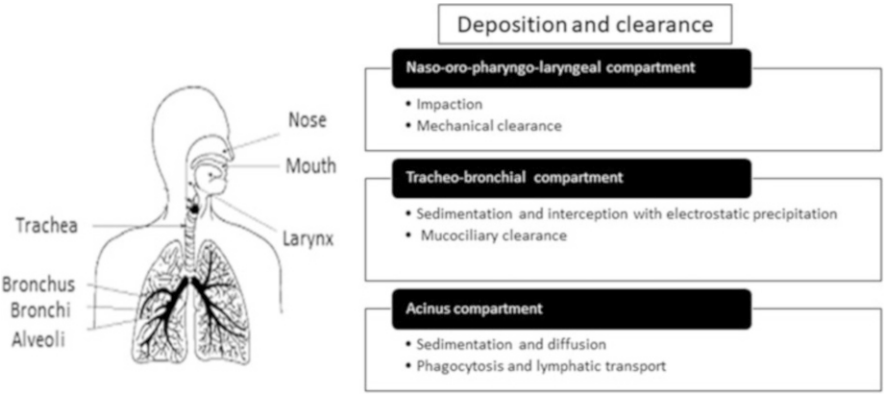

Fig. 10.2 Biodisposition of particles in inhalation therapy

eventually swallowed. Mucociliary clearance is predominant in the trachea bronchial region of the upper respiratory tract.

- *Mechanical clearance*: Inhaled particles of more than 10 μm diameter provoke coughing and are cleared from the upper airways. Sneezing and swallowing of particles are also seen with inhalation of large particles. The rate of mucociliary clearance decreases with age and in the presence of respiratory diseases like asthma, pneumonia, bronchitis, etc. The impaired mucociliary clearance results in coughing and removes larger particles from the upper airways.
- *Enzymatic degradation*: The drug-metabolizing capacity of lungs is substantially lower than that of the liver and does not impart major contribution to systemic clearance. Many small molecules have got better bioavailability via lung absorption compared to other routes of administration [43]. Drugs like theophylline, fluticasone propionate, budesonide, and salmeterol are substrates to enzymes present in lungs. The inhaled drugs are substrates for Cytochrome P450 enzymes (CYP1B1, CYP2B6, CYP2E1, CYP2J2, CYP3A5, and CYP1A1). Other biotransformation phase II enzymes present in the lungs are sulfotransferases, UDP glucuronosyltransferases, glutathione S-transferases, flavin monooxygenases, peptidases, cyclooxygenase, etc.
- *Alveolar clearance*: The poorly soluble drugs and the inhaled particles that remain in the alveoli are taken up by the macrophages by the process of phagocytosis. Slowly dissolving particles of 1.5–3 μm are taken up by this process. Phagocytosis remains the main obstacle in achieving controlled release of drug in the alveolar region.

10.3.2 General Consideration for Effective Inhalation Therapy

1. Pharmaceutical factors related to particle size:

- Particle size distribution:
  The distribution of inhalable particle/droplet size from a powder inhaler or aerosol can be expressed in terms of the mass median aerodynamic diameter (MMAD) and the fraction of fine particles, which is less than 5 μm.

  Therefore, in designing an inhalation product, the particle size variation is an inherent characteristic [44]. So, a mathematical representation of size distribution will be useful. The log-normal distribution fits the particle size data in terms of geometric mean diameter, standard deviation, and geometric standard deviation.

$$\text{Log Normal distribution } F = \left(1/\left[\sqrt{2\pi}D \ln \sigma_g\right]\right) \exp.\left(-\left[\ln D - \ln D_g\right]^2/2\left[\ln\ \sigma_g\right]^2,\right.$$

where D_g = geometric mean diameter, D = a given particle diameter, and σ_g = geometric standard deviation.

Considering from the log-normal distribution, cumulative frequency distribution function can be determined. D_g and σ_g can be estimated from cumulative frequency distribution vs diameter curve on a log probability graph paper. Using Hatch and Choate equations, volume mean diameter and surface mean diameter for a particle population can be measured.

These measures are used for the in vitro performance of different inhaler devices [45]. In general, the higher the fine particle fraction, the higher the proportion of the emitted dose that is likely to reach the lungs.

- Particle Density.
  Particle density influences the settling behavior of the particles. Two spherical particles with the same geometric diameter of 1 μm varies in a wide range if their density differs. The motion of particles in air with high density and low geometric diameter can have more Brownian bombardment [46]. Most aerosol particles have apparently low density than the corresponding bulk material due to porosity and the presence of voids in the particles.
- Electrical charge
  The presence of electrical charges on particles influences the deposition on the surfaces and rate of coagulation from an aerosol delivery device. Electrical charges can be generated by various reasons [47]. The triboelectric effect generated due to friction from an aerosol pump can lead to the acquisition of electric charge.
- Disruption of ion-containing liquid in an aerosol system leads to the generation of random motion of free ions of symmetrical or unsymmetrical distribution.

- Hygroscopicity.
  Hygroscopic substances absorb water within the warm and humid environment of the respiratory tract. The quality of hygroscopic particle determines the physicochemical property of an aerosol as particle size, composition, and residence time change with an increase in temperature and humidity. It affects the adhesive and cohesive properties, leading to irreversible aggregation in a dry powder inhaler system. It can affect the chemical and physical stability of the system.

  The change in particle size is the measure of hygroscopic growth. The most common parameter used to characterize the particle is the mass median aerodynamic diameter. Hygroscopic growth ratio is the ratio of diameter at a high relative humidity as in the lungs with the low relative humidity in an ambient condition [48].
- Surface area.
  The particle surface area in inhalation technology plays a critical role in determining airborne specific surface area, deposition in the lungs, rate of dissolution, and inhalation toxicology [49]. For a perfect sphere, the specific surface area (SSA) is calculated using the formula

$$\mathrm{SSA} = \frac{\text{Surface area}}{mass} = \frac{4\pi r^2}{\frac{4}{3}\pi r^3 \rho} = \frac{3}{r\rho} = \frac{6}{D\rho},$$

where r = radius of the particle, D = diameter of the particle, and ρ = density.

Asymmetric particles with rough surfaces, pores, cracks, or voids have a larger specific surface area compared to spherical particles.

As per BET theory, the gas molecules physically adsorb on specific sites of the sample surface and the desorption is a kinetically limited process. Therefore, the specific surface or surface area per unit weight determines the ability of adsorption of aerosol gases and thus the potential of the particles to be carried deep into lungs. It also determines the tendency of the particle surface to initiate the chemical reaction and the dissolution of the deposited particles from the lungs.

- Crystallinity and polymorphism.
  Many drugs exhibit polymorphism. Polymorphic forms differ in density, melting point, solubility, and hygroscopicity. Crystal habit is important as the change in the shape of the particles affects aerodynamic diameter and thereby lung deposition. Therefore, controlling crystallization is at the main part of particle engineering in the development of inhaler devices [50].

2. Excipients for different inhalation devices.
   The commonly used excipients for inhalation devices vary with their type, performance, and mode of action. The various excipients used are listed in Table 10.2.

Table 10.2 Common excipients used in inhalation formulations

Inhaler device	Excipients used	Function	Example
Nebulizer [51]	Isotonic solution	To prevent irritation To convert pro liposomes into isotonic liposomes	Sodium chloride and dextrose
	pH adjustment system	To prevent irritation	Sodium hydroxide, citric acid, and phosphate salts
	Surfactants	To aid dispersion	Polysorbates
	Preservatives	Preservation	Parabens
	Purging	To reduce oxidation	Nitrogen
	Chelating agents	To increase stability	Disodium EDTA
	Cosolvents	To improve solubility	Alcohol and propylene glycol
	Humectant	To balance moisture	Glycerin
Dry powder inhaler (DPI) [52, 53]	Carriers	To improve flow To increase the bulk for a potent drug To improve the taste	Lactose monohydrate Mannitol, and glucose
	Shell formers	Reduce attractive forces and to improve targeting	DSPC, DPPC, Leucine, isoleucine, and trileucine
	Hydrophobic additives	To protect drug from moisture	Magnesium stearate
	Surfactants	To make porous particles	Poloxamers and bile salts
	Biodegradable polymers	For sustained release	PLGA and chitosan
The pressurized metered-dose inhaler (pMDI)	Propellants	Energy source for production of aerosol plume	HFA and CFC
	Cosolvents	Formulation aid in HFA system	Ethanol and PEG 1000
	Surfactant	Solubility enhancement and wetting agent	Sorbitan trioleate (SPAN 85), oleic acid, and soy lecithin
	Organoleptics and stabilizers	Patient compliance and formulation stability	Flavors, sweeteners, antioxidants, and chelating agents

10.3.3 Nanomedicines for Targeted Therapy to Lung Cancer

Lung cancer is the leading malignancy for the cancer-related death among men and women. The conventional chemotherapy is the most widely used treatment strategy for lung cancer. The conventional therapy suffers from undesirable pharmacokinetics and pharmacodynamics, non-specific biodistribution, and improper specificity with chemical resistance [54]. The development of targeted therapy and novel nanoscaled delivery of drug have opened the discovery and identifications of new targets and novel therapeutic modalities for more effective treatment of lungs cancer. The theragnostics of nanomedicine are found to be largely effective due to its small size, enhanced permeability, retention effect, high drug loading, and good biocompatibility.

- Antibody-mediated targeted therapy.
  Nanoscaled drug delivery system decorated with antibody fragments can be used to treat various tumor conditions. Lin et al. investigated the utility of anticarbonic anhydrase IX antibody conjugated to the surface of triptolide-loaded liposomes in the treatment of lung cancer via pulmonary administration [55]. In another study, paclitaxel palmitate nanoparticles conjugated with cetuximab were engineered to achieve high efficiency and improved cellular internalization in lung cancer cells. ([56] In a recent report, Mukherjee et al. synthesized 19 bp synthetic CDC20 siRNA encapsulated guanidinylated cationic amphiphile with stearyl tails. The intravenous administration of the liposomal formulation inhibited B16F10 melanoma growth on lungs in a syngeneic C57BL/6 J mouse tumor model [57]. Nonsmall cell lung carcinoma (NSCLC) is any type of epithelial lung cancer, which accounts for about 85% of all lung cancers. Clinical trials proved the safety and usefulness of bevacizumab and cetuximab in combination with chemotherapy and radiotherapy in the treatment of NSCLC patients. The combination of mAb prolongs the plasma $t_{1/2}$ of about 20 days with high tolerability and very low toxicity [58].

10.4 Potential Limitations of Pulmonary Delivery

Conventional Pulmonary delivery has not stood up to its potential, particularly, for systemic actions [13]. Due to the thin epithelial barrier, even if the drug payload crosses into the systemic circulation, they are often degraded by the liver and blood enzymes, thereby limiting the duration of action. Hence, even though the onset may be hastened, the short biological half-life rendered via enzymatic degradation leads to the need of multiple dosing. This often leads to sub-optimal treatment efficacy. The drug is not retained for sustained action in the lungs, and depot does not form. This leads to the need of multiple dosing, thereby reducing patient compliance, though in emergency, inhaled systems are excellent performers. Further, in spite of being a nonoral route of administration, the drug reaches several organs and tissues where it exhibits unwanted pharmacological responses leading to possible adverse

effects. Particularly, the enzymatic barrier in pulmonary delivery prevents conventional delivery of several drugs including nucleic acids and proteins/peptides. The development cost and safety concerns are also vital when the pulmonary route is selected as an alternate established route for an existing drug. The glaring example of these issues is the quick withdrawal of Exubera (Pfizer)—the first inhalable insulin therapy—from the market, citing cost and safety concerns [59, 60]. Potentially, nanotechnological interventions in such contexts may rejuvenate hope for at least some of the missed opportunities for utilizing pulmonary delivery as an alternative port of entry into the human body.

10.5 Nanomedicines Used Diagnosis, Treatment, and Prevention of Respiratory Diseases

Although a plethora of literature and patent report various attempts to develop pulmonary nanoformulations with several drugs of different therapeutic categories for both local and systemic delivery; yet, very few have progressed to the clinical stage and ultimately to the market. In the following few paragraphs, we survey some of the marketed pulmonary nanomedicines and provide a glimpse of the various nonclinical attempts to paint the picture of progress in this field. The advances and the clinical evidence are categorized according to the different subtypes of nanometric dosage forms.

10.5.1 Nanoparticles

Nanoparticles are a large class of nanometric particulate systems lying within the size range below 1000 nm, although considerable debate exists in defining this range and no universally accepted range has yet emerged. A plethora of pharmaceutical dosage forms such as nanospheres, nanocapsules, nanosponges, nanocrystals, liposomes, nanoglobular systems, various "niosomes," etc. are often categorized under the term "Nanoparticles". However, classically rigid capsular or matrix systems made up of polymers and/or lipids with or without other excipients are expressed as nanoparticles in a pharmaceutical sense. Most commonly, these nanoscale constructs afford modulation of drug pharmacokinetics, especially residence time in biological milieu, and have the potential to target (both active and passive) specific biological tissues.

By virtue of their extreme fineness, nanoparticles have unique properties, often exploited pharmaceutically to overcome physicochemical and biopharmaceutical challenges of drugs in conventional dosage forms. The shape, size distribution, topological characteristics, charge, and material properties of nanoparticles play a significant role in altering the pharmacokinetics of drug payload. Further, ligand tagging, PEGylation, and surface decoration add functional properties, which are all useful in achieving the goals of nanoparticulate formulations.

Table 10.3 Some Clinically available Nanoparticulate formulations [62]

Paclitaxel (albumin nanoparticles)	Gold nanoparticles (colloidal gold and silica nanoshells)	Docetaxel (polymeric nanoparticles)	Leuprorelina (PEGylated polymeric nanoparticles)
Aprepitant (nanocrystals)	Paliperidone (nanocrystals)	Insulin (polymeric nanoparticles)	Megestrol (nanocrystals)
Pegaspargase (polymer-protein conjugated nanoparticles)	Denileukin (protein nanoparticles)	Rapamycin (nanocrystals)	Fenofibrate (nanocrystals)
siRNA (lipoidal nanoparticles)			

Broadly, the nanoparticles have been used for treatment and diagnostic purposes, and now, theragnostic applications are catching up. As carriers of drugs and contrast agents for efficient delivery, targeting of biological constructs, and imaging purpose in diagnosis, nanoparticles have been widely acclaimed. The potential of many drugs would not have been realized if the nanoparticles were not available [61]. However, for nanomedicine, the rate of translation from laboratory to clinics is not very encouraging due to various reasons—both technical and regulatory. A customary search using operators "nanoparticles" AND ("drug delivery" OR "drug targeting" OR imaging) yields more than 60,000 literature reports. Nevertheless, the actual number of clinically available nanoparticle formulations is limited (Table 10.3).

Of late, renewed interest is being shown in exploiting nanoparticulate systems for lung-specific and lung-mediated systemic delivery of pharmaceuticals. Advances in materials, particle engineering, and inhalation devices have enabled many drugs to be delivered to lungs directly. There are scopes in brain targeting, better management of tuberculosis and lung cancers, anti-infective and anti-inflammatory treatment, and vaccine administration [63]. It is now being recognized that the Mass Mean Aerodynamic Diameter (MMAD) of about 5 μ is not the only factor, but the fine fraction of particles in inhalation or nebulization plays pivotal roles in ensuring deep lung deposition of particles. Consequently, nanoparticulate systems appear to be full of potential for efficient drug delivery to the lungs.

Several drugs have been formulated into nanoparticles for pulmonary delivery such as salbutamol, fluticasone, doxorubicin, ofloxacin, moxifloxacin, azithromycin, Amikacin, rifampicin, isoniazid, pyrazinamide, ethambutol, tobramycin, ciprofloxacin, budesonide, tacrolimus, vancomycin, clarithromycin, cyclosporin A, tranilast, paclitaxel, Cisplatin, Silibinin, Methotrexate, Voriconazole, Itraconazole, Heparin, Exendin-4, IgG1, Sildenafil, Carvedilol, calcitonin, Pirfenidone, Indomethacin, Curcumin, other antioxidants, etc. Vaccines for norovirus, anthrax, and influenza using mucoadhesive nanoparticles have been evaluated for pulmonary delivery. Most of these nanoformulations are delivered via DPI and pMDI, which are ambulatory, and in clinical setup, the nebulizers are devices of choice [63, 64]. The recent approval by USFDA of Afrezza® for a recombinant Insulin inhalation system would

boost the research in the field of peptide delivery via lungs. However, several challenges need to be overcome before human trials become successful. The nonrespirable carrier particles often separate from drug bearing nanoparticles, particle aggregation leads to alteration of the intended lung deposition and pharmacokinetic profile, regulatory requirements are stringent, and formulation may fail at an advanced stage of development. Some of these technical limitations may be overcome by newer technologies such as effervescent nanoparticles, particle engineering, and supercritical fluid technology [63]. A particularly attractive option appears to be nanocrystals of drugs delivered via nebulizers (as nanosuspensions) or DPI. However, the safety of polymeric systems used in the production of the particles is a concern and only a handful of materials, such as PLGA and lecithin and its derivatives, are USFDA approved for such application. Certain proprietary technologies such as PulmoSphere® (Novartis) and AIR® (Alkermes Inc.) have shown great potential in developing successful pulmonary nanosystems and have since progressed into clinical trials [65].

Nanoparticles for pulmonary delivery have been fabricated from several different materials, headed by polymers, lipids, and proteins. The most common polymer reported for pulmonary nanoparticles is of course the FDA-approved biodegradable polylactic-co-glycolic acid (PLGA), having an excellent time-tested safety profile. Other polymers appearing in literature for the purpose include natural polymers such as mucoadhesive chitosan, dextran, and polyesters and synthetic polymers such as polyethylene glycols and their derivatives. They offer certain specific advantages over other materials available. Protein-based nanoparticles utilize gelatin that is a nonimmunogenic biodegradable polymer. Lipids are extensively studied as nanoparticulate systems as they are nonimmunogenic and their degradation products are often biocompatible. Apart from phospholipids and cholesterol, several others saturated as well as unsaturated lipid molecules are reported in the literature as pulmonary nanoparticles [66]. It has been found that stearic acid, palmitic acid, and Compritol-based SLNs and lipid-drug conjugates converted into nanoparticles bearing isoniazid, pyrazinamide, and rifampicin have an excellent safety profile [67].

10.5.2 Dendrimers

Dendrimers (Fig. 10.3) are homogeneous polymeric 3-D nanostructures with repeated molecular branches. They have multiple functional groups available on their surface. This results in their functional versatility and biocompatibility. By virtue of their structure, both hydrophilic and hydrophobic drugs can be loaded or conjugated to these structures.

Due to their unique structure, they have been exploited in several pharmaceutical formulations [68]. Naturally, Dendrimers have found pulmonary applications as well and Table 10.4 depicts some instances of success with dendrimer-based pulmonary drug delivery.

Dendrimers, particularly belonging to fourth generation based on polyamidoamine (PAMAM), have been found to be suitable in most advanced

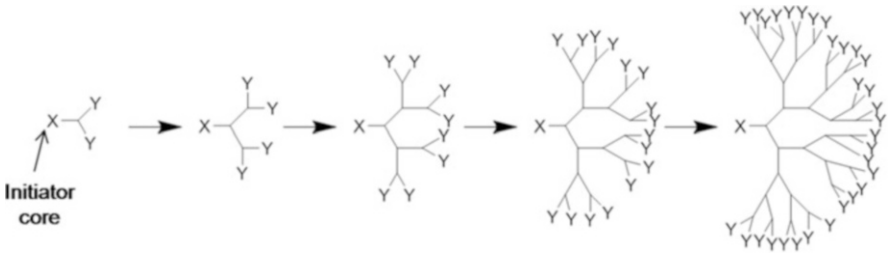

Fig. 10.3 Typical synthetic route and structure of a dendrimer

Table 10.4 Applications of Dendrimers in Pulmonary drug delivery [68, 69]

Drug/brand	Indication	Remarks
siRNA	Gene silencing, alveolar targeting, improved stability, and better efficacy	pMDI
Beclomethasone	Extended release	Nebulization
Doxorubicin	Increased pulmonary retention, pH-dependent drug release, better particle size control, and lower cardiac toxicity	pMDI, DPI, and nebulization
Methotrexate	PEGylated system, lower t_{max}, and increase in bioavailability	DPI
Rifampicin	Extended release and improved plasma profile than IV route	DPI
Enoxaparin	Low MW heparin and improved bioavailability	Rodent model

studies. PEGylated PAMAM dendrimers have shown promise. The pulmonary biodisposition has been encouraging in both in vitro and in vivo results. By virtue of their size and chemical characteristics, dendrimers have been found to be compatible with both formulation excipients and biological milieu, affording better internalization, transport, and drug delivery. A few dendrimer-based formulations are in various phases of clinical trials (Table 10.4). Though not pulmonary dosage forms, their success would definitely widen the path for translation of pulmonary formulations from lab to bedside. However, cationic dendrimers have potential of membrane destabilization as the biomembranes are negatively charged and need long-term safety studies within the current regulatory framework before clinical acceptance [69].

10.5.3 Liposomes

Liposomes were the first clinically used novel nanometric systems in the form of Amphotericin B (AmBisome)- and Doxorubicin-loaded formulations (Doxil) [62]. Although the initial formulations and most of the currently marketed liposomal formulations are injectables, progress in pulmonary liposomes has also been significant. The most prominent case of pulmonary liposome in clinics is Arikayce by

Table 10.5 Pulmonary liposomes reported in literature [73]

Drug/brand	Indication	Remarks
Amphotericin (Abelcet)	Fungal infections postlung transplantation	Human trial
Lung phospholipids (Survanta)	Respiratory distress syndrome (neonates)	Commercialized
Amikacin (Arikayce)	Antipseudomonal inhalation	Commercialized
Beclomethasone	Anti-inflammatory in asthma	Human study
Ciclosporin A	Immunosuppressant	Human study
Interleukin-2 (IL-2)	Anticancer, safety, and efficacy study	Dogs and humans (phase I)
9-nitrocamptothecin (9-NC)	Anticancer, safety, and efficacy study	Phase II clinical trial and animal studies
Insulin	Safety and efficacy study	Animals
All-trans-retinoic acid	Safety	Mice
Nonviral gene delivery	Efficacy, stability, and safety (oncological and infection indications)	AERx® (Aradigm Corp., Novo Nordisk)
Budesonide	Experimental asthma	Animal model
Ciprofloxacin (Pulmaquin®)	Lung infections	Clinical trials
Cisplatin	Lung cancer, toxicity reduction, and improved bioavailability	Clinical trials
Paclitaxel	Anticancer, safety, and efficacy study	Mice
Camptothecin	Anticancer and efficacy study	Mice
Doxorubicin	Anticancer, safety, and efficacy study	Mice

Insmed Inc., a formulation containing the antibiotic Amikacin for treatment of persistent pulmonary infections. It is an extended release inhalation formulation indicated for various threatening lung infections such as *Pseudomonas aeruginosa* (in Cystic Fibrosis and nonCF bronchiectasis patients) and in nontubercular *Mycobacterium* infections. The product has witnessed good therapeutic response and patient acceptance due to its considerable safety profile available so far [70]. A number of reports suggest that liposomes are attractive tools for local antimicrobial treatments with various drug classes such as fluoroquinolones [71] and aminoglycosides as well as for antineoplastic agents [72]. There are other liposomal formulations in various stages of development (both clinical and nonclinical); however, most are administered via i.v. route and utilize the lungs' ability to filter particles based on size to localize the liposomes to the pulmonary region; direct pulmonary delivery seems to have very few takers, probably due to the anatomical, pathological, and immunological barriers associated with pulmonary route [73].

Table 10.5 lists some of the recent liposomal formulations attempted for pulmonary delivery and which are at various stages of development.

In most cases of reported liposomal pulmonary formulations, the efficacy and bioavailability of the hydrophobic drugs have improved, often, significantly and reduction in side effects or adverse effects. In spite of potential general limitations of liposomes, particularly, in view of their stability and storage issues, liposomes have been given most attention in exploiting nanomedicine through pulmonary route. It should be kept in mind while developing such dosage form, the role of the delivery device and its relation to physicochemical properties of the formulations, which have a direct bearing on the efficacy of the product. This makes pulmonary liposomal formulation exercises complicated. Unless a sizeable number of clinically available such formulations come to the market and remain there for a sufficient time, the apparent huge potential of these products cannot be ascertained.

10.5.4 Lipid-Based Nanoparticles

In the 1960s, parenteral fat emulsion was found to be the first dosage form where lipophilic drug was incorporated in lipid droplet, which is nowadays used for poorly water-soluble drugs [74]. Various research groups such as Muller's group, Gasco's group, and Westesen's group developed the Solid Lipid Nanoparticles (SLNs) that are considered as the first generation of lipid nanoparticles [75–80]. Lipid matrix of SLN was converted to solid by replacing the oil of the fat emulsion by a solid lipid or a blend of it where lipid (0.1–30% w/w) dispersed in aqueous solution of surfactant (0.5–5% w/w) as stabilizing agent at room temperature or body temperature [81]. The mean diameter of SLN was found to be in a range of approx. 40–1000 nm [82]. If biodegradable lipids are chosen, then SLN can be well tolerated in the airways. Due to the smaller particle size, nanoparticles can easily be entrapped or aerosolized into droplets with aerodynamic preferable properties, which ensures that the active compound is deposited sufficiently in lungs. Moreover, nanoparticles can adhere to the mucosal surface of the lungs for a longer period of time [83, 84]. Due to the property of particle adhesion and accumulation and retention results in longer dosing interval, thus, they can be used for the treatment of chronic diseases and help to achieve better patient compliance [85]. Regardless of their advantages, Lipid Nanoparticles have to meet desirable properties (biocompatibility, sterility, isotonicity, and neutral pH value of 3–8.5 as lungs have limited buffering capacity) for their applications [53]. The sterilization procedure is carried out by different methods like autoclaving, gamma ray irradiation, and sterile filtration. Due to physical stability issues (like increase in particle size due to the melting of lipid matrix and recrystallization during cooling leads to a change in the structural matrix, aggregation, or gelation), autoclaving is not an appreciable technique for sterilization. Gamma ray irradiation also leads to the formation of free radicals, which ultimately results in the chemical modifications of the active moiety loaded to the carrier system [86]. Nonionic isotonization agents preferably glycerol or carbohydrates and ionic isotonization agents like NaCl or other salts can be used to isotonize a lipid nanoparticle formulation. The electrolyte addition reduces the electrostatic stabilities due to reduction in zeta potential. Lipid nanoparticles can be

given as suspension or in a dry powder form for inhalation. In both cases, they should have to show good aerodynamic properties in the desired airway regions for deposition. Aerodynamic behavior cannot be explained only by the geometric diameter of a particle, and it comprises geometric diameter along with density and shape of a particle as, for instance, big particle with a small density can move with the same velocity as a small particle with a high density. Deposition of particle basically depends upon the optimal aerodynamic size (range of 0.5–10 μm), and the mechanisms it follows are impaction, sedimentation, and diffusion [87]. Impaction leads to the deposition of particles on bifurcations and narrowing as it basically occurs when a heavier particle cannot follow the rapid changes that occur in the air stream of upper airway due to the inertial forces. It is more likely to happen with an increase in air velocity, density, and size of particles and rate of breathing. Particles of nanometer range are more likely to deposit in the alveoli and bronchiole due to the gravitational forces, termed as deposition by sedimentation. Decrease in breathing rate and longer residence time lead to an increase in deposition of particle by sedimentation. Deposition by diffusion occurs when the smaller and lighter particles (submicron-range) deposit in the small airways and alveoli during the movement of particles in the surrounding gas molecules by Brownian motion as the air velocity is too low [88]. Particles are generally exhaled out because they do not deposit in one breathing cycle. Currently available devices for inhalation that use nanoparticulate formulations follow the mechanism of impaction and sedimentation rather than diffusion as particles will not be inhaled as aggregates or droplets in the range of micrometer or upper submicrometer rather than nanometer range. Clearance of particles mainly depends upon many factors like their size, site of deposition (barrier thickness increases from lower to upper airways), their solubility in airway fluid, surface morphology, and physiological conditions of individuals. Insoluble particles eliminate out by mucociliary clearance within 24–48 hrs, which are deposited in the conducting airways as it is the main mechanism for clearance in the conductive zone; particles that are able to cross mucus layer can only escape mucocilliary clearance. Expectoration and swallowing help to remove the mucus carrying particles that is being transported toward glottis through cilia [89]. If the interaction of particles with the airway generation number and interaction with inner lung surface increase due to the increase of pathway length, then the retention time of inhaled particles increases. Clearance from alveolar region is bit complex and usually slower. In general, two types of clearances are there: absorptive and nonabsorptive. Particles, which are soluble in respiratory fluid, can easily penetrate the epithelium and are rapidly taken up into the lymph or blood within minutes, and in the case of removal by dissolution for poorly soluble particle, it may take months or years. Insoluble particles, which are deposited in the alveolar regions, follow the macrophage phagocytosis mechanism for clearance. After the deposition, if macrophages internalize the particles, then they are disintegrated by lysosomal enzymes or are taken up into lymph or are removed by mucocilliary clearance [89]. If clearance does not occur from the lung surface, then the retention in the lungs will be prolonged, which contributes to accumulation of the inhaled particles in the airway tissues, which ultimately results in systemic uptake. Smaller particles can generally penetrate epithelial cells as well

as interstitium by migration via lymphatics to hilar, pleural, and more distant lymph nodes or by absorption into the blood capillaries, and the particles of range 0.5–10 μm remain on the alveoli as well as epithelial surface [90]. As beta-2 receptors are located on smooth muscles (trachea down to conductive airways), beta 2-agonists for the treatment of asthma have to be deposited in these regions. For the treatment of COPD (Chronic Obstructive Pulmonary Disease), anticholinergics have to reach the central airways as the receptors are located there with decreasing number from smooth muscle of large airways down to the smaller bronchial airways. On the other hand, steroid receptors are found throughout the respiratory system (including small peripheral airways). However, the release profile of carrier system defines the site of deposition. Prolonged release features are limited after bronchial deposition as the clearance is more efficient and faster in bronchiole than in alveoli. Due to unintended adverse effects and low availability at the site of action, the absorption of a locally administered drug is not desirable. Drugs of low lipophilicity and high molecular weight have low absorption rates. Combination of lymphatic uptake and local effect of a pulmonary applied anticancer agent could be an area of interest. Lung cancer cells generally spread to mediastinal lymph nodes and intraparenchymal pulmonary. Therefore, the lymphatic uptake of an anticancer drug might inhibit metastatic progression to the lymph nodes. Moreover, the cases related to tuberculosis remain enormous. According to survey, it has been found that 1.4 million people died from among 8.7 million new cases of tuberculosis (TB) in 2011 [91]. With the local effect of antituberculosis drug as well as systemic uptake, the systemic effect of the drug can be a promising therapy approach [92].

10.5.5 Lipid-Polymer Hybrid Nanoparticles

Polymers can be used as an alternative to lipid nanoparticles and lipid-based nanocarriers by forming lipid-polymer hybrid (Fig. 10.4) nanoparticles [93]. Polymers are enormously gaining interest for pulmonary drug delivery. Numerous advantages are associated with them, such as high encapsulation of drug, modified surface properties, protection from degradation of drug, long shelf life, and prolonged drug delivery.

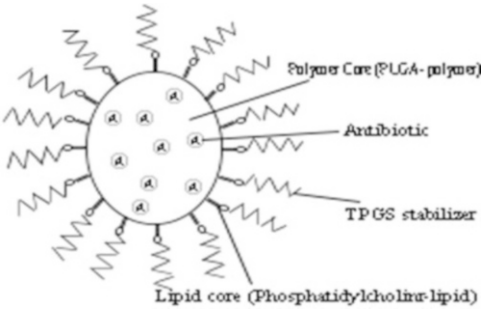

Fig. 10.4 Antibiotic-loaded lipid-polymer hybrid nanoparticle

Several polymers are used for therapeutic actions, but some are tested for inhalation delivery, which include Poly(lactic-co-glycolic acid), poly(lactic acid), poly (ε-caprolactone) (PCL), gelatin, alginate, and chitosan base. They are basically chemically modified in order to make them biodegradable. A lipid-hybrid nanoparticle can be prepared by enveloping poly(lactic-co-glycolic acid) with PC-stearylamine or Phosphatidylcholine (PC) layers for inhalation delivery. The shape of the resulting nanoparticle was spherical, and the particles were adsorbed on the carrier (like chitosan particles). It was suggested that the aerodynamic diameter of particles should be in a range of 1–5 μm as if the diameter is below 1 μm, then there is a probability of particles to be exhaled back and if it is larger than 5 μm, then there could be a chance of deposition of particles in the throat and mouth regions instead of lungs. According to a literature report, Beck-Broichsitter et al. [94] have performed a study to see the influence of polymer-based nanoparticles on pulmonary surfactant and its surface characteristics and also compared the effect of biodegradable and synthetic polymeric nanoparticles. They observed dose-dependent changes in surface tension of pulmonary surfactant [94]. Apart from this study, there are numerous studies performed on polymeric-nanoparticles. As an instance, Paclitaxel (an anticancer drug)-loaded polymeric nanoparticles were prepared by combining a polymer poly(ethylene oxide)-block-distearoyl phosphatidylethanolamine (DSPE) and Polyethylene glycol (PEG5000) where it was observed that the drug shows a better absorption in intratracheal instillation route as compared to IV administration [95]. According to few studies, it can be stated that encapsulation efficiency of a drug can be improved by polymeric nanoparticles along with an increased uptake by the modification of particle surfaces. For instance, PEGylation of particles can improve the encapsulation efficacy, improve uptake, and prolong the release of the drug as PEGylation evades the macrophages and, therefore, avoids engulfing by phagocytosis [96]. Apart from anticancer drugs, multiple studies were performed on antioxidants and anti-inflammatory agents incorporated into polymeric-based nanoparticles. As an example, an anti-inflammatory agent, HydroxyBenzyl Alcohol (HBA)-incorporated Polyoxalate (HPOX) nanoparticles (formulated using PLGA-polymeric-based nanoparticle), was administered through intratracheal route in a group of ovalbumin-induced asthma mice models, and they found attenuation in inflammatory responses in the group due to a decrease in the level of pro-inflammatory cytokines. Therefore, polymeric nanoparticles might have sufficient potential for treating asthma and airway inflammation [97].

10.5.6 Micelles

A promising drug carrier system comprises efficient drug loading capacity, release properties, low toxicity, and long shelf life. Colloidal systems (like vesicles, liquid crystal dispersions, micellar solution, and nanoparticle dispersions) proved to be a good carrier in pulmonary delivery. In micelle, drugs are trapped in the core and transported at a concentration even more than their intrinsic water solubility. Micelle is surrounded by a hydrophilic shell that protects the contents, and meanwhile, the

chemistry of the shell prevents the recognition by reticuloendothelial system, which further avoids early elimination from the bloodstream. Stability and spatial and temporal control of the micelles can be improved by using cross linking molecules through chemical techniques. Hydrophobic drugs, proteins, and DNA can be encapsulated into polymeric micelles (formed by an amphiphile macromolecule self-assembling to nanoscopic core in the aqueous environment) to deliver the drug to show their efficacy to their target site. These polymeric micelles resemble the functional and structural characteristics with natural transport system (like lipoprotein and virus). Multifaceted chemistry of polymeric micelles provides opportunities to formulate appropriate polymeric carriers of nanometric range for individual delivery requirements. Formulating nanoengineered polymeric micelles can be used against drug resistance problem by modifying their chemistry to manipulate encapsulation, to modulate release pattern, and for biodistribution and cellular interaction of P-glycoprotein substrate to resistant tumors. Asthma and chronic pulmonary obstructive disease can be treated by polymeric micelles as they have an ability to evade mononuclear phagocytic system because of their heavy hydrophilic outer shell and can also prolong the drug release [98]. For treating chronic pulmonary obstructive disease, hydrophobic corticosteroids (like beclomethasone dipropionate), which are unable to pass through mucus layer, can also be delivered by polymeric micelles as micelles of nanometric size range can easily penetrate via the mucus layer associated with bronchial inflammatory diseases directly to reach the receptors present in the epithelial cells, and supporting to this context, an additional report was found where it was stated that a mammalian-secreted Phospholipase A2 has an ability to degrade pegylated phosphatidylethanolamine [99, 100]. To this context, Gaber et al. [101] studied the efficiency of using poly-(ethylene oxide)-block-distearoyl phosphatidylethanolamine (mPEG-DSPE) polymer to formulate beclomethasone dipropionate-loaded micelles with high entrapment efficiency and less than 5 μm mass median aerodynamic diameter and also elaborated their prolonged release properties. The physicochemical properties, entrapment efficiency, outcomes of drug-polymer molar ratio on particle size, in vitro inhalation pattern, and release profile of polymeric micelles were also evaluated and allowed them to conclude the pharmacological interest of this kind of nanocarrier [101].

10.5.7 Magnetic Core-Shell Nanoparticles

Aerosolized techniques in the treatment of different respiratory diseases such as asthma, respiratory infection, lung cancer, and chronic obstructive pulmonary disease were tried by many researchers [102–105]. Verma et al. [106] also investigated potential of magnetic nanoparticles (MNPs) for delivery of quercetin in the treatment of lung cancer through nebulization technique. Quercetin, a flavonoid compound, which can inhibit the growth of cancer cell including lung cancer, was loaded inside the magnetic core of nanoparticles, and outer shell was fabricated from the biocompatible polymer, poly(DL-lactic-co-glycolic acid) (PLGA). Coating of polymer not

only improved biocompatibility of magnetic nanoparticles but also concealed iron oxide or magnetite from oxidation. Due to coating, size of MNPs increased from 9.6 to 53.2 nm, whereas the hydrodynamic diameter of MNPs increased from 54.3 to 293.4 nm. Images had been analyzed by transmission electron microscopy and dynamic light scattering, respectively. Human A549 lung epithelial cells were treated with magnetic core-shell NPs to check their cytocompatibility and intracellularization ability. Cell-based automated microscopy revealed no abnormalities found in morphology and in structure of cytoskeletal protein actin of treated cells. Intracellularization of MNPs was time-dependent process. However, MNPs at a concentration of 250 μg/ml was found to be toxic with reducing number of viable cells up to 25%. In vivo biocompatibility study was performed on mouse model for both coated and uncoated MNPs. Glutathione level of homogenized lung samples enhanced dramatically after 1-day exposure to this new drug delivery system probably due to nature of invasiveness through intratracheal pipe. Whether drug delivery through nebulization method has affected the particles has been investigated by Verma et al. [106]. It has been found that particles remained intact as prepared, which was confirmed by photoluminescence spectra at 380 nm before and after nebulization. Therapeutic efficacy of these drug delivery systems was carried out on human A549 lung carcinoma cells, and significant reduction of lung carcinoma cells was detected. So, it was confirmed by the researchers that necessary surface engineering on MNPs can deliver the drug even through narrow intratracheal path via aerosol therapy and maintains their biocompatibility and therapeutic efficacy. This kind of system can be used to treat different respiratory diseases where a systemic approach may not be feasible.

10.5.8 Mesoporous Silica Nanoparticle

Recently, silica nanoparticles have been not only gaining importance to be used through the intravenous route but also recognized as a useful drug delivery carrier for use through pulmonary route to treat respiratory diseases [107]. Bioerodibility, biocompatibility, controllable particle size, large surface area, and different surface functional ability make them suitable for use through pulmonary route. Due to hydrophilic property, dispersion of these particles is possible in aqueous media, and also, surface functionalization property enhances this property, which is essential for in vivo application. The large surface area of these porous nanoparticles enables them to load a high amount of drug in this carrier [108]. Gulin-Sarfraz's group in the year of 2019 worked on mesoporous silica nanoparticles (MSPs) to deliver dexamethasone through pulmonary route to treat airway inflammation. The experiment was performed using mouse model [109]. At first, both large MSPs and small MSPs were synthesized to load maximum drug even up to 1:1 ratio of drug and carrier. Then, this core of drug and MSP was coated with polyethylene glycol–polyethylene imine (PEG–PEI) copolymer. The coating was done with the aim of enhancing the biocompatibility and aqueous dispersibility of this system. Enhanced biocompatibility will preclude any kind of unwanted interaction of these drug

delivery systems with pulmonary route, and enhanced dispersibility helped in the nebulization process. A similar approach of coating the core with similar co-polymer (PEG-PEI) was taken by another research group [110]. In Gulin-Sarfraz's [109] study, melphalan (MEL)-induced airway inflammation mouse model and lipopolysaccharide (LPS)-induced airway inflammation mouse model were exposed to this nanoparticle-based drug delivery system in order to know its therapeutic potential. Through Aeroneb™PRO, SCIREQ® nebulizer, drugs with its carrier were aerosolized. As a control group, mouse was exposed only to free drug without MSP nanoparticles. Electron microscopy images revealed that synthesized particles were monodispersed and spherical with sizes of 1 μm and 200 nm for large MSPs and small MSPs, respectively. In vitro release kinetic study showed enhancement of dexamethasone solubility due to molecular dispersion of drug particles on the silica matrix. This similar consequence was observed by another group, Martin et al. [111] working on silica nanoparticle with entrapped prednisolone. Cellular inflammation and neutrophil counts of both mouse models were reduced due to administration of dexamethasone-loaded silica nanoparticles, and the response was the same as free drug. Though only half the amount of the drug was delivered through this delivery system, the effect was similar to the mouse model treated with free drug. This study has shown the immense possibility of this nanocarrier to exploit in the treatment of respiratory diseases through the pulmonary route.

10.6 Limitation/Potential Risk of Nano-Based Formulations

Nanotechnology involves the concept of design and exploitation of materials at nanoscale levels to form products that exhibit novel properties [112]. Without enough categorization and characterization, it is truly challenging to analyze any individual study results by several researchers. It is nearly impracticable to compare the results from different studies, even in cases where the identical nanoparticle has been explored. Hence, the aptitude to categorize significant parameters that might manipulate pulmonary toxicity is being difficult. Even though there is no universally recognized standard set of parameters that is deemed compulsory for nanoparticle estimation, its method of synthesis, shape, size and its size distribution, crystal structure, composition, purity, aggregation and agglomeration status, dissolution, surface area, and other surface characteristics play a vital role. Unfortunately, including all these parameters in publications relating nanoparticle pulmonary toxicity studies appears to be rare.

In general, phospholipid-based pulmonary formulations (particularly liposomal systems) have been found to be physiologically acceptable in numerous studies, primarily due to the similarity of the phospholipids used with pulmonary surfactants. This is a good sign for supporting the future development of pulmonary formulations.

Occupational or nonoccupational exposure to nontherapeutic nanoparticles is rising with time. Our existing domain of knowledge regarding the potential health effects of nanoparticles may be limited, but is sufficient to suggest that they may

bring to bear adverse effects at their portal of entry, marking lungs at high risk. Airborne nanoparticles are known to have genotoxic or carcinogenic effects because of the certain metals, carbons, etc used to make them. Some metal particles can lead to reactive oxygen species (ROS) generation that causes oxidative stress and DNA damage in the body [113–115].

When inhaled, the therapeutic nanoparticles are found to be distributed to the lungs, heart, spleen, liver, and even in the brain [116]. It is the basic body physiological system to clear any such inhaled nanoparticles present in the alveolar region via phagocytosis by macrophages due to chemotactic attraction of the alveolar macrophages to the deposition site [117, 118]. The average half-life ($t_{1/2}$) for nanoparticles in the respiratory tract is ≈700 days in humans, making it a site of accumulation depending on their size and shape [119]. Once inhaled, nanoparticles slowly show lung toxicity, like generation of oxidative stress, cytotoxicity, DNA damage, and inflammation leading to fibrosis and pneumoconiosis. A major change of systemic toxicity arises due to passive targeted drug delivery of therapeutic nanoparticle by pulmonary route of administration. This is because nanoparticle by virtue of its small size can penetrate the physiological barriers and even the blood–brain barrier (BBB).

Prudence dictates that nanomedicines might have different toxicity profiles from their macromolecular counterparts. Nanomaterials, by virtue of their extreme fineness, often exhibit novel properties, and therefore, it may be extrapolated to their toxicity potential. Limited prediction capability may exist today in delineating nanotoxicity, but risk evaluation and management will be the most important agenda in the short-term canvas of goals in the development of nanomedicine. Therefore, long-term studies on existing and future nanomedicines are a necessity to clearly assess their risks and benefits.

10.7 Summary and Future Prospects

In the present chapter, we have described and focused on several types and aspects of pulmonary nanomedicines and shown how they may be useful in modifying the outcome of pulmonary diseases. Despite having potential upsides in using medicines through pulmonary route, the industry seems to be reluctant to take the risk of development of clinical nanoscale products for pulmonary delivery. This is perhaps due to the lack of success in several clinical trials and poor response of a few marketed products. The most successful pulmonary nanomedicine appears to be those utilizing antimicrobial drug payload for local inhalation delivery. However, a lot needs to be done, particularly, in the field of oncological products for pulmonary delivery and for utilizing the route for systemic delivery of therapeutic agents. The regulatory framework is also quite incoherent, and only recently, FDA and other regulatory bodies have been waking up to the need for proper guidelines in the field of nanomedicine. In our opinion, a sleeping giant in the form of nanoscalar pulmonary delivery platforms is awaiting exploration and exploitation. It is hoped that

future researchers, in both academia and industry, would take advantage of the pulmonary route for translational research in this nascent field.

Acknowledgments The authors acknowledge the Management of Dr. B. C. Roy College of Pharmacy & AHS, Durgapur, WB, for support and Dr. Paulomi Pal, for help through sharing her experience in the field of pulmonary drug delivery during the preparation of this manuscript.

Conflict of Interest The authors declare that there are no conflicts of interest. All figures and tables are original and self-made.

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Nanoemulsion Delivery of Herbal Products: Prospects and Challenges

11

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Abstract

Herbal extracts from selected medicinal plants are frequently used to treat specialized health conditions and are preferred over synthetic medicines because of their increased therapeutic efficacy and fewer adverse effects. Nanoemulsions are presently gaining popularity as convenient carriers for the delivery of herbal lipophilic bioactives as they have the capability to dissolve huge quantities of poorly soluble drugs, provide extended release of the encapsulated drugs, and can deliver these drugs through different routes like oral, transdermal, topically on skin and mucous membranes, etc. Studies conducted previously found varied applications of herbal nanoemulsions in passive and active tumor targeting; transdermal, oral, ocular and nose-to-brain drug delivery, management of

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M. K. Das, Y. V Pathak (eds.), *Nano Medicine and Nano Safety*,
https://doi.org/10.1007/978-981-15-6255-6_11

vector-borne diseases, and in areas of food technology, etc. Safety considerations during production and quality control phases of herbal nanoemulsions particularly, and all nano-based formulations, in general, are an issue that needs urgent attention. Thus, looking into the prospects of nanoemulsion-based delivery of herbal drugs; it might absolutely have a probable future in enhancing the activity as well as surmounting the difficulties correlated with plant-extracted medicines. The present review focuses on the significance of nanoemulsions in the delivery of herbal bioactives and emphasizes on the various aspects of their formulation development, applications in drug-delivery systems, advantages and challenges, safety and regulatory issues associated with it, and future prospects of nanoemulsion-based herbal drug delivery.

Keywords

Nanoemulsion · Herbal · Drug delivery · Solubility · Bioavailability

11.1 Nanoemulsion Systems for Drug Delivery

Nanotechnology-devised formulations presently seem to be an attractive area for researchers as a hopeful alternative for the treatment of different diseases [1]. Nanoemulsions, among other nanoformulations, are thermodynamically more stable liquid dispersions of oil, water, surfactant, and co-surfactant with a droplet size of 20–200 nm and an appearance of a translucent or transparent liquid [2, 3]. Nanoemulsions were first formulated in the 1940s and are classified as water-in-oil (w/o), oil-in-water (o/w), and bicontinuous nanoemulsions. In w/o nanoemulsions, water droplets are distributed within the oil phase whereas o/w nanoemulsions have oil droplets distributed within the aqueous phase and bicontinuous nanoemulsions consist of oil droplets and water interdispersed within the system (Fig. 11.1) [4]. Pertaining to their smaller particle size, enhanced bioavailability, ease of preparation, bioefficacy, and kinetic stability, nanoemulsions are currently attaining popularity as suitable carriers for the delivery of lipophilic materials [5–7]. The water in nanoemulsions being bound in the structure itself,

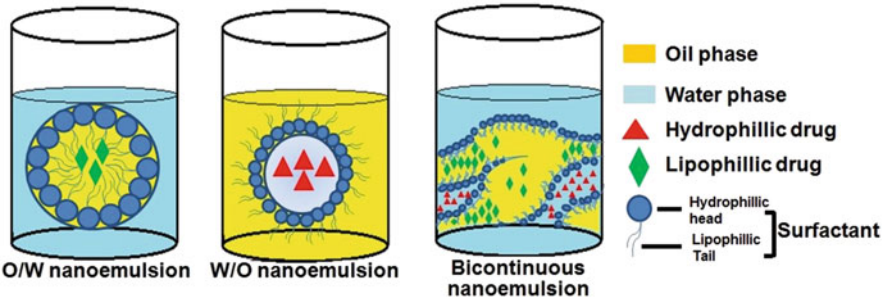

Fig. 11.1 Types of nanoemulsions

there is no water available for microbial growth, and hence, they are considered self-preserving antimicrobials [8]. They can deliver drugs through various routes like oral, transdermal, topically on mucous membranes, etc. They serve as excellent vectors for drug delivery as they have the capacity to dissolve huge quantities of poorly soluble drugs and also an ability to mutually shield and secure drugs from hydrolysis and enzymatic degradation. Nanosized emulsions have applications in food and cosmetic technology, vaccine delivery, cancer therapy, cell culture technology, as prophylactics in disinfectant cleaner, bio-terrorism attack, for improved oral delivery of formulations containing poorly soluble drugs, ocular, otic, intranasal, parenteral, and pulmonary delivery of drugs [9, 10]. Herbal medicines have enjoyed immense popularity throughout the globe since ancient times [11]. Herbal bioactives have innumerable health benefits but restricted therapeutic potential due to their short half-life and little bioavailability profile. These plant-derived bioactives are either hydrophilic or lipophilic by nature. Hydrophilic molecules have little absorption via lipid membranes, which reduces their biological potency and pharmacokinetics. A large molecular size and little membrane permeability are the main factors that limit their therapeutic utilities. Since last decade, nanotechnology has started playing a pivotal role in producing specific nanocarriers to intensify the therapeutic effect of herbal drugs. It offers several advantages over conventional drug-delivery platforms [12]. Nanoemulsions, are an ingenious platform, among other novel nano approaches, as they can be fabricated with an extensive range of liquid lipids and surfactants [13]. Nanoemulsions direct herbal bioactives to a specific target site and enable blood-plasma concentration for longer periods, something that conventional drug-delivery systems have failed to achieve. They preserve the bioactives from gastric degradation and increase their stability by enclosing them within water in oil (w/o) or oil in water (o/w) nanodroplets. They extend the bioavailability and permeability of less-bioavailable phytopharmaceuticals across the dermal and gastro-intestinal membranes [14, 15]. The limited solubility of hydrophobic herbal molecules in aqueous media is another crucial challenge while developing an appropriate formulation. In such cases, nanosized droplets of nanoemulsions provide an advantageous environment for the uptake of lipophilic herbal bioactive molecules [16, 17]. Thus, nanoemulsions are better options in the evolution of innovative drug-delivery systems with increased relevance. These lipid-surfactant-based formulations, by virtue of their composition and functionality, are capable of communicating across the body's natural barriers thereby enabling maximum drug absorption [18]. The aim of this chapter is to present a brief perspective of the delivery of herbal drugs with nanoemulsion as the carrier system and the challenges and prospects associated with such applications.

11.2 Herbal-Based Nanoemulsion System

Since the beginning of history, herbal preparations like medicinal plants and their essential oils have held burgeoning interest for researchers. Herbal preparations from selected medicinal plant parts are generally used to treat specific health conditions

[19, 20]. Examples of some major plant-derived drugs include quinidine and quinine from Cinchona spp., digoxin from Digitalis spp., atropine from *Atropa belladonna*, codeine and morphine from *Papaver somniferum*, vinblastine and vincristrine from *Catharanthus roseus,* etc. Around 60% of anti-infectious and anti-tumor drugs currently in the market or under clinical trials are estimated to be of natural origin [21]. In addition, drugs such as cannabinoids, physostigmine, muscarine, yohimbine, colchicines, forskolin, etc. all obtained from plants are principal tools used in physiological, pharmacological, and biochemical studies [22].

Pharmacology of medicinal plants has been extensively reported in reviews and there has been a growing enthusiasm on the advancement of novel drug-delivery systems for herbal drugs [20, 23]. Herbal medicines are used more because of their increased therapeutic effects and fewer adverse effects when compared with modern synthetic medicines [24]. Multifunctional properties like antiaging, moisturization, photoprotection, astringent, antioxidant, antimicrobial, anti-irritant activities, etc. are correlated with each other and occur naturally in certain botanical extracts. Antiaging properties are commonly obtained from centella, pycnogenol, boswelia, tetrahydrocurcuminoids, and oleanolic acid extracts [25–27]; moisturization from retinoids, alpha hydroxy fruit acids, soy, black cohosh, aloe vera, and calendula extracts [28–30]; antioxidant and photoprotection from vitamins C and E, tea, polyphenols, curcumin, silymarin, resveratrol, ginkgo, genistein, and pomegranate fruit extracts [31–34]; astringent properties from arnica, cucumber [29]; and anti-irritant and anti-inflammatory properties from coriander seed oil and bisabolol [34]. Thanks to these characteristics, a herbal drug can now be formulated both as an aqueous solution or as a nonaqueous extract diluted with water sufficiently before administration, or even administered as such. An innovation always improves both the aesthetics and accomplishment of any pharmaceutical product [35]. Poor absorption, solubility, bioavailability, stability, and high metabolism cause many herbal drugs and extracts to have a low in vivo activity.

Formulation of herbal drugs by nanodelivery systems represents enhanced bioavailability, and hence, is a favorable and acceptable tool. Novel nano-based herbal formulations have impressive advantages over traditional formulations, which include increased solubility, bioavailability, stability, enhancement of intracellular uptake, modification of pharmacokinetics and bio-distribution, sustained delivery, etc. Herbal nanoemulsions are stable, transparent, highly dispersed, and easy to prepare. The stability of herbal oils when formulated as nanoemulsions is enhanced when exposed to high temperatures, by oxidation by atmospheric oxygen, and electromagnetic radiation, thereby decreasing losses due to decomposition and evaporation of the active constituents. Furthermore, they are better absorbed by cell membranes because of their nanoscopic dimensions [24, 36–40]. Therefore, the inclusion of herbal extracts into lipophilic bioactive nanoemulsion systems is thought to have comprehensive value in pharmaceutical, agricultural, cosmetic, food, and beverage products. Designing herbal nanoemulsions with formulation potency has significance both in terms of industrial and academic research [23, 41]. Apart from its intended sustained release, formulating the herbal drug into a nanoemulsion will also improve skin and mucous membrane penetrability

of drugs, increase the stability of hydrolyzed matter, and reduce the tissues' stimulus to the drugs [23].

11.2.1 Previous Research Studies on Herbal Nanoemulsions

Nanoemulsion formulations containing phytoactives such as Camptothecin, Coixenolide oil, *Brucea javanica* oil, and zedoary oil have been previously reported [23]. Shen et al. [42] demonstrated enhanced in vivo absorption of Colchicine nanoemulsion on the human intestinal milieu. Silva et al. [43] have tried incorporating Genistein, possessing anticancer properties, into topical nanoemulsion formulations composed of water, egg lecithin, and triglycerides with spontaneous emulsification and improved pharmacological activity. Wang et al. [44] have demonstrated increased anti-inflammatory activity of Curcumin formulated as an oil-in-water nanoemulsion. Zulli et al. [45] showed nanoemulsion enhances encapsulation of Coenzyme Q10 or Ubiquinone concentration in the dermis as compared to traditional emulsion formulations. Several other bioactive- and plant extract-based nanoemulsions of berberine, capsicum oleoresin, citronella oil, eucalyptus oil, neem oil, triptolide, etc. have previously been reported [3, 46, 47]. Some additional herbal nanoemulsion formulations are depicted in Table 11.1.

11.3 Challenges and Advantages of Herbal Nanoemulsion Products

Herb-derived medicines have now become a core field of research for inventors and scientists all over the world due to their superior therapeutic value and lesser adverse effects as compared to contemporary medicines. Though herbal bioactive molecules have tremendous advantages over synthetic drugs, there have been common limitations due to their low solubility, low permeability, low bioavailability profile, and shorter half-life, which ultimately lead to lesser therapeutic potential [14]. Plant-derived molecules are either hydrophilic or lipophilic in nature. Due to weak absorption via lipid membranes, hydrophilic plant bioactives show decreased bioavailability and a pharmacokinetic profile. On the other hand, larger-sized bioactive molecules have limited bioavailability due to their low membrane permeability. This is a major barrier for the effective use of such herbal bioactive compounds against several health complications and conventional drug-delivery schemes have failed to attain these particular requirements for herbal products [24]. There is a possibility that these challenges might be overcome by utilizing the nanoencapsulation process of nanoemulsions that entraps the active herbal drug inside the lipid core. Gastric degradation of a herbal drug is another major disadvantage to the conventional oral delivery of such drugs. In such cases, nanoemulsions might be a good choice for formulation experts to protect plant bioactives and enhance their membrane diffusion to get an extended release of the encapsulated product. Nanoencapsulation of herbal drugs provides a large surface to volume ratio, which improves drug-tissue

Table 11.1 Some herbal nanoemulsions and their properties

Sl. no.	Herbal nanoemulsion formulation	Biological activity	Method of preparation	Route of administration	Reference
1	Phyto nanoemulsion containing black seed and wheat germ oil	Antioxidant capacity, wound healing, and radioprotective activity	Homogenization and emulsification	Dermal and oral	[20]
2	Neem oil nanoemulsion	Used in medicine, soil agriculture field, aquaculture, etc.	Emulsification, homogenization, and sonication	–	[3]
3	Rosemary, laurel, thyme and sage oil incorporated nanoemulsion	Antimicrobial, antioxidant, and preservative for fish fillets	Emulsification, homogenization, and sonication	–	[7]
4	Vitex agnus-castus extract-based nanoemulsion	Mastodynia or mastalgia and menstrual cycle disorders	Emulsification	Oral	[24]
5	Silymarin nanoemulsion	Hepatoprotective	High-pressure homogenization	Oral	[48]
6	Piplartine nanoemulsion formulation	Anticancer	Emulsification and homogenization-sonication	Oral	[49]
7	Rutin nanoemulsion	Anticancer	Aqueous titration	Oral	[50]
8	*Opuntia* nanoemulsion	Herbal cosmetic	Emulsification	Dermal	[51]
9	Tarragon nanoemulsion	Larvicidal	Emulsification	–	[52]
10	Neem oil nanoemulsion	Larvicidal	Emulsification	–	[53]
11	*Foeniculum vulgare* nanoemulsion	Antidiabetic	Emulsification	Transdermal	[54]

distribution and enhancement in the reticuloendothelial system (RES) uptake as well as enhancement in the permeability and retention (EPR) effect. As compared to the micro and conventional emulsions, nanoemulsions bear further viscous or gel-like texture with very low fat and droplet concentrations [5].

Nanoemulsion as a herbal drug-delivery carrier affords advantages in improvement of drug solubility and the absorption profile, increase in drug loading, controlled drug release with the ability to protect the drugs from hydrolysis and enzymatic degradation, reduced patient variability, and makes them ideal

drug-delivery carrier through encapsulation [10, 55]. Nanoemulsion enhances the adequacy of herbal drugs to that specific target site and manages the blood-plasma level concentration for a longer period with a minimized frequency of dose and side effects [56]. Development of multidrug resistance (MDR) against conventional and novel cancer chemotherapeutic agents is a primary challenge in the current medicinal scenario [57, 58]. Nanoemulsion of curcumin-paclitaxel encapsulated in flaxseed oil (*Linum usitatissimum* L.) enhanced apoptosis and led to downregulation of the MDR protein, P-gp, and inhibited the nuclear factor kappa B (NFκB) pathway, against the drug-resistant ($SKOV3_{TR}$) human ovarian adenocarcinoma cells [59, 60].

11.4 Applications of Herbal Nanoemulsions

11.4.1 In Drug Delivery

11.4.1.1 Passive and Active Tumor Targeting

Nanoemulsion is a substantial tool in the nano-technological field, outlined for clinical and therapeutic operation. Among other nanocarriers, nanoemulsions are now broadly anticipated as effective delivery systems for the targeted delivery of lipophilic neoplastic drugs. Advantages of such nanoemulsion systems include biocompatibility, drug encapsulation and controlled release, biodegradability, thermodynamic stability, etc. [61, 62]. With the droplet size ranging within submicron dimensions, colloidal nanoemulsions can surmount the anatomical and physiological obstacles during drug delivery in severe complications such as cancer [63]. Herbal chemotherapeutic drugs, through active targeting, improve the selectiveness of cellular uptake and cytotoxicity via receptor-mediated endocytosis. The nanometric size of the nanocarriers first allows passive targeting to the inflamed tissues and consequently to tumors by extravasation, which is called the EPR effect [64]. Enhancement of drug selectivity to cancer cells so as to avoid side effects in normal cells, enhancement of drug accumulation and drug efficiency, and control of drug release are the major advantages of nanoemulsions while formulating conventional chemotherapeutic drugs when compared to a nontargeted nanoparticle platform [65]. Li et al. [66] developed an oil-in-water nanoemulsion incorporating berberine hydrochloride, which significantly improved the absorption and oral bioavailability of the drug berberine in a rat model. The nanoemulsion helped to increase the transport and decrease the efflux on Caco-2 cell monolayers under permeability study. The absorption mechanism of nanoemulsion in rat intestines may be passive transport [66]. However, the limitation in active targeting is that it acts only on certain types of cancerous cells that express specific receptors on its surface. Hence, selection of nanoparticles to the target site depends on the types of target proteins or receptors available on the cancer cell surfaces [67].

11.4.1.2 Topical/Transdermal Delivery

Due to abundant skin surface for absorption as well as the limited first-pass effect, transdermal delivery remains a highly enviable route of administration [68]. Stratum

corneum is the principal barrier for delivery of transdermal drugs. In topical/transdermal drug delivery, nanoemulsions offer extended release of herbal drugs and boost viscoelasticity and hydration and of the skin [69, 70]. Rice bran oil (*Oriza Sativa*) containing high levels of antioxidants is a widely used component of anti-ageing and sunscreen creams and also in nanoemulsions by virtue of its anti-irritant effect [71]. Rice bran oil nanoemulsions possess great hydration and moisturising properties and help in maintaining the normal skin pH in the patients suffering from psoriasis and dermatitis [72]. Sharma et al. [73] successively prepared a nanoemulsion gel system, incorporating resveratrol, a type of natural phenol, to enhance the permeability and antioxidant activity to the ultraviolet (UV)-induced oxidative skin damage. Nanoemulsion of Paclitaxel with 5-Aminolevulinic acid showed promising antipsoriatic effects and improved transdermal permeation in vivo, in vitro over topical formulations such as ethosomes and liposomes [74]. Permeation enhancers like anethole, menthone, and eugenol are reported as highly effective, safe, and proven to rupture the skin barrier for nanoemulsion-based topical delivery of the drug, Valsartan. This nanoemulsion has potential application as a new transdermal therapeutic system for the management of hypertension [75]. Nastiti et al. [76] investigated the follicular delivery of caffeine with eucalyptol, a penetration enhancer in the form of nanoemulsion. This eucalyptol-based nanoemulsion increased the penetration of caffeine 43 folds as compared to control [76]. Numerous examples depicting transdermal/topical use of herbal nanoemulsions come from the cosmetic industry. Nanoemulsion systems have constituted hydrating creams, hair coloring products, etc. L'Oreal, a highly popular cosmetic company, already has dozens of proprietary technologies based on nanoemulsions alone [77]. A table (Table 11.2) in this regard has been given below:

11.4.1.3 Oral Delivery

Oral delivery is considered to be the biggest challenge for herbal drugs due to their degradation in the gastric pH, poor permeability, and water solubility and rapid metabolism [42, 86, 87]. Andrographolide is a herbal drug well known for its anti-inflammatory property. Its low water solubility and low oral bioavailability are a major hindrance to its therapeutic potential. When incorporated in nanoemulsion, andrographolide is effective for improving the oral bioavailability and thus exhibits greater potential in the management of inflammatory bowel disease [88]. Another example of improved oral bioavailability upon nanoemulsion-based encapsulation includes the drug Silymarin, a mixture of flavolignans having hepatoprotactant activity, whose oral bioavailability in rats increased by six and four folds as compared to silymarin nanosuspension and other commercial silymarin products [89]. Oral bioavailability can be achieved by preventing the nanoencapsulated drug with an efflux transporter such as P-glycoprotein [90, 91]. Curcumin-loaded nanoemulsions led to interesting therapeutic potentialities in Alzheimer's disease by inhibition of the Amyloid beta peptide oligomerization [92, 93]. Eugenol, being rich in terpinoids, inhibits the P-glycoprotein mediated transport. Hence, eugenol-loaded nanoemulsion has been proposed for enhanced oral delivery of colchicines, a P-glycoprotein substrate [42, 77].

Table 11.2 Proprietary nanoemulsion-based technologies by L'Oreal, a French personal care and cosmetic company

Sl no.	Technology	US Patent no.	Reference
1	Nanoemulsion based on phosphoric acid fatty acid esters and its uses in the cosmetics, dermatological, pharmaceutical, and/or ophthalmological fields	US 6,274,150 B1	[78]
2	Nanoemulsion based on ethylene oxide and propylene oxide block copolymers and its uses in the cosmetics, dermatological, and/or ophthalmological fields	US 6,464,990 B1	[79]
3	Nanoemulsion based on oxyethylenated or nonoxyethylenated sorbitan fatty esters, and its uses in the cosmetics, dermatological, and/or ophthalmological fields	US 6,335,022 B1	[80]
4	Nanoemulsion based on glycerol fatty esters, and its uses in the cosmetics, dermatological, and/or ophthalmological fields	US 6,541,018 B1	[81]
5	Nanoemulsion based on sugar fatty esters or on sugar fatty ethers and its uses in the cosmetics, dermatological, and/or ophthalmological fields	US 6,689,371 B1	[82]
6	Transparent nanoemulsion less than 100 nm based on fluid nonionic	US 5,753,241 A	[83]
7	Translucent nanoemulsion, production method, and uses thereof in the cosmetic, dermatological, and/or ophthalmological fields	US 6,902,737 B2	[84]
8	Aqueous photoprotective compositions comprising hydrophilic metal oxide nanopigments and vinyl pyrrolidone homopolymers	US 2010O254920A1	[85]

11.4.1.4 Ocular Delivery

In the case of ocular drug delivery, maintaining drug concentration at the site of administration remains challenging. Drug delivery into the ocular mucosa is an efficient strategy to extend ocular residence time with limited drainage, and to increase the bioavailability at the site of action [77]. Characteristics like drug penetration through corneal, precorneal, and blood-ocular barriers, frequent drug washout, frequency of dosing are the primary concerns associated with ophthalmic drug delivery in achieving therapeutic efficacy and patient compliance. Side effects such as miosis (cataractogenic) are associated with multiple dosing. In such cases, nanoemulsion-based drug-delivery systems might offer an improved interaction with the ocular mucosa and might also effectively penetrate the corneal and conjunctival epithelia [94]. A pilocarpine nanoemulsion formulation was developed by Naveh et al. [95] for the diagnosis of glaucoma. This nanoemulsion has been reported to prolong hypotensive action in normotensive rabbits for 11 hours (h) initially post instillation, which was further increased up to 29 h later [95].

11.4.1.5 Nose-to-Brain Delivery

Nanoemulsions have been thought to enhance the nose-to-brain delivery of drugs by virtue of their smaller particle size, easy preparation rate, better solubilization capability, and thermodynamic stability as compared to the other nanocarriers [96]. Excipients contained in nanoemulsions should have to facilitate permeation across the nasal epithelia to the brain. Chitosan is known to increase the permeability of nanoemulsions based on its transitory opening of the tight junctions in the brain. It has been reported that chitosan-coated nanoemulsion extends the nasal residence time of a drug, which facilitates high drug influx from nose to brain [50]. Kaempferol is a natural flavonol with anti-inflammatory, anti-oxidant, anti-tumor, and neuroprotective properties that might be beneficial for curing brain tumors such as gliomas. Kaempferol-loaded nanoemulsions have been reported for nose-to-brain targeting recently [97]. Thymoquinone, a volatile oil obtained from the *Nigella sativa* seeds, is well known for its antioxidant properties and is widely used in the treatment of cerebral ischemia. Recently, thymoquinone nanoemulsion was reported to have increased bioavailability and water solubility in the brain after nasal administration, as compared to intravenous application in Wistar rats with focal cerebral ischemia [98, 99].

11.4.2 Management of Vector-Borne Disease

Synthetic insect repellents or insecticides seem to produce persistent toxicity along with insect resistance, which has led to this immense shift of support from synthetic to green pesticides. Herbal repellents like eucalyptus, citronella, and cymbopogon have a great future in drug-delivery formulations because of their efficacy, safety, environmental sustainability, and pleasant aesthetics [100]. Nanoemulsions protect the plant essential oils from oxidation and increase their longevity. Leishmaniasis is an endemic infectious disease spread by the female phlebotomine sandfly. Conventional therapy with glucantime, pentostam, and pentamidine seemed to be ineffective in managing this disease [101]. Nanoemulsion-encapsulated Copaene, an antileishmanial agent has been recently reported as a new insight [1]. Most dengue control approaches include larvicidal drugs suspended or diluted in water. Hence, preparation of an active lipophilic natural product almost contemplated a technical challenge. In such circumstances, nanoemulsions appear to be feasible alternatives in solving this major problem. Development of Sucupira oil (*Pterodon emarginatus*)-based nanoemulsion is an alternative integrative practice for dengue control as reported by Oliveira et al. [102]. The nanoemulsion formulation of castor oil ensures higher efficacy as a larvicidal agent against *Anopheles culicifacies* when compared to conventional emulsion [103].

11.4.3 In the Food Industry

Currently, the food sector is bursting with several applications of herbal nanotechnology since it advocates newer insights to generate safer and healthier foodstuffs. In food technology, nanoemulsions provide enhanced physical stability, functionality, and optical transparency, which makes them very attractive to formulate lipophilic-active food ingredients [104]. Herbal essential oils, incorporated in nanoemulsions, penetrate swiftly into microbial membranes by virtue of their increased area per weight unit and are thus, becoming more popular over conventional emulsions due to the consumers' insistence of food free from synthetic supplements [105, 106]. For example, eugenol nanoemulsion masks the smell and increases the stability of food systems and could be an efficient antibacterial agent against food-borne pathogens [107]. However, the fact that methods of processing herbal essential oil nanoemulsions will also determine their final antimicrobial activity is to be commemorated during formulation preparation. The food industry has lipophilic bioactive compounds such as carotenoids, omega-3 fatty acids, polyphenols, flavonoids, phytosterols, tocopherols, etc., which have health-promoting and fortifying properties and might benefit (in terms of stability) from such nanoemulsion-based incorporation into foods.

11.5 Safety and Regulatory Issues of Herbal Nanoemulsions

11.5.1 Bioassays and Standardization of Herbal Drugs

The biological models, health claim, chemical analysis, and bioassay of many popular herbs are limited, as the active ingredient responsible for the plant's activity has not been identified. In addition, if the active ingredient of a herb was known, it would remain ambiguous whether the crude herb would be preferable to its purified active principle or not. Unfortunately, standardization techniques such as those defined for the herb, Digitalis, are not applicable for many herbs. In this regard, the absence of definitive information for traditional herbal preparations as digitalis leaf and opium has led to their replacement by drugs like digoxin and codeine, respectively. How can a herb be standardized when its active ingredients are unknown and there is no appropriate bioassay [108]?

11.5.2 Safety Consideration

Nanoemulsions are composed of generally regarded as safe (GRAS)-grade excipients. Toxicities associated with a drug-delivery carrier pose a hindrance in drug delivery as well as pharmacological effects. Ideal carriers efficiently encapsulate and deliver drugs at the desired sites, which are mostly physiologically inert and show predictable clearance from the body [109]. The Organization for Economic Cooperation and Development (OECD), started a strategic agenda in 2006, which

arranges a global convention for the consideration of fabricated nanomaterials, particularly their safety and risk assessment, and to promote the culpable advancement of these nanotechnologies. OECD's Working Party on Manufactured Nanomaterials (WPMN) advocates international collaboration on human wellbeing and environmental safety features of manufactured nanomaterials and centres on developing suitable approaches and methods to guarantee the safe utilization of nanotechnology [110]. More research regarding the development methods, safety and efficacy verification of nanoformulations, implementation of product labeling for transparency, and a better obtainment of quality data for regulatory functions are much needed [111].

11.5.3 Production and Quality Control

The Food and Drug Administraion (FDA) currently implemented a new frame work Quality-by-design (QbD) for the manufacturing and quality control of pharmaceuticals. The design of experiments (DOE) and method analytical technology are routinely employed as part of QbD principles from the raw material procurement stage to finished product [109]. It is essential to develop sturdy and documented process data for the preparation of nanoemulsion and other nanotechnology-based delivery systems to confirm QbD requirements [112]. The FDA provides guidance for nano-based products in terms of Pharmaceutical Development, Quality Risk Management, and Quality System for pharmaceutical products directed by the QbD [113]. In other legislative sectors such as food and cosmetics, there are scientific committees and expert groups, which address sector-specific risk assessment of nanomaterials. The scientific committee of the European Food Safety Authority (EFSA), which manages its food and feed sector, has refined the instructions on risk assessment and application of nanoscience as well as nanotechnologies in various food operations [114].

11.6 Future Prospects of Nanoemulsion-Based Herbal Drug Delivery

Phyto-formulation research, nowadays, popularly involves the development of nanodosage forms like nanoemulsions, polymeric nanoparticles and nanocapsules, solid lipid nanoparticles, phytosomes, liposomes, etc. Enhancement of solubility, bioavailability, pharmacological activity, stability, minimum toxicity, sustained delivery, enhanced tissue macrophage distribution, etc. are some advantages of the nanoemulsion-based delivery of herbal drugs. Thus, it is evident that the novel nanodelivery of herbal drugs has a probable future in enhancing the activity as well as surmounting the difficulties correlated with plant-extracted medicines [23]. Future development and research in the following areas might eventually provide newer and more fruitful methods for an enhanced therapeutic approach by nanoemulsion-based herbal drug-delivery systems.

11.6.1 Macrophage-Targeted Vaccine Delivery

Future advancements in immunogenomics and prognostic gene-derived toxicogenomics might, in the long run, provide new methods for evaluating an individual's sensitivity to nano-based drug delivery hence reducing the risk of possible immune-mediated side effects [115]. The human reticuloendothelial system consists of macrophages that play critical roles in determining immunogenicity and generate appropriate immune responses. Macrophages are known to rapidly recognize and clear particulate matter. This fact has contributed toward a realistic approach to design macrophage-specific drug targeting with nanocarriers. In this context, nanoemulsions can act as powerful adjuvants by either physical or covalent association with protein antigens [116–119]. Macrophages deteriorate the involved antigens and channel peptides into major histocompatibility complex (MHC) molecules (class I or II) after endocytic uptake of nanoparticles. Thus, the advancement of newer generation vaccines with nanoemulsions as adjuvants has considerable potential for either recombinant or fabricated peptide antigens that are both nonimmunogenic [74, 120].

11.6.2 Vascular Imaging and Drug Delivery

The National Cancer Institute in the USA has devised scientific programs with the goal of generating nanometer range multifunctional entities that can identify, analyze, and deliver therapeutic compounds and monitor the progress of cancer treatment. These include architecture and engineering of smart nanodevices and nanocarriers capable of consigning the biological and transformative diversity of the numerous cancer cells that constitute a tumor. Vascular imaging and drug delivery are two areas that nanotechnology is starting to modify the extent and methods of Nanoemulsion systems might come across as beneficial to obtain the full in vivo potential of nanotechnology in such targeted imaging and drug-delivery systems. A precise understanding of both the physiological and physicochemical framework of the drug as well as the nanoemulsion delivery process utilized might provide a pertinent realization of this [115, 121].

11.6.3 Nanoemulsions in the Food Industry

The food industry utilizes assorted lipophilic-active ingredients as antimicrobials and several bioactive compounds that might pose a barrier as they have instability and water insolubility when incorporated in food formulations with aqueous content. Low water solubility and sensorial detection thresholds, fast oxidation pose, and low bioaccessibility after digestion in the gastrointestinal tract are some disadvantages of lipophilic-bioactive ingredients during their incorporation into food. The composition and size of nanoemulsion droplets containing the lipophilic-active compound influences the bioavailability and amount of lipid digestion. The smaller the droplet

size, the greater is the rate of lipid digestion, which has been ascribed to the increased surface area of lipid exposed to lipase-containing intestinal juices [65, 122]. There is a compelling need for additional studies of active food ingredients loaded in nanoemulsions to exemplify the authentic benefits of nanoemulsions in food technology. Despite nanoemulsions showing a better digestibility pattern as compared to ordinary emulsions, further research in this area pertaining to their safety and toxicology for application in the food industry needs to be assured. The biological pathway of nanoemulsions, once they enter the human gut, should also be thoroughly studied to assess their tissue location and likely toxicity.

11.6.4 Nanoemulsions as Antiageing Formulations

Nanoemulsions are finer than normal emulsions and can be sprayed on. It is claimed that as compared to normal emulsions and microemulsions, nanoemulsions transport beneficial compounds deeper into the skin. Newer approaches and technologies are advancing the field of nanotechnology to improve the cosmetic market even more, although a lot of research and human studies in this field is required to obtain subsequent real-life data [123]. Herbal plant extracts and essential oils with anti-ageing and antioxidant activities are a big favorite nowadays even in skin care formulations [124]. Some examples of such oils obtained are from the seeds of red raspberry (*Rubus ideaus*), blueberry (*Vaccinium corymbosum*) [125], soya (*Glycine max*), sunflower (*Helianthus annus*), corn (*Zea mays*), grape (*Vitis vinifera*), flax (*Linum usitatissiumum*), hemp (*Cannabis sativa*), pumpkin (*Cucurbita pepo*), rice bran (*Oryza sativa*), olive (*Olea europaea*) [126], and moringa (*Moringa oleifera*) [127], to name a few. For cosmetic use, it is necessary to deliver the anti-ageing actives into the dermis where their targeting site is. The transepidermal route (due to its large fractional area) is the most favored route for transporting anti-ageing actives across the stratum corneum to the dermis. For better penetration of the cosmoceutical actives, the barrier function of the stratum corneum is overcome by the utilization of several strategies such as modification of its structure, vehicle manipulation, and even electrically assisted methods [128]. Nanoemulsions serve as advantageous vehicles in this regard. Although previous in vitro data signify that nanoemulsions could be potential candidates for anti-ageing drug-delivery systems, their in vivo efficacy and toxicity data are still to be further studied [124, 129].

11.6.5 Fundamental Toxicology Research

The disease type, developmental stage, and location determine the design and targeting approaches for any particular nano-based carrier. Very often, toxicity issues are particularly ignored. Therefore, essential fundamental research that signifies successful and adequate application of these technologies should be used to address toxicity issues. Rational design of nanoemulsion-based tools and technology based on accurate and comprehensive knowledge of biological mechanisms will

work better for the future of nanomedicine rather than enforcing applications of materials that are presently in vogue [115].

11.7 Conclusion

Herbal medicines have been widely recognized and explored since ancient times. They are less costly, nontoxic, and freely available as compared to their synthetic counterparts. Due to certain limitations like poor penetration power, rapid oxidation, slow absorption pattern, degradation in gastric pH, low solubility, etc., herbal drugs cannot entirely treat ailments or take longer time to cure diseases. Nanoemulsions are gaining attention as novel drug carriers highly recommended for herbal drugs to minimize their wastage and induce specific drug targeting at the desired site. Nanoemulsion is appropriate for practically all routes of drug delivery and the limitations of plant-based active pharmaceutical ingredients could easily be conquered when formulated as a nanoemulsion-based delivery system. The spreading interest in herbal drugs as alternatives to synthetic drugs needs more exploration of the safety and efficacious therapy study of their delivery systems, i.e., nanoemulsions. Such novel formulations are expected to reign the commercial market even more in the near future. Therefore, it is also necessary to obtain regulatory foundations that accurately represent and especially manage the risks associated with nanoemulsions as well as other relevant nanotechnology-based drug-delivery systems.

Acknowledgements Hemanga Hazarika and Harshita Krishnatreyya are thankful to the Defence Research and Development Organization (DRDO), Ministry of Defence, Govt. of India, for providing financial support in the form of research fellowship. Authors would akin to convey their special appreciation to Defence Research Laboratory (DRL), Tezpur, Assam, India; Administration of Dibrugarh University (DU), Dibrugarh, Assam, India; and Administration of Department of Chemical Technology, University of Calcutta (CU), Kolkata, India for providing all necessary amenities required for the doctoral work. Authors are also thankful and grateful to the Editor and anonymous reviewers for their valuable suggestions that helped us to improve this chapter.

Conflict of Interest The authors declare no conflicts associated with this work. All figures and tables are original and self-made.

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Stimuli-Responsive Polymers for Cancer Nanomedicines

12

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Abstract

Chemotherapy involves many anticancer drugs, which have mild to severe adverse effects, when administered in conventional dosage forms. However, the lack of adequate supply of drug to the target site, as well as biodistribution to irrelevant body compartments has limited such drug-delivery systems. The major challenge in the delivery of such drugs is the site specificity. This task can be made simpler to a remarkable extent by employing stimuli-responsive polymers for designing nanocarriers. The stimuli may be physical, chemical, or biological, but should be sufficient enough to elicit structural changes in those polymers at the local site to enable the release of drugs in a controlled manner. The cancer cells exaggerate some biological phenomena as compared to the normal cells, which can be utilized as site-specific stimuli. Nanocarriers fabricated by using these polymers respond to stimuli like pH, temperature, redox potential light, etc. Multiple stimuli responsiveness can also be exploited for more specific drug delivery. This area of research seems to be very promising by exploring the physiological environment minutely. This chapter highlights the recent trends in different stimuli-responsive polymers, especially for delivering anticancer drug and challenges on the way of developing nanoformulations as well as their clinical translation.

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M. K. Das, Y. V Pathak (eds.), *Nano Medicine and Nano Safety*,
https://doi.org/10.1007/978-981-15-6255-6_12

Keywords

Chemotherapy · Stimuli-responsive polymer · Site-specific drug delivery · Nanoformulations · Nanomedicine

Abbreviations

AIBN	Azobisisobutyronitrile
CRP	Controlled Radical Polymerisation
CST	Critical Solution Temperature
DOX	Doxorubicin
HLB	Hydrophilic Lipophilic Balance
LCST	Lower critical solution temperature
MMPs	Matrix metalloproteinases
MTX	Methotrexate
PEG	Poly Ethylene Glycol
PEtOx	Poly(N-ethyl oxazoline)
PMVE	Poly(methyl vinyl ether)
PNIPAM	poly (*N* -isopropylacrylamide)
PNP	Polymeric Nanoparticles
PNVC	Poly(N-vinylcaprolactam)
POZ	Poly(Oxazoline)
RAFT	Reversible-Addition Fragmentation Chain-Transfer
ROS	Reactive Oxygen Species
SRPs	Stimuli-Responsive Polymers

12.1 Introduction

Cancer has become the leading cause of mortality across the globe responsible for nearly one in six deaths. The complexity of cancer lends itself to establish new challenges in developing new drugs as well as delivering the existing chemotherapeutic agents efficiently. The major challenge in treating cancer is to specifically treat cancer-affected cells without any harm to healthy cells/tissues. Anticancer drugs also possess different pharmacokinetic limitations like short half-life and distribution to healthy tissues. It is also reported that practically an inadequate amount of drug reaches the target site. The conventional drug-delivery methods fail to overcome these limitations. Vital organs that redistribute the drug often get affected due to the non-specific drug distribution by conventional drug-delivery systems. The non-specific distribution of anticancer drugs sometimes leads to life-threatening side effects like excessive vomiting, immune suppression, nephrotoxicity, hepatotoxicity and severe anaemia. Therefore, a need for such a drug-delivery system is felt, which could eliminate the limitations and contribute to the improvement in the efficacy of anticancer drugs [1].

Nanotechnology has paved a path towards the successful delivery of Anticancer drugs; especially the Polymeric Nanoparticles (PNP)s, which has become an area of comprehensive research work and gathered sufficient interest of researchers for delivering Anticancer drugs. Again, the selection of polymers for developing PNP is crucial, as some traditional polymers have got systemic side effects, which may be due to their distribution in different body tissues and lack of perfect control over drug release [2]. Therefore, ultimately the requirement of such polymers is constantly felt, which could release the drug when it is sensitized by stimuli. Nanoparticles can be developed by using certain Stimuli-Responsive Polymers (SRPs) from which both drug targeting and controlled release upon sensitisation are achievable. Such developments may also reduce the frequency of dosing by accumulating the payloads adjacent to the tissue or cells of interest over a prolonged period. Nanoparticles by using SRPs maintain the steady level of plasma drug concentration, minimize adverse effects, and toxicities increasing the efficacy of drugs [3]. This specificity to respond against a stimulus makes such polymers Smart or Intelligent macromolecules. Some stimuli-responsive polymers for developing Nanomedicine against cancer have been described in this chapter. The stimuli, which have been studied are Physical (Temperature, Light, Ultrasound and Magnetic), Chemical (pH, Ion and Redox responsive) and Biological (Hypoxia, ROS, Glucose Enzyme, etc). Many SRPs have been developed to date, but due to the lack of a standardized manufacturing technique most of them have not reached clinical evaluation stages. Regulatory and ethical challenges are still there for such polymers due to their toxicity [4].

12.2 Physically Dependent Stimuli-Responsive Polymers

12.2.1 Temperature-Responsive Polymers

This category of polymers works with the fact that the cancer-affected tissue maintains a little higher temperature than the healthy tissue adjacent to it. Nanoformulations can be fabricated using such polymers, which preferably utilise the temperature difference as a stimulus. The polymers possess a temperature-dependent phase transition property called critical solution temperature (CST) [5]. Those polymers that solubilise in water at low temperature and become insoluble upon increase in temperature have a low CST (LCST). These LCST polymers are fit to be used against cancer cell. These polymers once get temperature more than the LCST initialise a collapse of the polymeric network, which ultimately triggers the release of drug. The beauty of such polymers is that the drug release can be triggered by externally inducing a local rise in temperature by using suitable means like ultrasound, magnetic field, etc. In general terms, thermosensitive polymers can be used for designing nanocarriers, which retain the drug at physiological pH and start releasing drug when exposed to a little rise in temperature. Examples of some polymers, which elicit the property of thermoresponsiveness, are poly-N-isopropylacrylamide (PNIPAAm), pluronic/PEI and trimethylchitosan-gpoly

(N-isopropylacrylamide (TMC-gPNIPAAm). Delivery of nucleic acid was also possible by suitably manipulating the structure of certain polymers. The major challenge that appears here is maintaining the safety along with an efficient swelling change to deliver the drug [6].

12.2.2 Light-Responsive Polymers

The light-responsive polymers can be utilized for both Photothermal therapy as well as Photodynamic therapy. The tumour cells possess a very high interstitial pressure and the extracellular matrix is also quite thick, which makes the penetration of nanocarriers difficult. The photoresponsiveness of such a class of polymer allows them to absorb light of different wavelengths like near-infrared and ultraviolet. The polymers convert light energy into heat. This local heat causes tumour ablation and helps in penetration of drug deeper into the cell layers [7]. It should be noted that the applied light must be absorbed maximally by the polymers and not by the local cells. Optic fibres can be used to convey the stimulus to distant tissues. The frequency of the light used must comply with the safety limits for biological application. Structurally, the light-responsive polymers contain light-absorbing chromophores like the azobenzene group, nitrobenzyl group, spironopiran group, stilbene, triphenylmethane 2-nitrophenylalanine, etc. Some polymers containing the chromophores are PAA, PHPMAm PNOPAM, etc. A less invasive method to utilize light-responsive polymer is the photodynamic therapy. In this method, the light-responsive polymers produce ROS, which are potentially toxic to the tumour cells. Studies suggested that a better targeting and effective killing of cancer cells can be achieved by this method. The internalisation of drug into the cell by disrupting the endosomes with the production of ROS and further disruption of cells can be achieved by photodynamic therapy [8]. Endosomal enzymes generally degrade the drug. The ROS preferred mostly is the 1O2. The 1O2 generated by the polymers disrupts the endosome as well as it triggers the release of the therapeutic agent. However, photodynamic control of drug release is a promising and challenging task. ROS-sensitive aminoacrylates have been used by researchers for delivering drugs [9, 10].

12.2.3 Electro-Responsive Polymers

A controlled release of drug near the tumours can be achieved by using such polymers, which swell, shrink or show bending and recoiling, when exposed to Electrical and Electrochemical stimuli [11]. Precise control over the drug release is achieved by tuning the parameters like the magnitude of current, the electrical pulse duration and the time duration between consecutive electrical pulses. The feature required for utilising such polymers is their conducting nature. The mechanism behind drug release from such a polymer is the influx of solvent and counter ions, which cause an increase in osmotic pressure and cause an expansion in volume [12]. The polyelectrolytes are loaded or adsorbed on the porous material in such a

manner that it can precisely control drug release. Sometimes, drug release is controlled by the formation of multilayers of redox-active polyelectrolytes, which leads to shrinkage of the polymers after the electrochemical movement of oppositely charged ions, where the polymer contributes mostly negatively charged ions [13].

12.3 Chemically Dependent Stimuli-Responsive Polymers

12.3.1 pH-Responsive Polymers

Cancer cells possess an extracellular acidic pH ranging approximately from pH 6.5 to pH 7.2 due to excessive production of lactic acids. This pH may be down to around pH 4 or pH 5 in the endosomes and lysosomes present in cancer cells [14]. This microclimatic change around the cell is being exploited by many researchers to deliver anticancer drugs to the specific site by utilizing pH-responsive polymers. To respond to pH as stimuli, the polymer needs to carry some ionisable groups. It means the polymer molecule should be a polyelectrolyte containing weakly acidic or basic groups with pKa values ranging from 3 to 10 [15]. These groups should be ionisable. This range of pKa value allows the acidic or basic group to exhibit a pH-dependent ionisation behaviour that means under a certain pH condition these groups will ionise to different extents. After being ionised, electrostatic repulsion is observed between different generated cations and anions. This repulsion leads to change in coiling of polymer and the polymer dramatically exhibits structural changes/swelling as depicted in Fig. 12.1. The ionised groups may influence further ionisation of the pendant groups due to the electrostatic effect [16]. Some ionisable groups showing this mode of responsiveness are carboxylates, sulphonates amino groups, etc. Another mechanism of pH responsiveness has been put forward by some researchers where the polymers undergo protonation and deprotonation changes with the distribution of charges to the different groups, which are ionisable upon pH change. The amino groups and carboxyl groups are known to exhibit such events. The major drawback of this system is that the transitions in the polymer are quite rapid, which respond to the minute pH change (0.2–0.3 U of pH). Some typical pH-responsive polymers include gelatin, albumin, poly(ethylene imine) (PEI), Chitosan, poly(acrylic acid) (PAAc)/chitosan poly(methacrylic acid-g-ethylene glycol) [P(MAA-g-EG)], etc. Polymers containing acid-labile or base-labile linkage can also be used as pH-responsive polymers, more preferably the acid-labile linkage. These polymers degrade after

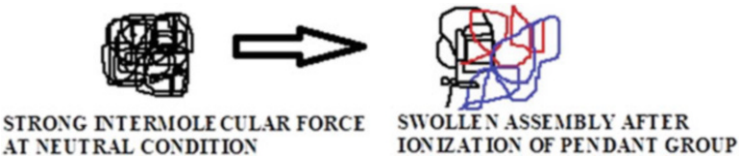

Fig. 12.1 Swelling behaviour of pH-responsive polymer

entering a certain microclimate pH and show structural changes, thereby controlling the drug release. This mode of pH responsiveness has been used by many researchers to deliver anticancer drugs. Hydrazone, acetal, ketal and boronate esters are some linkages reported to exhibit pH-dependent cleavage [13].

The pH-responsive behaviour is tuneable and is required many a times to ensure development of nanomedicines with least off-target effect and toxicities. The polymers are generally selected with a pKa value around the pH of the site of interest of drug delivery. This ensures a 50% ionisation of the polymer. The pH responsiveness can be manipulated by adding hydrophobic groups to the polymer chain or changing the hydrophobic chain length. Copolymerisation with ionisable and non-ionisable polymers can also give rise to newer polymers with a desired pKa value [17].

12.3.2 Ion-Responsive Polymers

The presence of ionisable groups can also be exploited for another stimulus other than pH, i.e. the ionic strength. The oppositely charged ions attract each other with their static Coulombic charges. This leads to changes in the rheological properties of the polymer at the target site. The change in ionic strength leads to lengthening of the polymer chain. The polymer solubility also varies. A change in fluorescence quenching kinetics may also occur when the chromophoric groups are attached to electrolytes [13, 18].

12.3.3 Redox-Responsive Polymers

To elicit redox responsiveness, a polymer preferably should contain some labile groups. The redox reaction succeeds to change the hydrophobicity and lipophilicity of the polymers leading to swelling of the polymers. The normal redox potential for a cell is oxidising extracellularly and reducing intracellularly. The reducing nature of the intracellular environment is due to the presence of glutathione. In cancer cells, the glutathione level is much higher than normal cells, so it makes the cytosol even more reducing. That is why Redox-responsive polymers can be exploited for the delivery of anticancer agents to cytosols [19]. The polymers with disulphide cross-linking have attracted sufficient interest of researchers because the linkage degrades when encounters any cellular environment with glutathione or cysteine content [20]. Disulphide-functionalised polymers have been successfully prepared by RAFT polymerisation. RAFT agents help in preparing bimolecular polymer conjugates, which show responsiveness towards redox stimulus [21]. A few examples of this category of polymers are poly (NiPAAm- co-Ru(bpy), (ethylene glycol)-b-poly(lactic acid) (MPEG-SS-PLA) diblock copolymers, hyaluronic acid (HA)-polycaprolactone (PCL) block copolymer Poly(ethyleneglycol)-b-polycarbonate-b-poly(ethyleneglycol) triblock co-polymer, etc. [22].

12.4 Biologically Dependent Stimuli-Responsive Polymers

12.4.1 ROS-Responsive Polymers

ROS concentration in mucosa has been reported to increase up to 100 times in colon cancer than any healthy cells. ROS in body are hydrogrn peroxide, superoxides, singlet oxygen, and hydroxyl radicals [23]. These reactive species can be exploited for controlled and targeted delivery of anticancer drug [24, 25]. Gene delivery to cancer-affected cell has been reported by Suk Shim and Xia [26]. They delivered complex DNA into the cell by utilising the ROS-responsive thioketal system. The thioketal linker containing a diblock polymer of PEG and poly (lactic-co-glycolic acid) has been used to deliver Doxorubin-loaded nanoparticles in Cal27 cells [27]. Selenium and Tellurium containing block polymers have also gained interest to deliver anticancer drugs by exploiting the ROS responsiveness [28].

12.4.2 Hypoxia-Responsive Polymers

Hypoxia indicates a pathological condition where the cells experience lack of adequate oxygen, with a fall of oxygen partial pressure from the surface to the interior. This reduced oxygen partial pressure may reach an alarming value of 0–5 mmHg in cancer-affected cells. The hypoxia leads to huge biochemical changes in the cells distinguishing them from normal cells. These changes offer the chance to develop target-specific drug-delivery systems. Hypoxia-responsive polymers show their responsiveness against this reduced partial pressure [13]. Son et al. developed Carboxymethyl dextran-black hole quencher 3 with an azo linker for targeted delivery of Doxorubicin to cancer cells. The drug release was controlled as the azo linkage was reduced eventually under the oxygen-deficient environment [9]. Thambi et al. developed polymeric nanoparticles with a hypoxia-responsive polymer, which they fabricated by conjugation of the 2-nitroimidazole derivative with CMD for delivering Doxorubicin [29]. In 2016, Thambi et al developed a micelle of amphiphilic block polymer consisting of PEG and poly (ε-(4-nitro)benzyloxycarbonyl-l-lysine) as the constituent to deliver Doxorubicin intracelluarly under hypoxic conditions [30]. Biomolecules like *si*-RNA have also been delivered under hypoxic conditions, where PEG, azobenzene, polyethyleneimine and phospholipid have been used for developing a nanocarrier system [31].

12.4.3 Enzyme-Responsive Polymers

Enzymes are proteins, which are necessary to carry out several biochemical reactions in the human body basically as biocatalysts. These are molecules, which respond to even marginal changes in the level of biochemical or function of any organ. These are specific towards a particular substrate. Their specificity and sensitivity to respond to the changes make them suitable stimuli. Especially in cancer cells, the catalyst

activity has its own importance to promote rapid cell division and growth of the cell. Enzyme-mediated drug delivery can be preferred to target the cancer cells where enzyme-responsive polymers can be utilized.

Several reports are there where the Matrix metalloproteinases (MMPs), an endopeptidase group of enzymes, have been explored by many researchers to target cancer cells by using enzyme-responsive polymers [32, 33]. Cathepsin-B, a lysosomal cysteine protease enzyme, has been reported to be expressed on epithelial cells of cancer-affected tissue. Both MMPs and Cathepsin-B together have been used as stimuli for delivery of Gemcitabine in a study. Hyaluronidase enzyme breaks the Hyaluronic acid, which plays an important role in cell proliferation in cancer. This enzyme is utilised by researchers as stimuli for delivery of drug-like 5-FU. Azoreductase enzymes are responsible for reducing nitroaromatic group-containing molecules. This enzyme has also been exploited as stimuli for delivering anticancer drug for targeting colon cancer [34].

12.5 Dual or Multiple Stimuli-Responsive Polymers

Macromolecules exhibit their biological activities mostly due to a series of environmental changes. To elicit a biological function or process, multiple changes occur in the microclimate of cells or tissue at a time [35]. The multi-stimuli responsiveness in polymers may, therefore, be exploited for delivering a drug via polymeric carriers to target the cancer cells [20]. Many reports are there where this strategy has proved to be successful in delivering anticancer drugs. The mechanism by which a drug-delivery carrier releases the drug near the cell or inside the cell is crucial. The premature drug release by an incompetent drug-delivery system may cause systemic toxicity. The multi-stimuli responsiveness may help in reducing the systemic toxicity by more specific mode drug delivery at the desired site. The cancer cells create an acidic extracellular environment suitable for metastasis. This reduced pH can be used along with other stimuli to exhibit target-specific drug delivery. The combined stimuli responsiveness may be designed in ways where it takes place simultaneously or in a sequence [34, 36].

12.5.1 pH- and Temperature-Responsive Polymers

The elevated temperature and acidic environment of cancer cells has been utilised as dual stimuli for designing polymer-based nanocarriers. Poly (N-isopropylacrylamide- co-N, N-dimethylacrylamide-co-10-undecenoic acid), Copolymer of Poly (caprolactone) and poly (Nisopropylacrylamide-co-acrylic acid), Copolymer of poly (tert-butyl acrylate) and poly (N-isopropyl acrylamide) and Copolymer of N-methyldiethanolamine and poly (ether urethane) backbone are some reported polymers for dual stimuli responsiveness. Some of these polymers have shown poor drug loading in the nanocarriers [20, 37].

12.5.2 pH- and Redox-Responsive Polymers

Polymeric NPs have been developed by using such polymers, which respond to both these stimuli simultaneously and result in disassembling of the chain. In cancer-affected cells, both these stimuli can be found. The drug release can be achieved by GSH reduction and sustained release can be achieved by the pH sensitivity [38]. Cysteamine-conjugated chitosan and dextran sulphate, acrylamide-based linear copolymers and poly (2-(pyridine-2-yldisulphanyl)ethyl acrylate) are some of the polymer systems, which have been utilised for this particular set of stimuli. The cross-linking of polymers is done by both imine and disulphide linkage to ascertain pH and Redox responsiveness [39].

12.5.3 Triple Stimuli-Responsive Polymers

The development of such systems, which respond to triple stimuli, is fascinating and also the drug targeting by such system is more specific. The stimuli like pH, Thermal, UV, Magnetic, and redox are utilised in suitable combinations as per the requirement at the desired site and feasibility of application. Drugs like Doxorubicin have been reported to be delivered to target specifically by utilising triple stimuli-responsive polymers [20, 40]. The effect of different stimuli on hydrogel has been depicted in Fig. 12.2.

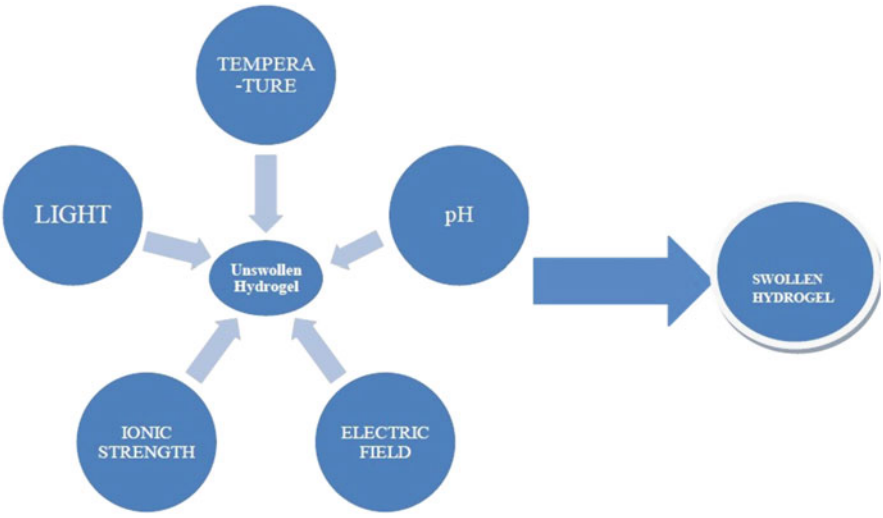

Fig. 12.2 Effect of different stimuli on hydrogels prepared with stimuli-responsive polymers

12.6 Brief Description of Some Common Stimuli-Responsive Polymers

12.6.1 Poly (N-Isopropylacrylamide) (PNiPAAm)

PNIPAM is the most commonly studied thermoresponsive Polymer (Fig. 12.3). The widespread acceptance of PNIPAM is not only based on the lower critical solution temperature (LCST) that is close to the body and room temperature (LCST 32–33 °C) making it very useful for controlled release application, but also on the robust phase behaviour. The LCST position of PNIPAM does not shift with changes in molecular weight, chain length and concentration, but changes can be observed due to the shifting of hydrophilic/hydrophobic balance. PNIPAM turns out to be an expanded coil form below the LCST and above it starts becoming more hydrophobic as it collapses to a globular state [41, 42, 43]. The *N* -isopropylacrylamide (NIPAM) monomers are polymerised by the process of free radical polymerisation of the vinyl group utilising a common radical initiator, such as azobisisobutyronitrile (AIBN). Recently, the polymers of defined and narrow molecular weight distribution and defined end-groups were developed by controlled radical polymerisation (CRP) of monomers. This enables straight forward modification and conjugation towards biological species. The most important point in this regard is that the vinyl group of NIPAM is activated by the amide group. The reversible-addition fragmentation chain-transfer (RAFT) polymerisation of NIPAM can be achieved with RAFT-agents including dithiobenzoate or trithiocarbonate groups [44, 45]. A triply triggered supramolecular nanocontainer was prepared by Loh et al. having potential chemotherapeutic applications. It is observed that in his article that the PNIPAAm was used as a temperature-responsive and this block is utilised for the release of loaded doxorubicin. It also acted as a pH-responsive segment. The temperature-responsive portion was suitable for triggering Doxorubicin (DOX) release, the pH-responsiveness has triggered the release within endosomal and lysosomal vesicles at an acidic pH of 4 [35].

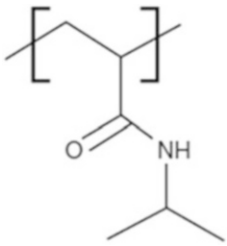

Fig. 12.3 Structures of poly (*N* -isopropylacrylamide) (PNIPAM)

12.6.2 Poly (N-Vinylcaprolactam) (PNVC)

The other polymer of thermoresponsive polymer is Poly (N-vinyl caprolactam) (PNVC) (Fig. 12.4). It is a nonionic amphiphilic polymer that has exceptionally fascinating properties both for therapeutic and biotechnological applications. It shows various properties like solubility in water and organic solvents, biocompatibility, high absorption ability and a transition temperature within the settings of these applications [42].

It is comprised of seven-membered cyclic amide in which the carboxyl group and an amide group are polar hydrophilic groups connected directly to a hydrophobic vinyl chain. It shows solubility at normal body temperature but it also shows phase transition behaviour at 32 °C. It is observed that in aqueous systems below its LCST, the polymer forms a chair-like conformation and achieved syndiotactic configuration. Due to amide linkage between the lactam ring and the carbon backbone chain, it shows stability on hydrolysis and it does not form any toxic compounds. The polyelectrolyte behaviour is observed in the presence of surfactants because of this the adsorbed counter ions inside the polymer matrix along with surfactants, leading to an increase in osmotic pressure triggering extensive swelling of polymer chains. The transition temperature increases with increasing surfactant concentration until it levels out at a certain surfactant concentration [46, 47].

NVC is polymerised by a free radical polymerisation process and the versatility of this polymerisation process of NVC provides straightforward access to a wide range of copolymers based on the large variety of vinyl monomers that are commercially available [48, 49]. PNVC has received considerable attention because of the phase transition induced by the alternation of the external environment and its biphasic transition via two different transition states, one at 31.5 °C and another at approximately 37.5 °C. With thermoresponsive nanoplatforms, the present challenge is to maintain the safety of the platforms without compromising their sensitivity to minor temperature changes.

12.6.3 Poly (Methyl Vinyl Ether) (PMVE)

This polymer has a transition temperature exactly at 37 °C, governed by the hydrophilic hydrophobic-balance (HLB). Such a polymer is sometimes referred to

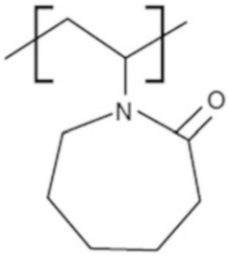

Fig. 12.4 Structures of poly (N-vinylcaprolactam)(PNVC)

as 'thermo-shrinking, and gets particular interest because of the abrupt nature of its phase transition and the fact that the transition is reversible, which allows repeated 'thermal-switching'. This property makes it very interesting for biomedical applications. It exhibits a typical type III demixing behaviour, which quitely differs from the thermal behaviour of PNIPAM. There are few limitations for this polymer that it cannot be synthesised by using nucleophiles such as alcohol or amino group so it has to be synthesised by cationic polymerisation using inert conditions [50, 51].

12.6.4 Chitosan

Chitosan is one of the most widely researched pH-sensitive polymers of natural origin belonging to the aminoglucopyran family (Fig. 12.5). It is a type of cationic polysaccharide, which is obtained by the partial deacetylation of chitin. Chitin is a structural element of crustaceans and it is a part of the exoskeleton of crustaceans and the cell wall of fungi. Due to its versatile properties such as pH sensitivity, biocompatibilility and biodegradable behaviour, it is widely accepted for various biomedical and pharmaceutical applications. It is suitable for designing various drug-delivery systems [52–54].

It is utilised in the preparation of various nanoformulations due to the presence of acid-swellable groups like the amine (-NH_2) group. These are acid-swellable groups, in contrast to the alkali-swellable carboxyl group. In acidic environments, the internal charge repulsions between neighbouring protonated polybasic groups are increased. As the pH value increases, the groups become less ionised, and the polymer-polymer interaction increases due to a reduction in charge repulsion, which is ultimately responsible for decreases in the hydrodynamic diameter of the polymer. As it is comprised of primary amino groups in the main backbone making its surfaces positively charged in biological fluids. Therefore, we can easily prepare nano/microparticles by treating chitosan with various types of polyanionic substances like sulphate, citrate, and tripolyphosphate, so we can utilise chitosan in a wide variety of drug-delivery approaches [55].

Tamoxifen-loaded pH-dependent chitosan nanoparticles were prepared for the treatment of breast cancer. In this research, it was observed that intracellular drug concentration was higher than in the unloaded drug during in vitro testing with human breast cancer cells, and its anticancer efficiency was enhanced. [53] It is also accepted in the field of wound healing as it is capable of promoting the dermal regeneration and triggering the wound-healing properties. Generally, it shows a

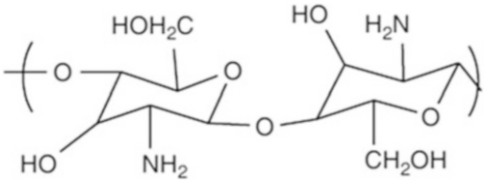

Fig. 12.5 Structure of Chitosan

minimal foreign body reaction and the typical course of healing with formation of normal granulation tissue, often with accelerated angiogenesis [56–58].

12.6.5 Pullulan

Pullulan is a fungal linear exopolysaccharide produced from starch by *Aureobasidium pullulans* (Fig. 12.6). It consists of α–1, 6-linked maltotriose residues. Pullulan has unique properties that make it more suitable such as it is biodegradable, impermeable to oxygen, non-hygroscopic and non-reducing and also has high adhesion, film-forming abilities and structural flexibilities. The nonionic polysaccharide and is blood compatible, biodegradable non-toxic, nonimmunogenic, non-mutagenic and non-carcinogenic behaviour of pullulan has increased its importance [59].

As nowadays, the polysaccharides play an important role in developing controlled drug-delivery systems, pullulan also gains a lot of attraction towards the formulation of pH-sensitive-derivatised pullulan nanoparticles. The negative charge in pullulan is introduced by Carboxymethylation. The liver uptake clearance of pullulan was decreased by more than a hundredfold. This derivative was then investigated for application in chemotherapy. The authors conjugated doxorubicin, a known chemotherapy drug used in various cancers, via a peptide linker to carboxymethylated pullulan [60].

Na et al. found that the nanoparticles of succinylated pullulan acetate/sulphonamide (PA/SDM) conjugates may provide some advantages for targeted anticancer drug delivery due to the particle aggregation and enhanced drug release rates at tumor pH. The drug release rate from the PA/SDM nanoparticles was pH-dependent and it was significantly enhanced below a pH of 6.8 [61].

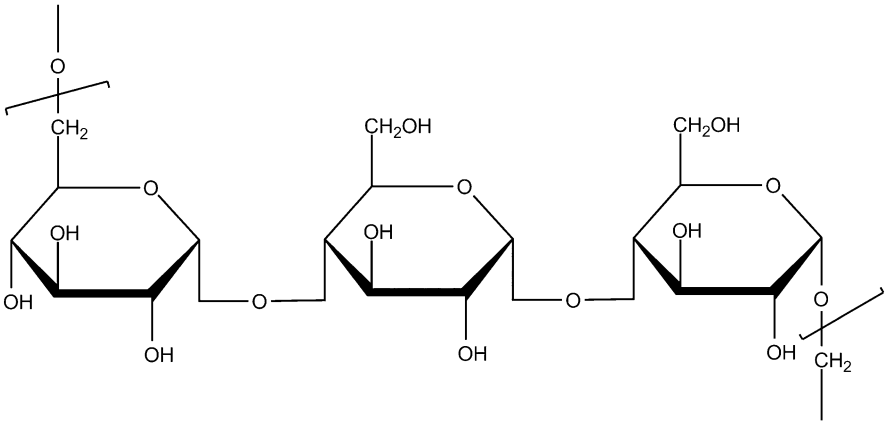

Fig. 12.6 Structure of pullulan

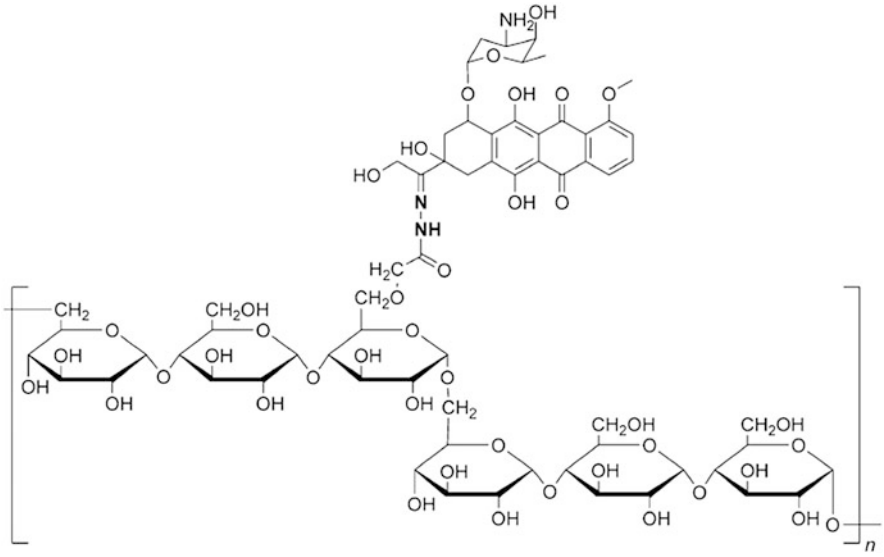

Fig. 12.7 pH-sensitive pullulan–DOX conjugate

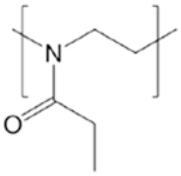

Fig. 12.8 Structure of poly(N-ethyl oxazoline) PEtOx

The anticancer drug doxorubicin (DOX) was attached to the backbone of pullulan, through a pH-sensitive hydrazone bond (Fig. 12.7) and it was observed that drug release was significantly enhanced at pH 5. The same linkage, but to an artificial recombinant chimeric polypeptide (CP), was also used successfully to deliver DOX in a pH-dependent fashion, as reported by Mackay et al in 2009. The CPs spontaneously self assemble into sub-100-nm-sized PNPs on the conjugation of diverse hydrophobic molecules, including DOX [62].

12.6.6 Poly(N-Ethyl Oxazoline) PEtOx

Poly (N-ethyl oxazoline) has been explored as a hydrophilic segment in amphiphilic block-copolymer and has a transition temperature around 62 °C, which is too high for any drug delivery application (Fig. 12.8). Rueda et al. prepared a double thermoresponsive system by graft polymerisation of EtOx onto a modified PNIPAM backbone. Poly (oxazoline) was also used in preparing liposomes and shown to be comparable to PEG in stealth effects. Currently, these systems are explored for their

potential in drug delivery, because they tend to aggregate micelles above the LCST. Unfortunately, the poly (oxazoline) chemistry has the disadvantage that it is not very tolerant against unprotected functionalities [50].

12.7 Stimuli-Responsive Polymeric Nanoformulations for Cancer Therapy

There are various nanoformulations have been prepared using different stimuli-responsive polymers by various researchers as presented in Table 12.1.

12.8 Challenges in Developing SRP-Based Nanomedicines Against Cancer

The stimuli-responsive nanopreparations show much more specificity for controlling the drug release at a desired location, time and quantity by responding to local stimuli or dual or multiple stimuli in the microclimate around the cell and sometimes within the cell after the internalisation. However, the stimulus-sensitivity of the current nanoformulations still needs fine tuning. The most prominent challenge is the off-tumor-targeting possibly by non-specific stimuli raised as a result of up-regulation of stimulus proteins in healthy cells due to drugs or harsh conditions. The polymeric nanoformulation offers more complexity in design aspects than the conventional technology, which causes difficulties in sticking towards Good Manufacturing Practice (GMP) standards during scale-up and quality control. Out of the plethora of research reports by several researchers, only a handful of reports are there with relevance to safety and efficacy of such polymers in preclinical studies as well as at clinical levels. A regulatory guideline must be framed specially for nanomedicines by collaborative efforts for successful translation of laboratory research to the clinical level [75].

The competition of anticancer agents in clinical trials is at its limit and the chance of failure is also highest for such formulation. For some cases, the root cause of the failure has been found out. The paclitaxel-polyglutamic acid conjugate Opaxio™ entered phase III clinical trials as a medication for non-small cell lung cancer. The results showed that survival benefit was observed for females only, but not males [76]. Opaxio™ relies on enzyme as stimuli for the activation. The enzyme cathepsin B mediated activation resulted in an interaction with the oestrogen levels [77]. This results in the exclusion of female with oestrogen levels beyond marked threshold. This puts an example for the researcher to study further a few things like drug delivery barriers and the desired clinical outcome. The physico-chemical property and rate and extent of stimuli responsiveness, the biodistribution property of the polymer and also the nature of stimuli must be thoroughly understood before formulating the nanomedicine in the beginning itself, so that less regulatory as well as clinical obstacle be faced later [67, 78]. A large number of patents have

Table 12.1 SRP-based nanopreparations for cancer therapy

Type of nano-medicine	Acting stimulus	Stimuli responsive polymers	Cargoes	Indication	Product name	References
Liposomes	–	Poly(ethylene glycol)	Doxorubicin	Kaposi's sarcoma	Doxil™	[63]
	Enzyme	Matrix metallo-proteinases (MMPs)	N4-octadecyl-1-b-D arabinofura nosylcytosine(NOAC)	Cancer treatment	–	[32]
Dendimers	Redox	PAMAM-SS-NAC	NAC	Cancer treatment	–	[64]
Polymeric conjugates	pH	Pullulan–DOX	Doxorubicin	Cancer treatment	–	[65]
	Temp	Poly(N,N-diethylacrylamide-co acrylamide)-block-poly (gbenzyl- l-glutamate	Paclitaxel	Cancer treatment	–	[20]
	pH	Amidised poly(l-lysine)	Camptothecin	Nuclear localisation of drug-enhanced cytotoxicity, compared with free drug, in carcinoma cells	Oncaspar™	[66]
Polymeric micelles	–	Poly(ethylene glycol)-cis-aconityl-chitosan-stearicAcid	Paclitaxel	Breast cancer	Genexol PM™	[67]
	–	Poly(ethylene glycol)-poly (mono-2,4,6-trimethoxy	Doxorubicin	Lung cancer	–	[68]
	pH	Benzylidene-pentaerythritol carbonate) [PEG-b-P(TMBPEC-co-AC)]	Paclitaxel	The micelles showed high intracellular drug release.	–	[69]
	Temp	Biotin-PEG-b-P(NIPAAm-co-HMMAm)	Methotrexate (MTX)	Triggered drug release	–	[70]

Polymeric nanoparticles	Enzyme & pH	(cathepsin B)	Doxorubicin	The enzyme (cathepsin B) was responsible for the in-vitro release of DOX concentration	–	[71]
	pH, magnetic	Poly(4-O-acryloyl benzaldehyde-b-oligoethylene glycol-acrylate)	Doxorubicin	Cancer	–	[72]
	Redox	mPEG-ss-CPP-SA	Curcumin	Triggered drug release	–	[64]
Nanogels	pH, magnetic	Magnetic chitosan	Doxorubicin	A pH-controlled release pattern was revealed by doxorubicin	–	[73]
Others	Temp., pH	Poly(NIPAAm-co-AA)/Fe_3O_4	Doxorubicin	The DOX-loaded hydrogel nanosphere rapidly releases at pH 5.3 37 °C with an enhanced anti-tumour effect	–	[74]

Table 12.2 Some recent patents on stimuli-responsive polymers for drug delivery

Sr. No.	Publication No.	Title	Researcher, year	References
1	US20190254302A1	Systems and methods for controlling the release from enzyme-responsive microcapsules with a smart natural shell.	Abbaspourrad, Ravanfar, 2019	[79]
2	WO2019133914A1	Method of treatment for solid tumours containing hypoxia and/or stroma features.	Sau, Iyer, Alsaab, 2019	[80]
3	US10188606B2	Expansible cross-linked polymerosome for pH sensitive delivery of anti-cancer drugs.	Liu, Yaszemski, Lu, 2019	[81]
4	WO2019136268A1	Modulation of extracellular vesicles with electrical stimulation.	Wang, Worrel, Lennon, Dong, 2019	[82]
5	US10406336B2	Adjustable rate drug-delivery implantable devices.	Davey, 2019	[83]
6	US20190083649A1	Cross-linked polymers nano-assemblies and uses thereof.	Thayumanavan, 2019	[84]
7	WO2018167618A1	Light-responsive quantum dot drug-delivery system.	Naasani, 2018	[85]
8	US10117837B2	Methods of preparing stimuli-responsive multi-functional nanoparticles.	Lin, Wang, 2018	[86]

been filed in this area of research by several scholars across the globe. A few recent important patents have been given in Table 12.2.

12.9 Conclusion and Future Perspectives

This article highlights that basketful stimuli-responsive polymers have been established to deliver the anticancer agents in response to endogenous (redox, pH, hypoxia and ROS) and exogenous stimuli (temperature, light and ultrasound). The endogenous stimuli-responsive system depends on the abnormal environments in pathological tissues for target-specific drug delivery, whereas the exogenous stimuli-responsive one desires previous information on the situation of the target site for effective therapy as a result of the variation within the physiological conditions. Different classes of polymers are mentioned here and varied SRP-based nanoformulations used for cancer have been highlighted. Nanoformulations containing such stimuli-responsive polymers have the potential to meet the challenges, and will result in approaches applicable to a range of cancers. The challenges in these ‘smart’ nanocarriers has led to the invention of recent and higher tumor-specific targeting moieties, and their integration into ‘smart’ nanocarriers that

exhibit self-assembly and adequate drug release inside the cytosol in response to certain stimuli. Theranostic approach makes the neoplasm diagnosis and therapy quite manageable and economical.

The ability of the stimuli-responsive polymers regarding their targeting efficiency is unquestionable and the dual or multiple stimuli responsiveness of polymers has taken the drug targeting to a new height. However, these polymers still need a thorough study for their tissue toxicity in organs like spleen, kidneys, lungs and liver. Somewhere in the chapter, we have also mentioned that the drug loading may also reduce to a certain extent by using some stimuli-responsive polymers. An interdisciplinary research area must be framed to know the physics of the polymer dynamics within the biological environment along with a chemical approach to it. Biologists and Physicians also must pay attention towards this to study the toxicity issues during the clinical translation phase of the respective formulations. Exhaustive preclinical studies need to be performed in desired orthotopic animal models to deliver a new formulation from the research laboratory to the clinical level. Future opportunities are open for these stimuli-responsive polymers towards the delivery of the combination of drugs, biological macromolecules like DNA, RNA Peptides, etc. by formulating them as nanomedicines. Problems like off-target accumulation, membrane permeability, releasing an adequate amount of drug at the target site and maintaining a wide therapeutic range also run parallel with the nanoformulation. Finally, the aim is to utilize the polymers in developing nanomedicines, which are cost-effective and manufacturers as well as researchers should pay sufficient attention to promote research in this area.

Conflict of Interest The authors declare that there is no conflict of interest. The Figures and Tables used in this chapter are original and prepared by the authors themselves.

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Carbohydrate-Derived Tailorable Interfaces: Recent Advances and Applications

13

Mehmet Can and Nurettin Sahiner

Abstract

Carbohydrates from simple sugars to complex polysaccharides comprise a large number of biomolecules that are readily available from replenishable sources. They perform crucial biological functions through diverse interplays with proteins and lipids on the basis of specific interactions such as modulation of immune response, cellular signaling, growth, and molecular recognition events. Due to their ability to reversibly bind through hydrophobic interactions and hydrogen bonding, carbohydrates have been exploited as intriguing substrates for the design of responsive nanovehicles and therefore hold great potential for a myriad of biomedical applications. Furthermore, functional groups that exist on carbohydrates such as amino-, hydroxyl-, and acetate groups provide facile modification sites for the prepared nanostructures and render additional functionalities, e.g., carbohydrate particles with fine-tuned particle size, shape, and surface properties. In addition to these advantages, carbohydrates are biologically safe, mostly biocompatible, degradable, and have stealth characteristics along with their affinity to specific cellular elements, which enables the active

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M. K. Das, Y. V Pathak (eds.), *Nano Medicine and Nano Safety*,
https://doi.org/10.1007/978-981-15-6255-6_13

targeting of the corresponding nanocarriers and the design of tailor-made, efficient, and long circulatory carrier systems. This chapter provides a brief overview of carbohydrates on the basis of structural and functional properties and highlights the cutting-edge advancements on carbohydrate-based polymeric materials in biomedical applications with a particular focus placed on the applications of polysaccharide-based micro−/nanohydrogels in nanomedicine.

Keywords

Carbohydrates · Polysaccharides · Biopolymers · Nanoparticles · Microgel · Nanogel · Hydrogel · Targeting · Stealth · Long circulatory · Protein repellent

13.1 Introduction

Carbohydrates are one of the most abundant biomacromolecules, mainly because of the existence of plant biomass and microorganisms. They constitute a ubiquitous group of compounds playing structural and pivotal functional roles in a plethora of biological processes and pathophysiology of various diseases [1, 2]. They are greatly involved in numerous tasks such as signal transmission, intercellular communications, molecular recognition, endocrine and immune response modulation, as well as transportation, energy storage, and as components of cellular structures.

Carbohydrates have drawn immense attention through many years by scientific and commercial organizations due to their inherent physicochemical properties, including hydrophilicity, humectancy, stabilization ability, viscosity, unique flow behaviors, gel formation, and water holding capacity, as well as unique bioactive properties, abundance, and affordable availability from renewable sources [1, 2].

Recent advances in medicine and nanotechnology have caused a significant increase in the relevance of carbohydrate-based biomaterials and have altered the strategies in which they are designed, manufactured, and utilized. Much of scientific interest has been shifted to such natural biomolecules in designing nanocarrier-based colloidal particulate drug delivery systems (DDSs) [3–5] as alternatives to liposomes aiming to circumvent the storage-related instability issues [6]. In this context, carbohydrate-based micro/nanostructures, e.g., microgels and nanogels, have gained significant momentum as promising DDSs in the administration of various bioactive agents such as drugs, nucleic acids, hormones, proteins, and so on into the human body [4, 5, 7–9]. Due to their biodegradability, biocompatibility, and nontoxic nature, carbohydrate-based DDSs can afford enhanced therapeutic efficacy and safety by providing increased circulation and bioavailability, enhanced protection of drugs against oxidative or any kinds of degradations, decreased toxic effects, and control over the release of their payloads at specific sites, rates, and durations. Additionally, various bioactive properties of carbohydrate-derived nanomaterials, including higher loading capacity, ease of internalization, lower immunogenicity, and active targeting abilities promote the domination of their use as prominent

carrier systems [3–5, 8, 10, 11]. In this review, the importance of carbohydrates in biological operations will be outlined as a brief reminder on carbohydrate structure and classification. The substantial use of carbohydrates, especially of polysaccharides as natural biopolymers, in the design and targeting of nanostructures will be discussed.

13.2 Some Examples of Carbohydrates and Their Classification

The pharmacological and medicinal importance of carbohydrates is increasing in a rapid pace owing to the increased awareness and understanding of their unique bioactive features, some of which include modulation of lipid and protein functions, immune stimulation, and anti-inflammatory, antioxidant, antimicrobial, and antitumor effects [1, 11–18]. The colossal diversity and complexity of carbohydrates had hampered the elucidation of their structural and functional properties due to the absence and/or lack of experimental techniques and technology in the past few years. These difficulties have led to the lack of agreement in the precise classification of carbohydrates through their history. Although it is still quite challenging to accurately characterize the structure of complex carbohydrates due to their structural heterogenicity, recent studies and analytical techniques emerged by the accumulation of knowledge on the chemical, therapeutic, and pharmacological properties of carbohydrates, together with the ensuing progress in bioanalytical technology, have paved new ways for better understanding of their structural and bioactive characteristics, and thus opened up new possibilities to design carbohydrate-based multifarious biomaterials as potential vaccines, drugs, and DDSs [3–5, 8, 11, 19–21].

Carbohydrates also called glycans are the most abundant and widely distributed biomolecules in nature. They can be classified in many ways based on their structure, size, function, source, and nutritional properties such as caloric value, glycemic response, carcinogenicity, fermentability, and so on. Figure 13.1 illustrates a fundamental classification of carbohydrates based on the constituent monosaccharides and the number of monosaccharide units in their polymeric forms. Carbohydrates occur in the form of saccharides and glycoconjugates as bound to other biomolecules such as lipids, peptides, and proteins. They perform various cellular functions as hormones [22] and receptors [23, 24]. Moreover, saccharides can be classified into three subgroups including monosaccharides, oligosaccharides, and polysaccharides.

Monosaccharides are the smallest members of carbohydrates, which occur either as polyhydroxy aldehydes or ketones (aldose and ketose sugars, respectively) possessing three to nine carbon atoms in their skeleton. They are named based on the number of carbon atoms they contain such as trioses, tetroses, pentoses, hexoses, etc. They are also named as D- or L- sugars depending on the position of the hydroxyl group that is on the farthest chiral carbon atom from the carbonyl group e.g. for a ketopentose sugar ribose, if the farthest hydroxyl group that is on carbon number 4 is on the right-hand side, it is called D-ribose, or if it is on the left side, it is called L-ribose. The most commonly arising forms of monosaccharides are pentose

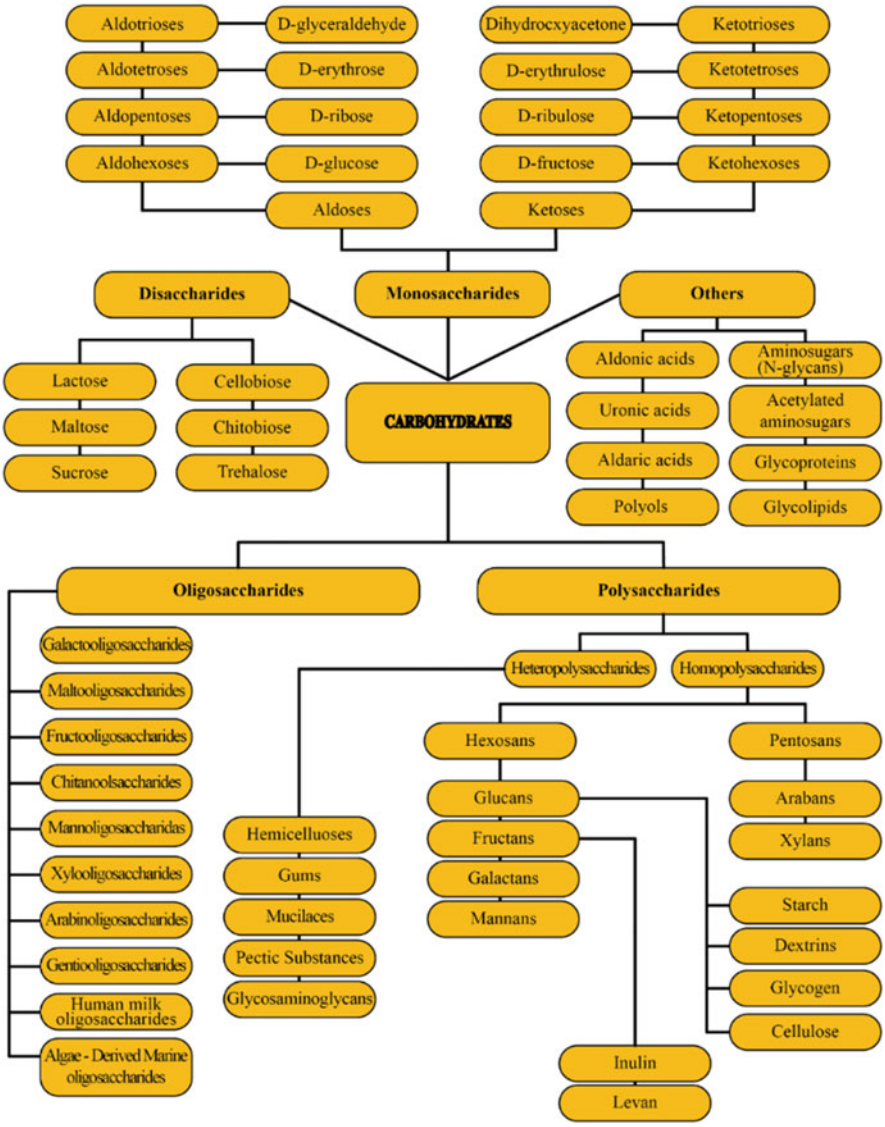


Fig. 13.1 Basic classification of carbohydrates based on the number and type of monosaccharides

and hexose sugars; xylose, ribose, and arabinose are some examples of pentose sugars and glucose, fructose, galactose, and mannose are some examples of hexose sugars. A large number of carbohydrate derivatives exist in nature because of the anomeric carbon atoms in the backbone and as a result of the reduction, oxidation, and substitution of hydroxyl groups with other functional groups e.g., amino, carboxylate, phosphate, and sulphate groups [1, 2, 12, 25]. Some of the

monosaccharide derivatives include amino sugars (hexosamines, e.g. glucosamine and N-acetyl glucosamine), acid sugars (aldonic acids, uronic acids, and aldaric acids), sugar alcohols (also called alditols or polyols), nucleotide sugars (adenosine, guanosine, ATP, etc.), and phosphate sugars that are phosphate esters of sugars produced as intermediate products during the sugar metabolism in glycolysis e.g., glucose 6-phosphate.

All monosaccharides are referred to as reducing sugars that can potentially act as reducing agents in basic environments since they all have free anomeric carbon atoms, that is, the aldehyde or ketone groups in their structure. Monosaccharides such as mannose, galactose, glucosamine, sialic acid, etc. are coupled with the cell surface proteins and lipids to aid in molecular recognition [23, 24].

Oligosaccharides are composed of 2 or more units of sugars joined together in a sequential progressive way from 2–10 monosaccharide repeats in their molecular chain. Disaccharides as members of oligosaccharides are formed by joining two monosaccharide units together via glycosidic bonds. Lactose, maltose, cellobiose, and chitobiose are examples of reducing disaccharides, whereas sucrose and trehalose are nonreducing disaccharide sugars. Oligosaccharides greater than two units of sugar molecules are classified according to the number of constituent monosaccharides in their molecular chain such as trisaccharides, tetrasaccharides, pentasaccharides, etc. up to decasaccharides [26]. They can be derived from disaccharides and obtained by enzymatic or chemical (acid-catalyzed) breakdown of polysaccharides into oligosaccharide-sized smaller units, e.g. cyclodextrins (CyDs) are the oligosaccharides formed by enzymatic breakdown of starch. They are composed of α (1–4)-linked glucopyranose residues with the most common forms ranging from 6 to 8 glucose units and are respectively called α-, β-, and γ-CyDs. They are cyclic oligosaccharides possessing a hydrophobic inner cavity and a hydrophilic outer surface with a truncated cone-shaped structure that is convenient for creating inclusion complexes for a broad range of molecules such as proteins, oligonucleotides, ions, and many drugs, particularly of low water solubility. Amphiphilic cyclodextrins have a tendency to form self-assembled supramolecular nanostructures including nanoparticles, micelles, and vesicles. Cyclodextrins are broadly used in biomedicine, e.g., in ocular applications as drug delivery systems [27].

Another type of classification of oligosaccharides can be made based on the origin of their formation, the ones derived from disaccharides or polysaccharides such as maltooligosaccharides (MOS), fructooligosaccharides (FOS), galactooligosaccharides (GOS), chitooligosaccharides (COS), xylooligosaccharides (XOS), mannooligosaccharides (MaOS), and gentiooligosaccharides (GeOS). Other types include human milk oligosaccharides (HMOS) found in the breast milk of humans and the algae-derived marine oligosaccharides (ADMOS) including the oligosaccharides of alginate, fucoidan, laminarin, ulvan, and so on [28]. Native oligosaccharides in free forms exist in small amounts in nature, and they are mostly found attached to other biomolecules such as lipids and proteins in the form of glycolipids and glycoproteins, respectively [2, 23, 24, 26]. The examples of which include oligosaccharide antigens bound to the lipids and proteins on the surface of

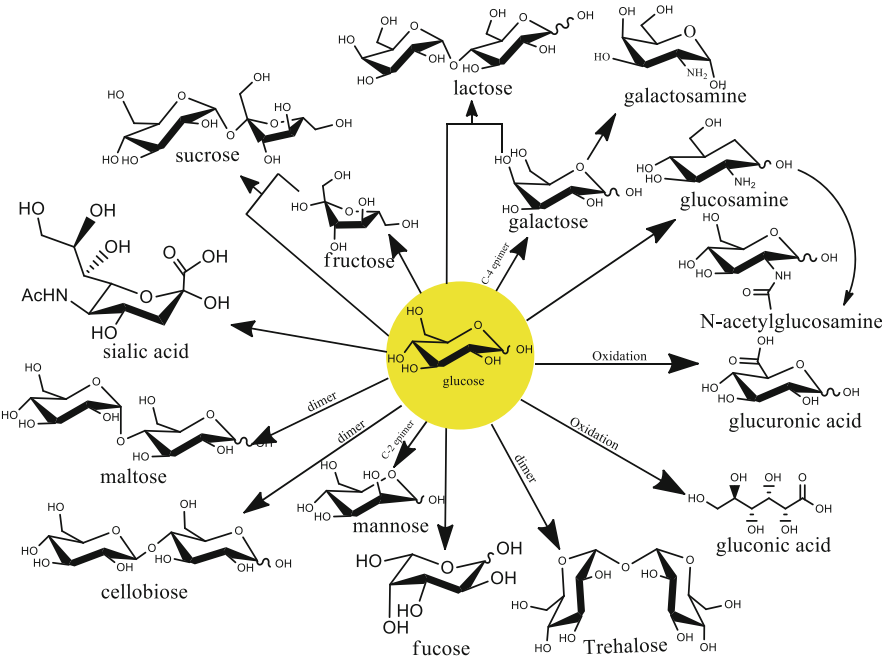


Fig. 13.2 Chemical structures of some of the selected monosaccharides, disaccharides, and sugar derivatives

red blood cells. They are involved in various processes including cellular recognition and formation of blood group serotypes [1, 2, 23, 24]. Oligosaccharides are resistant to enzymatic degradation and are commercially used as dietary prebiotic supplements. Many of the oligosaccharides are reported to exhibit antibacterial, antioxidant, immunomodulatory, and potential anticancer activities [28–33]. These versatile bioactivities of oligosaccharides were utilized in the design and functionalization of various oligosaccharide-based nanoparticles [3, 4, 27]. Polysaccharides are composed of long chains of polymeric sugars linked in a similar fashion to that present in the oligosaccharides. They contain up to hundreds of thousands of glycosyl repeats. The linear oligo- and polysaccharides have only one reducing end because of the nature of most glycosidic bonds e.g., from the anomeric carbon of one glycosyl unit to the nonreducing hydroxyl groups of other units between their glycosyl units [1, 2]. The degree of polymerization (DP) is a scientific term used for specifying the number of constituent molecules in polymers (monosaccharides in the case of carbohydrates). Figure 13.2 shows the structures of some selected saccharides.

Classification of polysaccharides has been challenging not only because they have complex structures but they also exhibit a vast number of distinct, biological, chemical, and nutritional characteristics [2, 5, 34]. Polysaccharides exist in a range of structures from linear, e.g., cellulose and amylose, to highly branched forms such

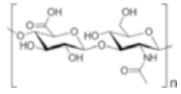
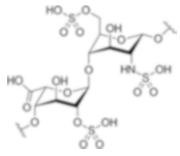
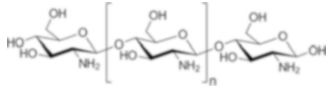
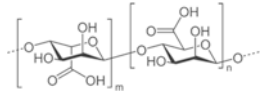
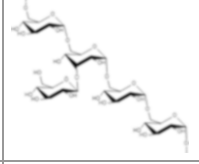
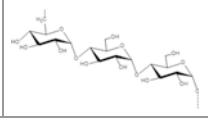
as amylopectin and glycogen. They can be obtained from plants, i.e., gum arabic, inulin, locust bean gum, from animals e.g., chitosan, hyaluronic acid, heparin, and heparan sulphate, from algae, i.e., agar, alginate, fucoidan, carrageenan, and from microbial organisms such as dextran, pullulan, xanthan, and gellan gums.

They are classified by composition as homo- and heteroglycans. Polysaccharides containing a single type of monosaccharide unit in their chain are called homopolysaccharides or homoglycans e.g. cellulose, fructan, araban, mannan, and galactan, likewise, those with more than two or more types of monosaccharide units are called heteropolysaccharides or heteroglycans e.g. hyaluronic acid, chitosan, guar, cassia, and locust bean gums. They can also be classified by their charge such as chitosan, hyaluronic acid, and cellulose that are, respectively, cationic, anionic, and neutral polysaccharides. Some of the bioactive polysaccharides that have caught significant biomedical interest in a wide range of biomedical applications are listed in Table 13.1.

The DP, number of repeating units, type of linking, molecular weight, and monosaccharide contents found in the composition of polysaccharides vary depending on their type and source. Additionally, branching, conformation, flexibility, hydrophilic/hydrophobic character, and electrical properties of polysaccharides are subject to many variations. Taken together, it can be attributable that these parameters are major factors for the physicochemical and bioactive properties of polysaccharides that are pivotal for homeostasis in cellular functions. A highlighting example on the relevance of distinct carbohydrate compositions and relation to their function can be given as the complex structure of the glycocalyx. It is the outermost layer that overlays the surface of the plasma membrane on most eukaryotic and prokaryotic organisms. It serves as a protective shield for prokaryotic organisms and contributes to the invisibility of the bacteria against the immune system detection of host organisms and also associated with bacterial adhesion to surfaces and biofilm formation [35, 36]. As the name implies, glycocalyx is composed of glycosylated elements called glycoconjugates including glycoproteins, proteoglycans, and glycolipids. The composition of the glycocalyx varies according to cell and tissue types. Beyond its role as a mechanical barrier against pathogen invasions and disruption of the plasma membrane by mechanical stress, glycocalyx has complicated kinetic functions that include cellular recognition by providing compartmentalization for transmembrane proteins and receptors, modulation of cell-cell adhesion, vascular homeostasis, and so on. Perturbations or redecoration of the glycocalyx composition is associated with many acquired diseases, such as vascular disorders caused by the deterioration of the glycocalyx of the endothelial cells, and increased aggressiveness or lethality of some cancer types upon decorating the composition of glycocalyx components [37–43].

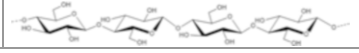
These attributes are crucial to take into consideration when designing carbohydrate-based particulate DDSs, as they will have a direct influence on the surface charge, size, shape, and textural morphology, bioactive and physicochemical properties of the derived nanoparticles (NPs), as well as their flocculation properties, protein adsorption, and interactions with other biological molecules [3–5, 25, 44, 45]. Furthermore, biological safety, extent of compatibility, toxicity, degradation,

Table 13.1 Some selected bioactive polysaccharides are listed by their name, source, and compositions

Name	Structure	Repeating unit	Linkage	Source
Hyaluronic acid		D-glucuronic acid and D-*N*-acetylglucosamine	Alternating β-1,3 and β-1,4	Animal origin
Heparin		2-*O*-sulfateiduronicacid 6-*O*-sulfate-*N*-sulfate glucosamine	α(1 → 4)	Animal origin
Chitosan		D-glucosamine *N*-acetylglucosamine	β-(1.4)	Animal origin
Alginic acid		β-D-mannuronic acid and α-L-guluronic acid	β-(1.4)	Algal origin
Dextran		Glucose	α-(1.6) linked with minor α-(1.3) branches	Dextran
Pullulan		Maltotriose	α-(1 → 6)-linked (1 → 4)-α-D-triglucosides	Pullulan

(continued)

Table 13.1 (continued)

Name	Structure	Repeating unit	Linkage	Source
Pectin		Galacturonic acid	α-(1.4)	Pectin
Cellulose		Glucose	β-(1.4)	Cellulose

duration of systemic circulation, and cellular internalization, alongside capacity of drug loading and release behaviors of the resultant nanomaterials are greatly determined by these parameters [46–50].

13.3 Applications of Carbohydrate-Based Functional Interfaces in Nanomedicine

Nanomaterials have been the subject of increased attention over the past few decades as intriguing vehicles for biomedical applications [51–53]. They have been used as drug delivery devices and in diagnosis, imaging, and treatments of various diseases such as infection, cancer, neurological, and inflammatory disorders [54–57]. The use of nanomaterials as DDSs provides several advantages in comparison to the administration of therapeutics by conventional ways.

Rapid clearance of circulating drugs via renal excretion, splenic, pulmonary, and hepatic elimination as well as by the action of mononuclear phagocyte systems (MPSs) can be reduced by tuning the size and surface properties of nanomaterials. They also decrease the toxic side effects of drugs and enhance their bioavailability by offering increased solubility and extra protection against harsh conditions, along with decreasing unspecific cellular uptake and potentially controlled/responsive release kinetics. Moreover, taking advantage of the small sizes, they can pass through even the smallest capillaries and easily penetrate the cells, thereby increasing the biodistribution and therapeutic efficacy of drugs [6, 20]. In a nutshell, the biodistribution and overall efficiency of a drug delivery system are drastically associated with the size, shape, and surface properties of nanomaterials [58]. In this viewpoint, a wide range of nanomaterials have been developed from a variety of sources and types including inorganic NPs, quantum dots, dendrimers, polymeric NPs, micelles, and so on. Amongst the multifarious materials that have been used to construct nanocarriers, polymeric NPs derived from natural and renewable sources have gained a great deal of interest and feasibility in a broad spectrum of applications such as in ophthalmic nanodevices [59–61], tissue engineering [60, 62], and delivery of many drugs [63, 64], proteins [65, 66], siRNAs [67, 68], and so on. Furthermore, carbohydrate-based nanocarrier systems have been the focus of intense research in recent years upon a significant rise in demand for designing natural, biodegradable, and effective drug delivery systems due to the objectives mentioned above. In particular, the unique properties of carbohydrates, such as biodegradability, biocompatibility, nontoxicity, protein repellent ability also called the stealth effect, the ease of modification, and tendency to form supramolecular networks, make them indispensable biomaterials for drug delivery and release applications [3, 4, 27, 69–71]. Besides, carbohydrates have been extensively implemented for their ability to balance toxicity and colloidal stability, functionalization, biocompatibility and biodegradation of various nanovehicles.

13.3.1 Functionalization of Carbohydrate Nanocarriers

Sugar-decorated cationic dendrimers are one of the examples addressing carbohydrate functionalization of dendritic nanocarriers that are hyperbranched multivalent nanostructures with a large number of terminal groups. However, they induce cytotoxicity and high hemolytic activity by disrupting biological membranes due to high cationic density in their periphery [72]. Functionalization of cationic dendrimers with carbohydrate moieties, e.g., maltose disaccharides are one of the prime methods that have been exploited to decrease their toxic effects. Maltose was used in functionalization of poly(propylene imine) (PPI-GX) dendrimers of second to fifth generations while "X" denotes the generation numbers of PPI dendrimers. Amongst the modified and unmodified PPI dendrimers, G2 and G5 generations of both modified and unmodified dendrimers were evaluated for their hemolytic activity at 3 and 6 mg/mL concentrations. The results of the hemolysis test indicated that while unmodified PPI dendrimers showed destructive hemolytic indexes at both concentrations in a generation-dependent manner, upon maltose functionalization, their hemolytic activity significantly decreased in both generations and dendrimer concentrations [73]. Similar studies have shown the effect of carbohydrate functionalization in reducing the toxic effect of dendrimers [74–77]. In another study conducted by Sun et.al, self-assembled dendron-like poly(ε-caprolactone) (PCL) and third-generation poly(amido amine) dendron (D3)-based diazonaphthoquinone (DNQ) conjugated dendritic nanomicelles (D3-PCL-DNQ) were prepared with about ~100–200 nm size ranges by Click chemistry [78]. The obtained nanocarriers with two near-infrared (NIR) photon sensitivity at 365 and 808 nm were functionalized with lactose (D3-PCL-DNQ-LAC) and glucose (D3-PCL-DNQ-GLC) as active targeting agents, respectively, with two types of lectins Concanavalin A and *Ricinus communis agglutinin* (RCA_{120}). The anticancer drug doxorubicin (DOX) was loaded onto the sugar-conjugated dendritic micelles by encapsulation with 6.2 weight (wt)% loading capacity of D3-PCL-DNQ-LAC and 6.8 wt% loading capacity of D3-PCL-DNQ-GLC. Drug release profiles from dual responsive sugar-decorated dendritic nanocarriers were controlled by changing the time of exposure to irradiation, and ~ 40% of DOX was released in 45 h for both dendrimers at pH 7.4 up to 10 min of irradiation in PBS. Lectin-binding abilities of the micelles were monitored by online DLS and optical density measurements, which showed successful targeting of the micelles upon sugar conjugation.

Quantum Dots (QDs) are another type of nanocarriers with superior optical properties aimed to be used as potential bioimaging and contrast agents [79, 80]. However, their toxicity and low solubility limit their use in biolabeling applications, increasing the need for modification and solubilizing and stabilizing them in aqueous solutions. Sugar functionalization can be applied to achieve the functional use of QDs in imaging applications. In a study conducted by Babu et al., sugar-functionalized CdSe-ZnS core-shell QDs (S-QDs) were produced with increased solubility and stability in aqueous environments for detection of lectins [81]. Lactose, melibiose, and maltotriose were used as sugar sources in the production of S-QDs. Water-soluble S-QDs exhibited reversible selective agglutination and

lectin specificities that promise marked potentials for analytical and nanomedical applications. Carbohydrate functionalization also provides stable and protective compartments for synthesis and reduces the toxicity of inorganic NPs [82–84]. Laminarin polysaccharide (LP), which is a seaweed-derived bioactive polysaccharide found in brown seaweed, i.e., *Laminaria digitata*, was used as a template in the stabilization of selenium nanoparticles (SeNPs) that otherwise were unstable with a tendency for aggregation. LP-decorated SeNPs (LP-SeNPs) with an average size of 60 nm was demonstrated to exert marked cytotoxicity against HepG2 cells by inhibition of autophagy and increased apoptosis [85]. Carbohydrate-functionalized NPs can be used in enhancing stability and dissolution of poorly water-soluble drugs [65, 86]. Vengala et al. prepared three-layered lactose-coated ceramic nanoparticles (aquasomes) with a median size of 92 nm to increase the dissolution rate of an antipsychotic hydrophobic drug pimozide. The aquasomes were prepared via the co-precipitation technique via sonication that was followed by lactose coating and adsorption of the pimozide drug. The results of drug release from the aquasomes exhibited first-order kinetics and improved dissolution of pimozide in comparison to the solubility of a bare, nonadsorbed drug [87].

Hyaluronic acid (HA) is an important type of glycosaminoglycan (GAG) in the extracellular matrix (ECM). It is composed of D-glucuronic acid and N-acetyl-D-glucosamine repeats linked by alternating β-(1–3) and β-(1–4) glyosidic bonds [88]. In a study, an antimicrotubule cancer drug paclitaxel (PTX) was conjugated to primary hydroxyl groups of hyaluronic acid from C-6 of the N-acetyl-D-glucosamine (GlcNAc) residue retaining the carboxylate groups on the glucuronic acid (GlcA) unmodified to reserve the bioactivity of HA [89]. The carboxyl groups of HA was revealed to be essential in maintaining its hydrophilicity and CD44 receptor interactions [90, 91]. Thus, PTX-conjugated HA (HA-PTX) was shown to contain 22% PTX in its composition with good water solubility and retained the CD44 receptor-binding capability. HA-PTX conjugates exhibited less than 12% and 20% release of PTX in 96 h at pH 6.0 and 7.4, respectively. Upon incubation with hyaluronidase (HAase) enzyme at pH 6.0, the release of PTX was increased from 12 to 42%. HA-PTX nanoconjugates were internalized by A549 and HepG2 cancer cell lines through CD44-mediated endocytosis. The cytotoxicity studies indicated that HA-PTX nanoconjugates showed increased apoptosis in both cell lines in comparison to free PTX, from ~7.1 to 30.5% for A549 cells at 0.1 μg/mL PTX concentrations and from ~10.9 to 22.1% for HepG2 cells at 0.025 μg/mL PTX concentrations. Mannose is a simple hexose sugar with complex and stringent regulation in the metabolism of humans [92]. It is a C-2 epimer of glucose possessing important roles in recognition and communication events, e.g., glycosylation of cell surface proteins [93]. Mannose functionalization was applied to target a C-type lectin receptor DC-SIGN also known as CD209 on the surface of dendritic cells. Gold nanoparticles were functionalized with thiol-linked (oligo) mannosides to block the binding of HIV envelope glycoprotein gp120 to the DC-SIGN receptor [94] . In another work, dimannose and lactose moieties were used in the functionalization of polyanhydride NPs via amine-carboxylic acid coupling to target various C-type lectins on bone marrow-derived dendritic cells, again aiming to

induce immune responses. Dimannose-coupled polyanhydride NPs were termed as "pathogen-like" nanoparticles to be used as a potential vaccine adjuvant for improving the efficacy of vaccines [95]. Furthermore, various nanocarriers are functionalized with different carbohydrate residues such as monosaccharides [9, 96–98], disaccharides [99–102], oligosaccharides [49, 50, 103–105], and polysaccharides [46–48, 106] for targeting, and distinct functionalities have been rendered in each case.

Besides the functionalization of nanocarriers, carbohydrates were also used in the functionalization and enhancement of therapeutic activities of native biological molecules including growth factors, peptides, and proteins [107, 108]. HA was conjugated to a peptide hormone, insulin. Its functionalization with HA enhanced the hypoglycemic activity of insulin and prolonged its plasma half-life [109]. In a similar rationale, a low molecular weight polysaccharide, dextrin, which is produced by hydrolysis of glycogen or starch, was used in the functionalization of trypsin. When it was conjugated to trypsin, its activity was masked by the polysaccharide in a restorable manner and could be recovered after degradation of dextrin by α-amylase enzyme [110]. This type of functionalization utilizing polysaccharides as protective and/or reversible activity-masking agents for proteins are termed Polymer-masked unmasked protein therapy (PUMT) [111].

13.3.2 Carbohydrates as Full Construction Agents: Synthesis and Applications of Nanogels and Microgels

Besides the use of carbohydrates in the functionalization of nanocarriers and biological molecules, they have been employed as construction precursors of complete nanostructures, i.e., micro/nano hydrogels [63, 112, 113]. Hydrogels are crosslinked hydrated networks of polymeric particles possessing remarkable superior properties such as high water absorption and surface area, adjustable particle size, modifiable surface properties, and enhanced drug loading capacities as compared to other nanovehicles [20, 64, 66, 70, 114, 115]. Various top-down and bottom-up approaches are employed for the synthesis of hydrogels, each with their advantages and limitations [116, 117]. Although diverse techniques have been developed as bottom-up synthesis of colloidally stable hydrogels, such as photoinduced crosslinking, radical polymerization, precipitation polymerization, and emulsion polymerization, and the following two main mechanisms dictate hydrogel formation:

1. Physical crosslinking via noncovalent interactions such as van der Waals forces, hydrophobic and electrostatic interactions, hydrogen bonding, and chain entanglements between polymers that result in the formation of self-assembled physically crosslinked networks.
2. Chemical crosslinking through intra- [118, 119] and intermolecular [118] covalent bonding under appropriate reaction conditions.

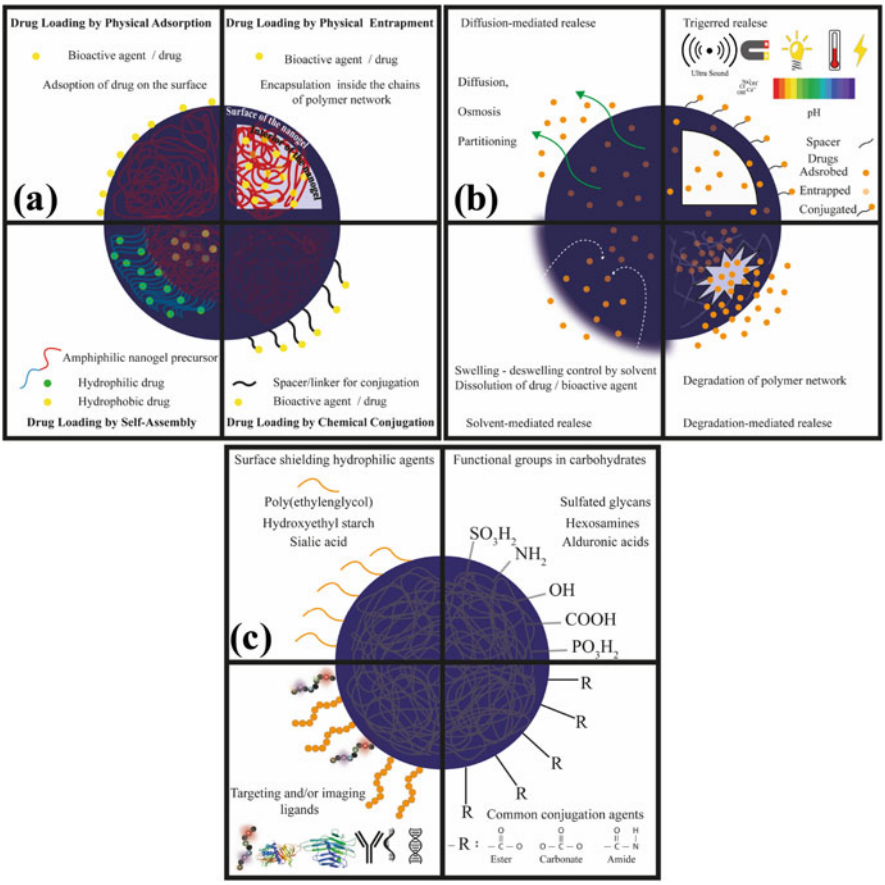


Fig. 13.3 Schematic illustration of (**a**) drug loading, (**b**) release, and (**c**) targeting techniques

Nanogels and microgels can be incorporated with various bioactive compounds and therapeutics. Different methods of drug loading have been developed and engineered to improve the kinetics of loading and release in terms of loading capacity, pattern, and rate of drug release. As illustrated in Fig. 13.3a, three major techniques have been employed for loading of drugs toward microgel and nanogel structures:

(a) Incorporation of drugs through physical adsorption and entrapment from solutions in appropriate solvents, and therefore, the interactions between functional groups carried by drugs and hydrogel particles as well as noncovalent interactions, for instance, van der Waals forces, hydrogen bonding, and hydrophobic and electrostatic interactions are the major decisive forces for the efficiency of loading.

(b) Chemical attachment of drugs to pendent groups on the surface of hydrogels through covalent conjugation that is mostly accomplished by using spacer molecules carrying certain types of functional groups that form labile and hydrolysable bonds, steadily degradable in biological environments, e.g., ester bonds, amide bonds, carbonate bonds, and so on.
(c) Self-assembly by simultaneous organization of drugs and polymer chains through noncovalent interactions, e.g., hydrophobic and/or electrostatic associations as main driving forces in thermodynamically favorable minima leads to the formation of stable molecular structures.

One of the fundamental goals in controlling the pattern of drug release is to manage and keep the plasma concentration of drugs in their therapeutic ranges with lesser fluctuations [120, 121]. As depicted in Fig. 13.3b, the mode of drug release from nanocarriers can be realized by several mechanisms such as

1. Diffusion-mediated drug release,
2. solvent-controlled drug release,
3. the release upon degradation of nanocarriers, i.e., nanogels and microgels, and,
4. triggered release that causes a change in the physical or chemical state of nanomaterials by sensing an external stimulus such as electromagnetic radiation, pH, temperature, ultrasound, magnetic field, electricity, or ionic strength and so on.

Moreover, there have been several methods of approach extensively investigated in modeling the pattern of drug release kinetics for various nanocarrier systems including

1. Statistical methods, e.g., exploratory data analysis and multivariate analysis of variance (MANOVA),
2. Model-independent methods, e.g., difference factor (f_1) and similarity factor (f_2)
3. Model-dependent methods, e.g., zero-order, first-order, Higuchi, Korsmeyer-Peppas model.

With regard to model-dependent methods, patterns regarding the rate of drug release can be summarized as follows:

- Zero-order, this type of release kinetics exhibit constant rates of release in a sustained manner over a period of time, which is the ultimate aim of most DDSs.
- First-order release is defined as the rate of the drug release that is dependent on its concentration.
- Rapid initial burst release followed by zero- or first-order release.

More information on the kinetics of drug release can be obtained from comprehensive studies in the literature [122–124].

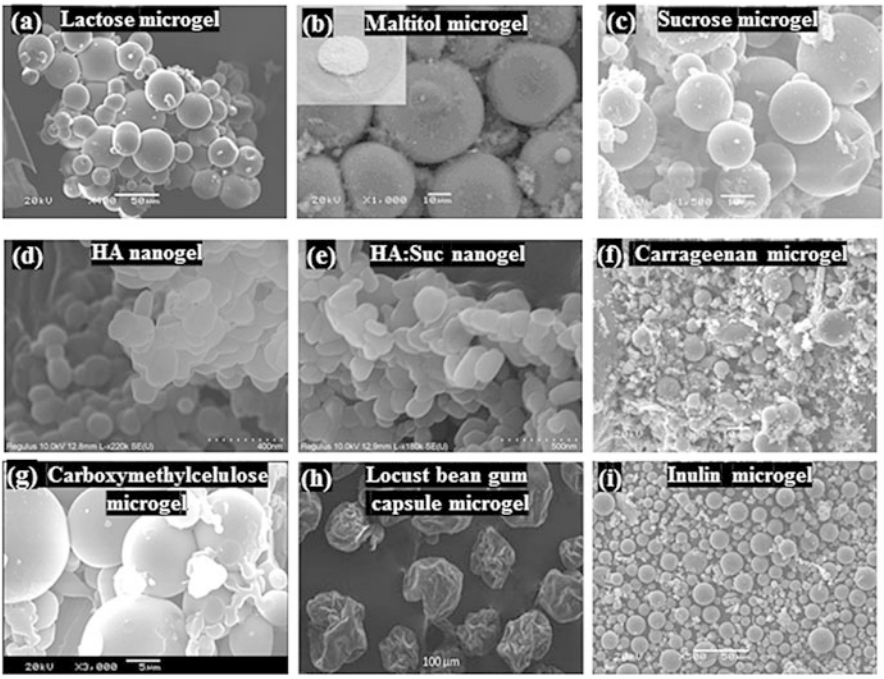


Fig. 13.4 SEM images of (**a**) sucrose, (**b**) lactose, (**c**) maltitol (**d**) HA, (**e**) HA:sucrose, and (**f**) carrageenan, (**g**) carboxymethyl cellulose, (**h**) locust bean gum, and (**i**) inulin microgels and nanogels. (**b**, **c**), and (**f**–**h**) was reprinted with permission from ref. 113, 115, 128, 133, 134, and, respectively

Carbohydrate-based DDSs have attracted remarkable attention owing to their inherent biodegradability and native or acquired responsiveness to external stimuli, as they are intrinsically affine to some biomolecules, e.g., lectins. As depicted in Fig. 13.3c, based on the multivalence of binding and plenty of functional groups that can be easily modified by which responsive molecules can be linked to the backbone of the polymer network and attain those behaviors [125–127].

Simple sugars such as lactose, sucrose, and maltitol were explored by our research group as hydrogel precursors to create crosslinked polymeric networks of micro- and nano sizes as shown in Fig. 13.4a-c [69, 113, 128]. Synthesis of crosslinked poly(lactose) nanogels and microgels were achieved using divinyl sulfone (DVS) as a chemical crosslinker [69]. Poly(lactose) micro/nano hydrogels were reported to be compatible for blood interactions at 2 mg/mL particle concentrations. Poly(lactose) particles with tunable surface charge and functional groups were shown to be used as natural potential DDSs. Moreover, poly(maltitol) micro/nano hydrogel particles were prepared as blood compatible, modifiable, and biocompatible DDSs that were shown to be loaded with ciprofloxacin (CPX) by physical absorption and chemical conjugation. They have exhibited almost linear release profiles; about 3.5 mg/g of drug was released within 3 h from p(maltitol)

particles upon adsorption-mediated drug loading, whereas it was almost doubled in amount, 6 mg/g with sustained CPX release up to 12 h from CPX-conjugated p (maltitol) particles [113]. Similarly, poly(sucrose) microparticles were synthesized by chemical crosslinking with DVS and tuned for their responsiveness to magnetic field [128]. More than 66% of the poly(sucrose) particles were shown to be degradable at pH 7.4 in 10 days. Besides, the simple sugars, polysaccharides of various composition, size, charge, and bioactivities were also rendered to form versatile networks for the delivery of therapeutic agents. The most common polysaccharides that have been recruited in the design of DDSs are hyaluronic acid (HA), heparin (HEP), chitosan (CS), alginic acid (ALG), dextran (DEX), and pectin (PEC) polysaccharides, where distinct properties were endowed for each of the nanovehicles. HA has been widely used in the preparation of various nanomaterial-based formulations in the delivery of drugs, nucleic acids, hormones, and proteins [129–132]. For instance, HA and HA:sucrose nanogels have been prepared by our research group within the size range of 50–200 nm. The nanogels attained spherical-like irregular shapes with rough and porous surfaces as illustrated in Fig. 13.4d, e. Owing to the high surface area, HA and HA:sucrose nanogels are amenable to surface decoration and/or modification for hydrophobic drug conjugation/adsorption studies. The prepared HA and HA:sucrose nanogels were found to be blood compatible up to 250 μg/mL and hold promising potential as sustained release systems with degradable and customizable characteristics [63]. Besides HA, HA:sucrose, and simple sugars, polysaccharides such as inulin, carboxymethyl cellulose, carrageenan, and locust bean gum-based microgels and nanogels were reported by our group as seen in Fig. 13.4d–i [69, 113, 115, 128, 133–135].

It was reported that the design of HA-based particles with the ability of on-site tracking and visualization upon coupling various fluorescent dyes to particle backbone is possible [136–140]. For instance, HA-based dual sensitive nanogels termed F-nanogels are labile to pH-mediated and enzymatic degradation. They were prepared as dual carriers for targeted co-delivery of DOX and nitric oxide (NO) to cancer cells [29]. In the design of this system, boronic acid-conjugated lactose-modified chitosan (chitlac-BOH) and dopamine and NO-conjugated partially carbonized fluorescent HA [NO/DA-FNP(HA)] was crosslinked through boronic chemistry. By means of the attached fluorescent dye and responsive degradability alongside the natural cancer targeting ability of HA, the F-nanogel system shows promising potential in theranostic cancer chemotherapy. The molecular weight of HA is one of the major parameters affecting physicochemical characteristics and in vivo performances of HA-derived carrier systems [141–144]. HA-based nanomaterials have been reported by different types of preparation methods, e.g., self-assembled HA-testosterone conjugates were prepared by Quinones et al. for sustained release of DOX and camptothecin (CPT) drugs [131]. In another study, HA-steroid conjugates were obtained by self-assembly of hydrazide-modified HA molecules and coupled with diosgenin and two types of brassionsteroids D131 and S7. HA-steroid conjugates exhibited sustained release profiles up to 72 h with a nearly constant rate of release in the first 8 h [145]. In another work conducted by Ji et al., biodegradable β-cyclodextrin-grafted HA-based nanocomplexes were

prepared by formation of inclusion complexes between β-cyclodextrin and phenyl pendant groups, phenylalanine-based poly(ester amide). The prepared HA-based supramolecular nanocomplex was used to improve the solubility of gambogic acid to combat multidrug resistance in melanoma cell lines [146].

Heparin (HEP) is a negatively charged GAG type polysaccharide composed of disaccharide units varying in sulfate composition. It interacts with proteins, modulates the localization in the extracellular matrix, and regulates ligand binding and proteolysis against proteins [147, 148]. HEP was employed in the preparation of self-assembled amphiphilic nanoparticles [149–151] such as self-assembly of thiolated-HEP and poly(ethylene glycol) (PEG) in dimethyl sulfoxide (DMSO), resulting in the formation of HEP-PEG nanocomplexes by means of hydrogen bonding. The HEP-PEG nanocomplex was crosslinked by ultrasonic treatment and recruited for redox-responsive intracellular HEP delivery [151]. Similarly, thiolated HEP-pluronic conjugates formed by self-assembly and crosslinked nanogels (DHP) were formed through disulfide linkages upon oxidation of the HEP-based conjugates [152]. RNase A was loaded into DHP nanogels by encapsulation in order to investigate the efficacy of protein stabilization due to the electrostatic interactions between proteins and HEP. DHP nanogels gained high-protein loading efficiency with increased stability and redox responsive release that can be used as intracellular redox responsive DDS. Moreover, low molecular weight HEP (LH) was prepared in the form of nanogels (LHP) upon pluronic F127 conjugation. LHP was tested for its role in liver fibrosis. It was preferentially internalized by liver tissue, compared to nonconjugated LH, and distributed in the site of liver injury. LHP nanogels were reported to exhibit hepatoprotective and antifibrotic activities by inhibition of transforming growth factor-β1 (TGF-β1)/Smad signaling. Thus, the LHP nanogels have a potential use in the treatment of cancers and injuries of the liver upon loading with different anticancer agents [153]. Long circulating HEP-based nanogels were prepared by disulfide crosslinking. Methacrylate-functionalized HEP was joined in disulfide-crosslinking via copolymerization with cysteamine bisacrylamide (CBA) in an aqueous environment, resulting in the formation of HEP nanogels with a size range of 80–200 nm [154]. Synthesis of HEP nanogels was done through amidation between the carboxylic acid groups of HEP. The prepared HEP nanogels were loaded with DOX from basic drug solution. Up to 30% drug loading content and 90% efficiency were reported. As seen in Fig. 13.5a, in vitro release profiles revealed slow drug release under physiological pH with ~10% at pH 7.4 and 20% at pH 5.5 for 240 h. Faster drug release was observed in the presence of 10 mM 3 mg/mL glutathione (GSH) ~60% at pH 7.4 and ~ 80% at pH 5.5, respectively. In vivo drug distribution was examined by near-infrared (NIR) fluorescence imaging dye, NIR-797, labeling through the hydroxyl groups on HEP and monitored as a function of time. DOX-loaded HEP nanogels have gained an extended systemic circulation with effective targeting of the tumor cells. As can be seen from Fig. 13.5b, a rapid and significant accumulation of HEP-based nanogels at the tumor site was observed clearly even at only 1-h post-injection (p.i.). After 48 h, the accumulation of HEP nanogels reached its maximum at the tumor site and lasted for 120 h before being eliminated. Figure 13.5c shows the normalized NIR fluorescence images using a

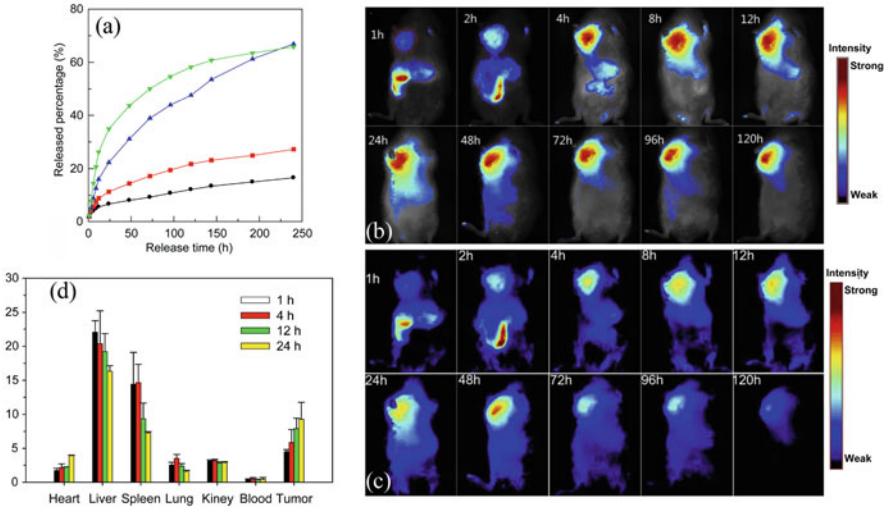


Fig. 13.5 (**a**) In vitro DOX release from heparin-based (HEP) nanogels, 0.01 M PBS at pH 5.5 and 7.4 with/without the presence of 3 mg/mL GSH in release media, (**b**) NIR fluorescence images of a hepatic H22 tumor-bearing mouse at different time points after injection of the NIR-797-labeled HEP nanogels via the tail vein. The circled region is tumor; (**c**) the images shown (**b**) after normalization using a constant exposure time. (**d**) Biodistribution of DOX in different tissues of hepatic H22 tumor-bearing mice at various time points after injecting DOX-loaded nanogels via the tail vein. The values were acquired as the percentage of ID per gram of collected tissues and based on three mice per group. Adopted from ref. 154 with permission

constant exposure time. In order to investigate whether the distribution of nanogels were parallel with the distribution of DOX in tissues, DOX was recovered from the tissues, and quantitative examination of the in vivo DOX distribution was performed by fluorescence spectroscopy. The results were expressed in average percentage of injected dose per gram of wet tissues (% ID/g) as shown in Fig. 13.5d. The maximum DOX concentration in liver and spleen was found to be 22% ID/g at 1 h p.i., and 14% ID/g at 4 h p.i., while it reached 4.5% ID/g at 1 h p.i. and increased up to 9.3% ID/g after 24 h p.i. at the tumor site.

As clearly seen from Fig. 13.5, DOX-loaded HEP nanogels show sustained release up to 240 h, sensing the pH and reductive stimulus of the medium. In vivo administration of DOX-loaded HEP nanogels to H22 tumor-bearing mice showed extended in vivo circulation times and successful accumulation around the site of tumor. 9.3% ID/g DOX was retained at 24 h post-injection, indicating that HEP nanogels can be used as long circulated, high loading, and effectively targeted sustained DDSs. Figures were adopted from ref. [154] with permission.

Chitosan is a deacetylated derivative of chitin under alkaline conditions. It is composed of N-glucosamine moieties linked by β-(1–4) bonds. CS has a nontoxic, biodegradable, biocompatible, and mucoadhesive properties [155]. It is soluble in dilute acid environments and insoluble under physiological conditions which can be utilized in site-specific targeting of drugs, i.e., for ophthalmic [61] and colonic drug

delivery [156, 157]. CS NPs formed by ionic gelation between CS and sodium tripolyphosphate (TPP) in the range of 90–200 nm sizes with a narrow size distribution and loaded with bovine serum albumin (BSA) to a maximum of 51 wt% [158]. In vitro BSA release from CS NPs was performed in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) at pH values of 1.2 and 7.5, respectively. The result of BSA release showed that 75–90% of the loaded BSA was released in 24 h in SGF. On the contrary, the BSA release in SIF was slower since the CS NPs experienced partial collapsing and the BSAs were entrapped in CSNPs. In another study, CS NPs were prepared by ionic gelation of CS with TPP ions and loaded with quercetin, which is an antioxidant phenolic compound of plant origin with low water solubility; CS-quercetin inclusion complexes formed to potentiate the bioavailability of quercetin [159]. Another study was conducted by Maestrelli et al., in which hydroxypropyl cyclodextrin (HPCyD) entrapped CS nanoparticles (HPCyD-CS) were prepared aiming to increase the bioavailability of class 2 and 4 drugs, triclosan and furosemide [160]. The ability of CyDs to form molecular inclusion complexes with drugs were utilized, which in turn were entrapped with CS NPs, providing extra protection and facilitated absorption of drugs. The release of drugs from HPCyD-CS NPs showed similar kinetics for both drugs, that is, fast drug release within 1.5 h at about 40–60% of the total release, and followed by delayed release to 24 h [160].

Alginic acid is a linear polysaccharide derived from brown seaweeds and some bacterial species. It is composed of β-D-mannuronic acid (M) and α-L-gluronic acid (L) residues through 1,4 glycosidic linkage [161]. The gel formation abilities of ALG make it feasible for encapsulation of macromolecules, e.g., proteins and peptides; however, the composition of ALG including viscosity and the ratio and distribution of constituent monosaccharides M and L has important roles in the swelling behaviors of prepared interfaces [162], for example, ALG beads were synthesized by the ionotropic gelation technique [163, 164]. The counter ions used in synthesis was reported to significantly change the release behaviors of ALG beads [165]. Alginate NPs were prepared to encapsulate exemestane (EXE), a cancer drug used for breast cancer, by controlled gelation and reported as a potential drug carrier for sustained release of EXE [166]. In another research, ALG-CS construct-loaded AgNPs were prepared with antibacterial and antitumor properties against HeLa cells, while the L929 fibroblast cells retained their viability [167].

Dextran is a linear bacterial polysaccharide composed of α-(1–6) linked glucose residues with an average of 5–10% α-(1–3) branching. It is not degraded by the upper GI tract and metabolized by colonic microorganisms. DEXs have been used as blood substitutes, scaffolds for cartilage tissue engineering [168], and conjugated with various drugs [169, 170]. DEX-based nanogels were reported in siRNA delivery and gene silencing applications [171]. Moreover, methacrylated DEX nanogels by disulfide crosslinking were prepared with pH and redox dual-responsive properties and internalized by Hela cells [169]. In a similar study, ovalbumin (OVA) was immobilized on cationic DEX nanogels through disulfide linkage [172]. While the release of OVA was significantly low at physiological pH without reductive stimulus, it showed a rapid in vitro release in the presence of GSH, being consistent with the intracellular release in D1 dendritic cells. It was concluded that thiol groups

provide controllable release for intracellular drug delivery. Similar studies were reported for protein and drug release from DEX-based nanogels [173–175].

Pectin is an acidic heteropolysaccharide found in the primary cell walls of plants. It is majorly composed of linear chains of α-(1–4) linked d-galacturonic acid moieties with hundred to thousand DPs. They are highly soluble in water but are not digestible by the upper gastrointestinal tract and degraded by microbial organisms in the colon. It has been used as a coating agent or excipient in ophthalmic [176], oral [177], and vaginal [71] drug delivery systems. Many attempts have been made to decrease the water solubility of pectin such as crosslinking with epichlorohydrin [178], amidation [179], and so on to design colon-targeted PEC-based DDSs [180–182]. In a study conducted by Majzoob et al., pectin was thiolated by attaching cysteine (Cy) amino acids to improve its mucoadhesive properties [183]. Upon conjugation of PEC with Cy, PEC-Cy conjugates were treated with pectinolytic enzymes to test its biodegradability. Consequently, thiolated PEC has been considered as a promising excipient possessing a biodegradable backbone with low toxicity and bead forming ability; moreover, the inconvenient properties of native PEC such as rapid hydration, swelling, and erosion were surmounted.

PEC and some other polysaccharides such as carrageenan, guar gum, and other gums are used as drug carriers, matrix tablets, and excipients. When used alone, they might not offer desired release profiles, e.g., zero-order release, although they are often used as polymer blends and/or conjugated to other carriers or residues, e.g., hydrophobic molecules to obtain desired dosage forms.

Furthermore, sulfated polysaccharides derived from algal and/or microbial organisms such as ulvan, laminarin, fucoidan, and so on have not been deeply investigated, yet there has been some studies reported, and they have a vast scope of potential in the development of nanocarriers due to their natural, degradable, and biocompatible properties [184–192].

13.4 Carbohydrate-Based Smart Delivery Systems: Basics of Targeted Delivery

Recently, emerging technology has arisen the quest of intelligent materials with well-defined, tailor-made architectures and controllable behaviors. Amongst the available materials, hydrogels and their smaller forms, nanogels and microgels are having remarkable customization capabilities that can be exploited in site-specific and/or target-oriented sensing of an external stimulus and release their contents accordingly. There are two fundamental ways employed in manipulating NPs, as passive and active targeting methods. Passive targeting encompasses the direction of nanocarriers to the target site through affinity or mode of binding, which are significantly influenced by experienced conditions in the present whereabouts of carriers such as pH, temperature, molecular site, and shape of the environment. Nanocarriers can be actively targeted to distinct tissues, cells, or biological molecules by engineering their surface [20, 70, 116]. Attachment of specific affinity ligands to the surface of hydrogels including oligosaccharides, polysaccharides,

aptamers, antibodies, lectins, and so on enables the molecular recognition of targets and hence selective binding [4, 81, 94, 95, 125]. As the stimulus has been detected by a responsive hydrogel system, an immediate corresponding response is triggered as a change in predetermined behavior, which could be the release of its therapeutic cargo by changing the swelling-deswelling dynamics, movement in a certain direction, a change in particle size, color, shape, and so on.

Efficient targeting, prolonged periods of circulation, and lower immunogenicity as well as sustained release of therapeutic cargo are the central responses aimed to be received from in vivo applications of smart carrier systems, but it is yet to be completely carried out. Although promising NPs formulated to achieve considerable plasma half-lives in circulation [107, 154, 193], majority of the DDSs suffer either from premature in vivo clearance by MPSs before they exert the therapeutic effects or they induce cytotoxicity [194, 195]. In this regard, besides serving as excellent functionalization agents, carbohydrates have been a great choice for the fabrication of carriers for particular several reasons. As nanocarriers are administered to the systemic circulation or a biological fluid, they interact with proteins in the environment, e.g., plasma proteins in blood will be adsorbed on the carrier surface due to the hydrophobic/electrostatic interactions and high surface energy of most nanocarriers. The absorption of proteins will result in the formation of a coating around NPs called protein corona [196, 197]. The composition and size of the corona will determine the subsequent fate of the nanocarrier system as it might cause phagocytic elimination of the NPs by MPSs, a process called opsonization. Opsonization makes in vivo drug targeting a quite challenging process. In order for a nanocarrier system to have a prolonged plasma half-life, the extent of opsonization should be reduced. This would be accomplished by the nanocarrier itself or by modification of its surface to create steric hindrance for protein adsorption.

Modification of NP surfaces with polyethylene glycol (PEGylation) by conjugation, coating, or adsorption has been extensively implemented to reduce surface opsonization. It has also decreased the uptake of nanocarriers by unspecific cells owing to the increased hydrophilicity and steric repulsions by PEGylated surfaces [47, 67, 68, 138]. However, the major drawback of PEG is that it is not a biodegradable polymer, as has been revealed by recent studies that PEG caused the occurrence of renal tubular vacuolization in animals [198]. This brings about some concerns that using PEGylated therapeutics during prolonged periods of time may cause the accumulation of PEG in the body especially in the kidneys [199, 200]. It has been reported that natural polysaccharides or some of their synthetic counterparts such as hydroxyethyl cellulose (HEC) were shown to exhibit low protein affinities [201–203]. Moreover, glycolipids and polysaccharides derived from microbial organisms, e.g., dextran were proven to decrease the uptake of nanocarriers by MPSs, which could potentially be used as substituents for PEGylation to evade opsonization of nanocarriers [204]. Another example of a carbohydrate with protein repelling properties is a monosaccharide, sialic acid (SA), found on the surface of erythrocytes. It naturally prevents erythrocytes from being phagocytized by MPSs and the red blood cells lacking SA moieties on their surface experience rapid

clearance from circulation [205]. This promising ability of SA was inspired for various nanocarriers to surmount premature clearance of nanoparticles [206–208].

13.5 Concluding Remarks

Carbohydrates as prominent biomolecules are highly complex, howbeit, quite functional elements in cells of living organisms. They function in diverse biological processes governed by inherent algorithms underlying their unique structural glycosides. The ubiquitous involvement of carbohydrates in the molecular recognition of biomolecules, signaling, and trafficking of cellular inputs and outputs as gatekeepers and/or transporters in the form of glycoconjugates such as glycoproteins and glycolipids as well as their use as primary instant energy sources and storage molecules has been briefly reviewed.

Realization of the biodegradability, biocompatibility, and unique bioactive properties of carbohydrates as well as their specific interplays in diverse cellular processes has sparked evergrowing interest in the design of carbohydrate-based-nanostructured interfaces as tailorable multifunctional bioplatforms for the construction of future therapeutics.

Nanocarrier systems provide exceptional feasibility from industrial to a large number of biomedical applications. For instance, nanovehicles improve the pharmacodynamic and pharmacokinetic properties of drugs in the following ways: They protect the cargo against acidic and oxidative degradation, decrease overdosing and related toxic side effects, show unspecific cellular uptake of drugs, and enhance their half-lives, solubility, and therefore therapeutic efficacy and plasma concentration in systemic circulation. The efficacy of nanocarriers is greatly dependent on the type, size, charge, and their composition. Inherently natural biomolecules from renewable resources are of paramount interest due to the lower immunogenicity and toxicity as compared to their inorganic or synthetic counterparts. Moreover, various synthesis mechanisms such as self-assembly, physical, and chemical crosslinking mechanisms were explored by employing different experimental techniques. Similarly, methods used for loading of distinct bioactive agents have been exploited to improve the efficacy of encapsulation and release kinetics. Taking inevitable inspiration from natural carbohydrates, nanocarriers were equipped with various moieties to target specific sites and undergoes mimicking to evade immune system phagocytosis.

Furthermore, computational studies coupled to experimental works capture envisaged snapshots to molecular interactions between drugs and biological interfaces that will lead to the development of next generation, more accurate, and easily controllable nanodevices, which will in turn hopefully enlighten new paths in diagnosis and treatment of persistent disorders such as HIV, malaria, diabetes, cancers, and genetic diseases. Researchers from glycobiology, nanotechnology, biophysics, and computational science peg away at understanding the source code of this secondary/auxiliary information network incorporating multidisciplinary approaches.

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Multifunctional Nanoscale Particles for Theranostic Application in Healthcare

14

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Abstract

The development of multifunctional nanoparticles has greatly expanded the application of nanomedicines for various health care ailments with better diagnosis and therapy. The combination of suitable imaging modalities with drugs and ligand-guided or external stimuli-guided site-specific delivery holds great potential for safer and efficient therapy. Various such nanocarrier systems have been discussed in this chapter such as the polymeric conjugates, dendrimers, micelles, gold nanostructures, Iron oxide nanoparticles, Quantum dots, carbon dots, and stimuli-responsive systems. In recent trends, these multifunctional systems involve a combination of photothermal, photoacoustic, and photodynamic effects along with the chemotherapeutic and other drugs. These systems hold great promise for better therapy for several difficult health care problems such as cardiovascular diseases, atherosclerosis, rheumatoid arthritis, Alzheimer, psychotic disorders, and inflammation and most importantly for the cure of various types of cancer and are presently under clinical manifestation.

Keywords

Theranostic · Nanoparticle · Healthcare · Multifunctional design

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M. K. Das, Y. V Pathak (eds.), *Nano Medicine and Nano Safety*,
https://doi.org/10.1007/978-981-15-6255-6_14

14.1 Introduction

In recent years, research has been trending toward the design of multifunctional nanocarriers, which can deliver the drug in a controlled manner to the targeted site and act as an imaging agent for simultaneous diagnosis and therapy. Such systems are termed as Theranostics, meaning a combination of Therapy and Diagnostics. This field has been growing since the last decade with the advancement of nanomedicines with a newer synthesis of nanostructures and due to the need of getting cured for diseases difficult to treat like rheumatoid arthritis, cardiovascular diseases, cancer, etc. [1]. Cancer is one of the major areas, which may be benefited from these theranostic designs. The major problem for successful clinical manifestations of various chemotherapy is the heterogeneity of the tumors [2]. This necessitates an approach for the combination of diagnosis and multifacet therapy using nanocarriers. Hence, the recent advancements in nanoparticulate theranostics have been an integration of diagnosis, drug monitoring, and targeted and controlled drug delivery [3].

Nanoparticles have been designed as theranostics as due to their large surface to volume ratio they can be multifunctionalized for diagnosis and controlled and targeted delivery. Moreover, many nanoparticles have intrinsic imaging properties (such as iron oxide based) or therapeutic properties (metallic nanoparticles), which can be cofunctionalized for better theranostic effect [4].

Most of these multifunctional designs are composed of a suitable imaging modality for advanced diagnosis. Molecular imaging is performed for the characterization of biological processes at the cellular and subcellular levels in intact organisms. These powerful techniques can help in simultaneous diagnosis with drug delivery for better therapy. Some of the multifunctional systems include these imaging contrast agents to achieve an image-guided more efficient site-specific delivery with reduced toxicity. Currently used molecular imaging modalities include MRI (Magnetic resonance Imaging), CT (Computed Tomography), US (Ultrasound), optical imaging (bioluminescence and fluorescence), single photon emission computed tomography (SPECT), and positron emission tomography (PET) [5]. Several multifunctional nanosystems have been designed containing these contrast agents alone or in combination with the drug. Besides the imaging agent and drug, these multifunctional systems also include suitable ligands for ligand-mediated targeted delivery or stimuli-responsive agents (pH, temperature, Ultrasound, etc) for stimuli-responsive targeted release of drugs. This has certainly helped in better designs of delivery systems for several diseases. Different nanocarriers have been designed successfully for such multifunctional approach, and recently, these systems have been used for the combination of chemotherapy, photothermal therapy, or photodynamic therapy and hold huge potential for better and safer efficacy for various health care problems.

14.2 Different Multifunctional Nanocarriers Used as Theranostic System

14.2.1 Polymer Conjugates

Polymer-drug conjugates using nondegradable polymeric carriers, particularly poly (ethylene glycol) (PEG) [6, 7] and N-(2-hydroxypropyl methacrylamide) (HPMA) copolymers [8, 9], have been used extensively, and some of these have also translated to use in the clinic. These functionalized conjugates are increasingly being utilized to obtain biodegradable, stimuli-sensitive, and targeted systems to increase the efficacy and safety of drugs particularly in cancer [10]. Numerous studies have been conducted in recent years on these conjugates for theranostic use for various health conditions. These theranostic agents help in monitoring the biodistribution and accumulation at the target site of therapy in a noninvasive manner [11]. Moreover, the extent of localization at the target site can be used as an accurate predictor of efficacy, which may help in relieving a patient from subsequent therapy that might not prove efficacious.

These polymer drug conjugates have been successfully designed for theranostic use by conjugating with suitable ligands for active targeting and contrast agents for imaging [12]. Besides, these polymer conjugations also result in increased circulation time, passive targeting, and reduced toxicity [10]. Chemical conjugation and copolymerization have been the two major schemes for functionalizing the polymers with the therapeutic and imaging agents [5].These polymer conjugates have been used for different types of imaging modality fluorescence imaging [13], MRI [14, 15], PET [16, 17], SPECT [18], and Ultrasound [19]. These polymer drug conjugates have been used for various ailments like inflammation cardiovascular diseases and particularly in cancer [20].

In a recent work by Etrych T et al 2018, HPMA–doxorubicin conjugate has been prepared for simultaneous malignant lymphoma treatment and lymphoma growth imaging. Two types of passively targeted conjugates were prepared, one using a single linear polymer chain and a *star*-shaped high-molecular-weight (HMW) polymer employing a dendrimer core. Both types of conjugates were designed as stimuli-sensitive systems by attaching the drug copolymer *via* a pH-labile hydrazone linkage and reacted with near IR dye Cy7-NHS-ester for fluorescence imaging. The effect of these conjugates was observed in murine models of malignant lymphomas including one cell line-derived xenograft (RAJI) and two patient-derived lymphoma xenografts (VFN-D1 and VFN-M2). They have better pharmacokinetics and uptake of the drug and polymer. Dynamically analyzed fluorescence intensity over subcutaneously xenografted lymphomas closely corresponded to changes in the lymphoma tumor volumes, thereby enabling a noninvasive assessment of treatment efficacy [21].

In a recent study by Gao X et al 2019, a novel theranostic probe (TPE-Man) has been designed by conjugating mannose with red emissive and AIE (aggregation-induced emission) active photosensitizers. The probe has been designed for application in TAM (Tumor-associated macrophages) targeting, photoimaging, and

photodynamic therapy. The prepared TPE-Man showed excellent targeting specificity for CD206, which is overexpressed on the membranes of TAMs. The study showed high detection efficiency due to the high fluorescence staining contrast between TAM and M0 macrophages and TPE-Man, which shall be a potentially useful rapid diagnosis. They also showed excellent tumor-killing efficiency by photodynamic effect by generating ROS upon exposure to irradiation by visible light [22].

In recent years, most of these works have been based on combined modalities for better detection along with targeted delivery. There have been works on polymeric conjugates involving a combination of optical imaging, PET, SPECT, MRI, and Ultrasound for better effects and to reduce the limitations of each other. In addition to providing diagnostic signals, these conjugates have also been used for minimally invasive therapeutic techniques of Photodynamic therapy (PDT), Photothermal Therapy (PT), and Photoacoustic Therapy (PA) [23]. In one such study, Du C et al 2017 have prepared all-in-one biopolymer drug conjugate theranostics. It was an attempt to integrate polymeric prodrug-induced chemotherapy and NIR light-mediated photothermal therapy along with combined modalities of self-fluorescence, Photothermal, and photoacoustic therapy. The study involved preparation of a pH-sensitive polydopamine-doxorubicin conjugate. The prodrug-induced chemotherapy, intrinsic Photothermal therapy on NIR irradiation, and combined PT-CT was observed in Hela cell-induced nude mice model. The study showed prolonged blood circulation time and Doxorubicin self-fluorescence imaging, which assisted in better tissue distribution and showed selective tumor uptake. The photothermal effect was observed using a thermal imaging camera during NIR irradiation, which showed hyperthermic effect on the tumor site due to the NIR absorbing Polydopamine. This NIR light was also found to be absorbed by the conjugate and converted to heat fluctuations that induced ultrasonic waves, resulting in Photoacoustic imaging. The combined modality successfully killed the tumors and showed a synergistic effect [24].

14.2.2 Dendrimers

Dendrimers are a new class of synthetic macromolecules and have been used as nanocarriers with size in the range of 10-100nm. These have unique tree-like branched architecture, which provides room for conjugation and incorporation of various agents. These have been used for encapsulating hydrophobic drugs and for site-specific delivery. The dendrimers have three parts: (1) A central core with two or more reactive groups, (2) Homocentric layers called generations constituted of repeated units, and (3) functional groups on the surface determining the physicochemical properties of the dendrimers [25]. The dendrimers are very good vehicles for diagnosis and targeted delivery of different diseases. The higher dendrimers (>5) have recently been used for designing multifunctional nanocarriers by simultaneous grafting of the outer terminals with site-specific ligands, diagnostic contrast agents, or their combinations for imaging along with the drug [26, 27] due to higher void

spaces and terminals. Hence, these dendrimers have good potential to be sued as multifunctional theranostics due to their monodispersity, low immunogenicity, modifiable surface functionality, water solubility, and multivalency [28].

PAMAM (Polyamidoamine) and Poly(propylene imine) (PPI) are the two most widely used types of dendrimers. However, the most successful and preferred in theranostic designs have been the PAMAM (Polyamidoamine) dendrimers due to their ease of preparation, surface versatility, and low toxicity. These dendrimers are synthesized with polyamide branches containing an ethylene diamine core with tertiary amines as focal points [29]. These dendrimers have been used in the design of different theranostic nanocarriers by conjugating with suitable contrast agents [30] such as ^{68}Ga and ^{64} Cu [29, 31] for PET imaging, ^{67} Ga [27] and ^{99Tc} [32] for SPECT, Gadolinium (Gd) for MRI [33], Cy 5.5 [34] and ^{177} Lu [35] for optical imaging, etc. These PAMAM dendrimer-based theranostic nanocarriers have been designed for various diseases like arthritis [34], atherosclerosis [31], myocardial infarction [36], malaria [37], neuroinflammation [38], and different forms of Cancer [39, 40].

The major concerns for use of dendrimers as nanocarriers in humans are their biocompatibility, circulation time, and degradation. Hence, there are efforts to design more biocompatible dendrimers with long circulation time and reduced toxicity [41]. There are efforts to achieve this surface or core modification of the dendrimers by PEGylation as well as glycosylation, acetylation, [42] and conjugation with amino acids. PEGlytaed dendrimers have been the most widely explored among these [43]. However, the toxicity due to its degradation product still remains a major concern and the small size of less than 10nm is not suitable for enhanced permeation and retention.

There are efforts to reduce this toxicity due to degradation by the design of Peptide dendrimers [44]. These peptide dendrimers contain peptide bonds in their structure and are synthesized from amino acids. These help in reducing the toxicity as these amino acids of the degradation are used by the cells in their metabolism. In such a study by Nigam S et al 2017, a peptide dendrimer was synthesized whose core was made of ethylene diamine and cationic amino acid constituted branching monomer. This peptide dendrimer was loaded with SPIO for MRI detection and coloaded with anticancer drug doxorubicin for theranostic therapy. The dendrimer showed efficient drug loading capacity. Moreover, a synergistic effect was observed on combination of doxorubicin and magnetic hyperthermia effect with Alternating current magnetic field (ACMF) for treating cervical cancers with this nanocarrier [45].

However, the major problem of the high-generation PAMAM and Peptide dendrimers is that they are not easy to prepare due to the steric hindrance to the chemical reactions, are costly, and result in latent toxicity due to slow degradation [43, 46, 47]. To resolve these problems with high generation dendritic theranostic carriers, recently, there has been work on the use of hyperbranched or dendronized polymer strategy [27]. These involve a combination of lower generation dendrimers or dendrons with Polymers particularly polysaccharides like heparin [48] and hyaluronic acid [45]. These dendronized polymer-based theranostic nanocarriers

have been found to be better in terms of ease of synthesis, biocompatibility, and biodegradability and considered more potential for clinical application (Table 14.1).

14.2.3 Polymeric Micelles

Polymeric micelles are another class of nanocarriers, which have been investigated for their application as a multifunctional nanocarrier system. These are composed of amphiphilic polymers having a hydrophobic core and a hydrophilic shell. Their uniform size, ease of preparation, enhanced circulation time, and high colloidal stability *in vivo* make them very promising nanocarriers [54]. These can be easily designed for attaining multiple functionalities by loading the hydrophobic drug in the core and the release controlling agents, ligands, and imaging agent in the structure [55]. In a majority of studies, the hydrophilic outer layer is composed of Polyethylene glycol, rendering a stealth nature, and prolongs its circulation time by preventing from uptake by RES [55, 56]. These polymeric micelles have been prepared with macromolecules of various shapes such as star-like, cycle-like, brush-like, or hyperbranched polymers [49].

The polymeric micelles are broadly of two types, macromolecular and Unimolecular [57]. However, the major problem with macromolecular micelles is their sensitivity toward thermodynamic parameters such as temperature, pH, flow rates, electrolyte concentrations, etc., which may lead to premature aggregation, resulting in instability [58]. Hence, in recent times, more work has been on the design of unimolecular polymeric micelles. There have been works on multifunctional polymeric micelles by combination with different types of imaging agents for theranostic applications such as Fluorescence imaging [59, 60], MRI [56, 61, 62], PET [63, 64], SPECT [65, 66], and ultrasound imaging and therapy [67, 68] (Table 14.2).

Recently, more work has been conducted involving the use of a combination of contrast agents in the multifunctional polymeric micelles for imaging as well as therapy. Particularly, NIR and Magnetic irradiation or Ultrasound has been used in combination for imaging as well as Photothermal or Photoacoustic therapy along with chemotherapy. In such a recent study by Zhang L et al 2018, they have designed a pH reduction dual-responsive polymeric micelles loaded with doxorubicin and decorated with folic acid on the surface. The micelles have also been loaded with Indocyanine Green (ICG) for NIR fluorescence imaging and to achieve chemo-photothermal combination therapy [69].

In another study by Pan G Y et al 2018, self-assembling nanomicelles were prepared by conjugating copolymer methoxypoly(ethylene glycol)5k-block-poly (l-aspartic acid sodium salt)10 (PEG-PLD) with hydrophobic near-infrared (NIR) heptamethine cyanine molecule IR825-NH2 by amine-carboxyl reaction. The resulting copolymers were amphiphilic in nature and could self-assemble into nanomicelles. These systems resulted in NIR fluorescence-guided photothermal therapy on irradiation, resulting in site-targeted delivery, enhanced cellular uptake, and extended circular retention. These nanosystems resulted in effective

Table 14.1 Recent developments in theranostic dendrimers

Imaging	Dendrimer	Drug	Targeting approach	Disease	References
MRI	PEG-G5-PAMAM Gold Nanoparticle	human ferritin heavy chain (hFTH1) gene	hFTH1-NK-92 cells	Breast Cancer	[40]
IRDye 800CW (NIR) Fluorescence Imaging	G4-PAMAM	siRNA ((siVEGFA)	Folic Acid	Head and neck squamous cell carcinomas (HNSCCs)	[41]
^{99}Tc SPECT/CT	G5 PAMAM	Doxorubicin	FC131 peptide- Chemokine receptor-4 (CXCR4)	Glioma	[49]
Fluorescence Imaging	DHP-bMPA, dendronized hyperbranched polymers	Chloroquine, primaquine, and quinacrine	DHP-bMPA -Plasmodium-infected red blood cells (pRBCs)	Malaria	[39]
(NIR) dye: S0456 Fluorescence Imaging	G4-PAMAM	Curcumin	Galactosamine-asialoglycoprotein receptors (ASGPRs)	Hepatocellular cellular carcinoma	[50]
(NIR Vivo Tag® 800 Fluorescence Imaging)	G3-PAMAM	Cysteine	Serine	Renal ischemia	[51]
^{64}Cu PET	PAMAM	Doxorubicin	Peptide F3-cellular nucleolin	Breast Cancer	[52]
Cy5-D Fluorescence Imaging	G6- PAMAM	Minocycline	D amino conjugate	Neuroinflammation	[38]
^{131}I SPECT	G6-PAMAM amine terminated	^{131}I Radiotherapy	Chlorotoxin-matrix metallopeptidase 2 (MMP2)	Glioma	[51]
Iron Oxide MRI	Lysine Dendrigraft	Doxorubicin	Arg-Gly-Asp peptide (RGD)-VEGFRs and α_v β_3	Hepatocellular carcinoma	[53]

Note: All references of the sources of information in the compiled table have been cited accordingly

Table 14.2 Recent advancements in theranostic polymeric micelles

Imaging	Polymer/copolymer	Drug/therapy	Targeting approach	Disease	Reference
CT	β CD-(PLA-PDMAEA-PEtOxMA)$_{21}$Gold Nanoparticle	Doxorubicin	Passive	HepG2 cells	[58]
DOTA-Gd MRI	β CD-based star polymer	Doxorubicin	Folic Acid –HeLa cells	Cervical Cancer	[72]
ICG-NIR Imaging	N-PEG-N-Octyl Chitosan	Doxorubicin	Folic Acid-Arginine rich CPP	Hepatocellular carcinoma	[73]
MHI-148 Cyanine Dye NIR Imaging	Glycol Chitosan	Paclitaxel	4T1 & SCC7 cells	Breast cancer Squamous cells carcinoma	[74]
SPION MRI	mPEG-PA-DIP-DBA copolymer	Doxorubicin	HepG2 cells	Hepatoma	[75]
Metal Oxide (Fe/Mn) MRI	DSPE-PEG-CREKA	CREKA Peptides	HMO-Ms-Fibrin clots	Atherosclerosis	[76]
DTPA-Gd MRI	Stearic Acid-grafted Chitooligosaccharide (COSSA)	Docetaxel	Passive	Pancreatic Cancer	[52]
SPION MRI & Nile Red NIR	mPEG-b dendritic-oligocholic acid (m-PEG-Lys3-CA4)	NA	Macrophage Passive targeting (Size below 40nm)	Raw 264.7 cells lymph node metastases	[77]
MRI	MPEG-PLA-PTMC (mPEG-Poly (Lactic acid)-Poly(Trimethyl carbonate) triblock copolymer	Zonisamide	Passive targeting	Acute Spinal Cord Injury(SCI)	[78]
High Intensity Focused Ultrasound (HIFU)	Epi-Conjugated PEG-Polyaspartate Co polymer (NC-6300)	Sonodynamic therapy	Focused Ultrasound	Human Pancreas Adenocarcinoma	[79]
Gold nanorod (GNR) Photoacoustic imaging	PLGA-b-PEG Copolymer	Adriamycin	Epithelial cell adhesion molecule (EpCAM) antibody-HePa 1-6 cells	Hepatocellular Carcinoma	[80]

Note: All references of the sources of information in the compiled table have been cited accordingly

photothermal effect as observed in He La cells and resulted in U14 tumor-bearing mice [70].

14.2.4 SPIONs

Superparamagnetic iron oxide nanoparticles (SPIONs) are the most widely used nanocarriers for multifunctional theranostic agents. These are based on inherently paramagnetic iron oxide, which acts by shortening T2, and are known as Negative contrast in MR Imaging, as discussed in an earlier section on MRI. Though Gadolinium (Gd) is widely used as a contrast agent in MRI due to its brighter images, however, it is potentially nephrotoxic by binding with ligands *in vivo*. Hence, because of the better biocompatibility, Iron Oxide-based Contrast agents have also been widely used in recent years. These iron oxide nanoparticles have iron oxide at the core, which is considered to be superparamagnetically coated with various macromolecular materials when the magnetic ions are aligned mutually and are known as Superparamagnetic Iron Oxide nanoparticles (SPIONs) [71]. The magnetic properties of these nanoparticles have also been used for targeted delivery of drugs using magnetic fields. Due to their low cost and versatile synthetic techniques, biocompatibility, less toxicity, magnetic properties, size, and surface versatility, these have been the most widely explored among all theranostic carriers.

The SPIONs can be prepared by several physical and chemical methods such as Coprecipitation, Thermal decomposition, Hydrothermal method, Thermal Parsing, Mild Reduction, Reduction precipitation, Micro/nanoemulsion, Polyol method, Sonochemical /thermal/microwave-assisted synthesis, etc. [71]

These SPIONs have been efficiently used for targeted drug delivery with the use of suitable ligands and diagnostics as MRI contrast agents because of their potential to be used for chemotherapy, Photodynamic therapy, Photothermal therapy, etc. [81] Multifunctional Surface-modified or conjugated SPIONs have been developed combining with pH-sensitive [81], PEGylated [82], active targeting ligands, and other imaging modalities like Optical imaging [83], PET [84] for chemotherapy [83], Photodynamic effect [85], or Photothermal Therapy [45], etc.

The SPIONs are the most versatile among the theranostic carriers and have been used for various health ailments besides cancer such as arthritis, AIDS, Tuberculosis, Atherosclerosis, brain disorders, etc. as can be observed in Table 14.3. In recent times, there have also been works on development of more advanced SPION-loaded multifunctional nanocarrier systems like liposomes [41], Polymeric micelles [96], nanomicelles [97], Microparticles [98], etc. for better efficacy in various critical conditions.

In such a recent study, SPIONs containing anticancer drug Paclitaxel have been loaded in a pH-responsive liposome [81]. The system contained pH-responsive peptide H7K $(R2)_2$ as targeting ligand, which acted as cell-penetrating peptide (CPP) at lower pH of the tumor region. The SPIONs acted as MRI contrast agent and assisted in image guided transport of the liposomes. The conjugate system was prepared by thin film hydration method. The *in vitro* and *in vivo* experiments in

Table 14.3 Recent advancements in theranostic SPIONs

Surface modification/ coating	Drug/therapy	Purpose of coating/ modification	Disease	References
Polyethyleneimine (PEI)	siRNA (Macrophage)	Increased RNA Delivery	Arthritis	[86]
Gold	MUC-1 Aptamers and Photothermal therapy	Photothermal effect	Colon Cancer	[87]
PEG	Relaxin	Increased circulation time	Hepatic Cirrhosis	[88]
Folic acid PEG PEI	PD LI-siRNA	Active targeting, Increased Circulation time, and Increased Transfection	Gastric cancer	[89]
Carboxymethyl Assam Bora rice starch	Doxorubicin	Stabilization	Breast Cancer	[90]
Carboxymethyl dextran-trimethyl chitosan	HIV-1 Nef siRNA	Stabilization and increased solubility	HIV Infection	[91]
Mesoporous silica	Doxorubicin Mucin -1 Aptamer	Modify biocompatibility and reduced toxicity	Breast Cancer	[92]
Lauric acid-HSA	Dexamethasone	Magnetic targeting to deceased vascular region	Atherosclerotic Plaque	[93]
Alpha-methyl-L-Tryptophan	1L-1β Monoclonal antibody	Increased uptake in epileptic focus	Epilepsy	[94]
NA	Curcumin/ Magnetic therapy	Increased neuroprotection	Schizophrenia	[95]
PEGylated Phospholipid IDG	Doxorubicin	Increased circulation andfluorescence Imaging	Glioma	[83]

Note: All references of the sources of information in the compiled table have been cited accordingly

MDA-MB-231-induced tumor-bearing mice showed better targeting effects, showing increased accumulation and cytotoxicity, MRI imaging, and antitumor effects with greater tumor inhibition and apoptosis. In another similar study, such a theranostic system was developed by Shen C et al 2019 to get access to BBB in glioma. The study involved the development of a hydrophobic SPION produced by thermal decomposition method, which is then coated with DSPE-PEG and anticancer drug Doxorubicin by thin layer hydration method. For fluorescence imaging,

further, a NIR contrast agent Indocyanine green (ICG) was coated on the lipid layers of the surface. This theranostic system achieved a synergistic effect of MR Imaging, Fluorescence imaging, EPR-based passive targeting, and chemotherapy of doxorubicin in glioblastoma cells. The MRI and Fluorescence imaging confirmed the successful crossing of BBB and sufficient accumulation of the nanocarrier at the tumor site. These resulted in higher efficacy, showing a high tumor inhibition rate in the C6 glioma bearing rats [83].

14.2.5 Quantum Dots

Quantum dots are fluorescent nanocarriers composed of semiconductors widely used in LEDs and Lasers and for biomedical application of size 2–10 nm. These semiconductor-based systems have been used in biomedical use for bioimaging and drug delivery and as multifunctional theranostics [99]. These are based on the Quantum Theory according to which the quantum effects resulting due to reduction in the size of the semiconductors contribute to unique electronic and optical properties of the resulting nanoscale particles. The reduction in size results in wide energy gaps, which generally results in emission and absorptions during transition from excited to ground state. By selecting specific composition and size, this emission of light can be controlled into the UV, visible, NIR, or Mid IR range [100]. The Quantum dots used in theranostics and drug delivery mostly involve emission in the visible (400–650 nm) or Near IR range (650–950 nm) and can be achieved by suitable adjustment of the size. The various types of quantum dots, which are mostly used, are based on binary alloys of II-VI and III-V semiconductor materials such as CuS (Copper sulphide) [101], CdSe (Cadmium Selenide), CdTe (Cadmium Telluride), Cd ZnS capped [102], etc. The Quantum Dots are prepared by both top-down and bottom-up methods; however, in recent years, green synthesis has been the most preferred method [101].

The major advantages of these Quantum Dots as nanocarriers in theranostics use are their tunable size, photostability, photophysical property, and biocompatibility [103]. Moreover, the surface to volume ratio and the ease of surface modification are advantageous in designing advanced multifunctional theranostic systems. The fluorescence obtained is used as a suitable contrast agent for imaging, and the surface modification with suitable conjugates and ligands can be sued for controlled and targeted drug delivery. Moreover, these QDs can also be used as a scaffold to shield both hydrophilic and hydrophobic therapeutic moieties. Lipophilic drugs can be embedded between the inorganic core and amphiphilic polymer coating layer, whereas lyophilic drugs can be immobilized onto the hydrophilic side of the amphiphilic polymer, through covalent or noncovalent bonds [102, 104]. These Quantum Dots have been used in conjugation with suitable ligands for targeted delivery such as folic acid [101, 105], hyaluronic acid [106], transferrin [107], peptides [108], etc. The Drug-loaded QDs are further loaded in magnetic nanoparticles [109], pH-sensitive NP [110], hydrogels [111], liposomes [112], implants [113], nanocomposite film [114], micelles [99], etc.

In recent years, the theranostic QDs have been mostly used for combined chemotherapy along with photothermal therapy [101] or photodynamic therapy [115] besides using as contrast agents for better imaging.

In such a recent work by Yu W et al 2019, a chitosan and folic acid-conjugated CuS QD was prepared for photoacoustic imaging-guided photothermal therapy. The CuS QDs were prepared by a suitable green synthesis method of co-precipitation using a chitosan surface conjugated with folic acid for increasing the biocompatibility and targeting efficiency. The QDs exhibited strong NIR photoabsorption and high photothermal efficiency of 47%. The inherent property of polymer-coated CuS QD was used for photothermal therapy, which resulted in efficient inhibition of tumor in 4T1 cell-induced mice [101]. A similar work has also been reported recently by Jin R et al 2019 where they have prepared a multifunctional polypeptide $PC_{10}A$-modified Ag_2S QD-based hydrogel loaded with paclitaxel for photoacoustic imaging-guided chemo and photothermal therapy. The combined approach system showed excellent biocompatibility, imaging, and drug delivery and achieved efficient sustained therapy against SKOV3 ovarian cancer in mice [111].

14.2.6 Carbon Dots (Graphene Quantum Dots)

Carbon dots are nanomaterials having quantum properties with size 2–8 nm and are also known as graphene quantum dots, carbon dots, or carbon quantum dots. These carbon dots are similar to graphene oxide in terms of their physical and chemical properties; however, they are different in terms of their size. These were accidenatly discovered by Xu B S et al 2004 during the arc discharge synthesis of carbon nanotubes [116]. These are actually clusters of carbon atoms along with considerable amount of oxygen and hydrogen and a trace amount of nitrogen [117].

Similar to quantum dots, these carbon dots also have similar properties of biocompatibility, less toxicity, and photostability. They are soluble in water, and their surface can be conveniently modified for multifunctional theranostic use [118].

Carbon dots are prepared by both top-down and bottom-up approach from a suitable carbon precursor (Organic or Bioorganic). Some of these simple synthetic processes are top-down methods such as laser ablation, chemical oxidation, electrochemical oxidation, ultrasonic synthesis, and bottom-up methods like hydrothermal synthesis, thermal decomposition microwave synthesis, etc. [119]. In recent years, there has been more work on the development of Carbon dots from organic sources without chemical exposure by green synthesis because of the availability of precursor, higher quantum yield, and self-passivation [120].

Some of the widely used organic sources are graphene, graphite, carbon nanotubes, glucose, glycerol, etc., whereas silk, lychee [121], curcumin [122], alginates [123], and honey are important bioorganic sources [118].

Due to their various advantages, these carbon dots are used widely for various theranostic designs like other quantum dots [119]. They are more comparatively preferred than the semiconductor quantum dots due to their excellent fluorescence, higher photostability, and more tunable emission spectra [120].

These have been designed for multifunctional nanocarriers for imaging-guided targeted delivery of various genes [123] and drugs [124, 125] and as antibacterial agents [126, 127]. They have also been designed for combined bioimaging and imaging-guided photothermal therapy [128], photodynamic therapy [129] or both for synergistic effect along with chemotherapeutic drugs. Some of these theranostic designs are added with controlled stimuli-responsive targeted delivery for better efficacy [130].

14.2.7 Gold Nanostructures

Gold Nanoparticles are most widely used theranostic nanocarriers next to SPIONs because of their high surface to volume ratio, good solubility, and, most importantly, readily tunable surface as most of the ligands have a high affinity for them [131]. Moreover, the inert and nontoxic gold core and ease of synthesis further add to their advantage [132]. Hence, these gold nanoparticles have been widely used for targeted delivery of anticancer drugs [133, 134], antibacterial [135], gene delivery [136], Cardiovascular drugs [137], etc. Another important application of these nanoparticles is their inherent efficacy in bone [131] and dental disorders [138].

These gold NPs have been designed into different shapes and structures as spheres, cubes, rods, stars, etc. Because of their sharp and spike edges in nanorods and stars, they have a tendency to enhance the electromagnetic radiation. This property has been efficiently used in SERS imaging-based theranostic design. Moreover, these NPs also have the property of absorbing X-rays strongly and hence are used as contrast agents for X-Ray-based imaging and radiotherapeutics [138].

These nanoparticles are synthesized by various simple top-down and bottom-up approaches like chemical, electrochemical, and thermal reduction. Turkevich and Brust methods are the most widely sued techniques. Turkevich method involves reducing the metal salt using sodium citrate as a reducing agent, although ascorbic acid, amino acids, and UV light have also been used [139]. Brust method is a two-step process involving transfer from organic to inorganic solution with the help of Tetrabutylammonium bromide (TOAB) as the transfer agent. Besides these two methods, the Method of Seeding is another technique for growing different structures like nanorods, nanocubes, etc., which have been used widely in recent years. These methods employ development of nucleation and then build the desired structure on it using ascorbic acid and hydroxylamine as the reducing agents [140].

Besides modification of their surface properties, the physical property of gold nanoparticles can also be exploited particularly in local hyperthermia on irradiation in NIR region of 800–1200 nm [132]. These designed gold nanostructures are especially preferred for photothermal therapy because of their high photothermal conversion and due to their surface plasmon response (SPR) effect, which can be easily tuned as required [134] with change in size and shape. Hence, there are a lot of efforts in designing gold nanostructures involving photothermal therapy. In most

cases, they are combined with a suitable contrast agent for additional photoacoustic effect and photodynamic therapy besides the chemotherapy [134] Moreover, there have been lots of work on theranostic designs using a combination of gold nanoparticles with SPIONs or MR-assisted [141] or FUS (Focused Ultrasound) agents [142] or loading into carbon dots [143] and pH-sensitive structures [133]. These combinations have resulted in much better therapeutic and diagnostic results.

Gold Nanorods have been found as the most preferred nanostructure for photothermal therapy due to their efficient thermal conversion, prolonged circulation, and accumulation. However, the major limitations of gold nanorods are their toxicity due to the use of CTAB (Cetyltrimethylammonium bromide) during synthesis, instability in physiological medium, and poor drug loading [144]. There have been efforts to address these by surface modification with various agents such as hyaluronic acid, peptides [145], PEG [146], chitosan, [147] etc.

In such a recent work on such multifunctional design, a PEGylated Gold Nanorod was developed having hydrazide linkage for pH-responsive combined triple therapy approach of chemotherapy, Photodynamic therapy, and Photothermal therapy [146]. The nanoplatform was designed by adding a hydrazide linkage and PEG coating. To this, the drug doxorubicin was added along with prophotosensitizer (6-Aminolevulinic acid) which on NIR irradiation produced the photodynamic effect and produced hyperthermia as the inherent property of gold nanorods. The nanoplatform showed efficient uptake by the MCF-7 cells, showed pH-responsive chemotherapeutic drug release, induced hyperthermia for the photothermal effect, and succeeded in generating large amounts of Reactive Oxygen species for achieving better Photodynamic therapy. This triple therapy approach was successful in completely site-specific suppressing of the tumor growth.

14.2.8 Stimuli Responsive

In recent years, there has been development of various multifunctional nanosystems employing some sort of stimuli-responsive behavior. These structures undergo a reversible or an irreversible physical or chemical transformation in response to change in pH, temperature, light irradiation, or use of ultrasound, etc. [148]. Most of these systems involve polymeric nanocarriers, hydrogels, Quantum dots, gold nanostructures, mesoporous silica nanoparticle, [149, 150] etc.

14.2.8.1 Temperature Sensitive

Thermoresponsive nanocarrier systems have been developed using materials that are safe and sensitive to temperature changes between 39 and 42 °C, which can sequester a drug until it reaches the target site, where temperature change can promote carrier extravasation and a localized triggered release.

Among the various temperature-responsive nanocarriers used for theranostic purpose are the fluorescence/temperature-sensitive hydrogels. These systems have a suitable fluorescence contrast agent for imaging in the form of fluorescent dye,

nanoclusters, or carbon dots. These are transformed to a thermosensitive hydrogel by using monomers like NIPAM (N-isopropyl acrylamide) [78], N-vinylcaprolactam [151], acrylamide [152], etc.

Similar thermoresponsive multifunctional nanogels have also been prepared using magnetic nanoparticles, mostly iron oxide nanoparticles [148, 152].

In such a study by Shakoori Z et al 2017, a magnetic, temperature, and pH-sensitive nanogel was developed for anticancer drug Cisplatin. The nanogels were loaded with the drug and iron oxide nanoparticles. A combined system was prepared by free radical copolymerization crosslinking technique using NIPAM as temperature-sensitive polymer, DMAEMA(N,N Dimethyl amino ethyl methacrylate) and 4-acrylamidofluorescein as the fluorescent agent. These nanogels showed a sustained and pH- and temperature-sensitive drug release at the specific site [153].

Besides these hydrogels, the other forms of thermoresponsive nanocarrier systems are liposomes [154], magnetoliposomes [155], mesoporous silica nanoparticles [150], gold nanostars [156], Polymeric matrix, etc. Most of these systems involve dual-responsive mechanism such as magnetic thermoresponsive or pH thermoresponsive, etc. for better efficacy [157].

These nanosystems have been used for various disease conditions mostly microbial conditions such as skin healing [156], AIDS [158], other microbial infections [159], and cancer.

Mild hyperthermia has been recently used as an effective strategy for anticancer drug delivery due to its cytotoxicity at temperatures of 40–42 °C and sensitizing of tumor cells to chemotherapy. Hence, in recent years, a lot of thermoresponsive theranostic systems have been designed for combined chemotherapy with photothermal therapy of various tumors [160] or radiotherapy [161].

In such a recent study by Zhao T et al 2019, they have prepared a multifunctional nanosystem for combined chemotherapy, photoacoustic imaging, ligand-mediated targeting, and photothermal effect. The basic nanostructure consists of a suitable temperature-sensitive fatty acid (Lauric acid, stearic acid) as phase change materials are core loaded with docetaxel. It constituted of a Polypyrrole shell, which is an organic conjugate polymer having both photothermal effect and photoacoustic imaging property. This was further surface modified with the conjugation of Hyaluronic acid for CD44 overexpressed receptor targeting. The designed nanosystem showed good cellular uptake, site-specific photothermal effect, and drug release in $4T_1$cell-induced tumor cells in mice. These synergistic effects resulted in significant cytotoxicity and tumor inhibition in the mice [162].

14.2.8.2 pH Sensitive

pH-responsive delivery systems are considered the most effective stimuli-responsive systems and hence are most widely used particularly in targeting the acidic environment of tumors and the microbes. There are numerous studies involving the development of multifunctional theranostic designs with a pH responsive drug delivery. Only the most recent ones have been outlined in this chapter.

Such a nanocarrier system has been designed for anticancer drugs like doxorubicin [128], methotrexate [163], bleomycin [164], 5-Fluorouracil [165], epirubicin [166], monoclonal antibodies [167], cinnamaldehyde, [168] etc. Besides anticancer drugs, these have also been used in recent times for Antimicrobial agents such as antimicrobial nanofiber films [169], AntiAlzheimer drugs Huperzine A [170], gene delivery [171], and other health care problems.

Different nanocarriers have been designed based on this approach such as graphene oxide nanostructures [172], β-Cyclodextrin-based supramolecular self-assembly or nanocomplex [173], Polymeric micelles [174], mesoporous silica nanoparticles [175], Carbon Nanotubes [163], hydrogels [164, 165], Quantum dots [176], Silver Nanoclusters [169], Polymeric nanoparticles [177], liposomes [178], etc.

Most of these nanocarrier systems are based on the combination of a suitable imaging contrast agent such as fluorescent agent [166, 176], MRI agent [179], Ultrasound [174], etc. These theranostic systems are mostly intended for synergistic effect due to combination of chemotherapy with Photothermal therapy [69, 176, 180], Photoacoustic effect [166], or Photodynamic therapy [174] and ligand-mediated targeting [181] along with pH-responsive drug release or uptake. Such synergistic multifunctional systems have shown successful drug delivery with better efficacy and reduced toxicity.

14.2.8.3 Ultrasound Responsive

Ultrasound has been one of the effective agents in imaging, selective site-specific delivery of drug, or therapy. The use of ultrasound results in cavitation, local hyperthermia, enhanced poration or permeability of membranes, and coagulative necrosis. These help in imaging and assist in site-specific drug delivery or tissue ablation used in treating various health disorders [182]. The most widely used form is the focused Ultrasound (FUS) or the high intensity focused ultrasound (HIFU) technique [183] in combination with other functions in the newer theranostic designs.

Similar to the other stimuli-sensitive multifunctional systems, several theranostic designs have been developed responsive to ultrasound such as Microbubbles [184], nanodroplets [184, 185], hydrogels [186], Gold nanostructures [184, 187], Polymeric micelles [188], polymeric nanoparticles [189], Gas-generating NP [190], SPIONs [191], ligand-conjugated NP [176], etc.

Effecting imaging has been the major purpose of ultrasound in this multifunctional system-assisted drug delivery [192]. It is also used for controlling the site-specific drug release [185, 188], thermal ablation of the tissue [191, 193], and enhanced permeation [184, 193].

These ultrasound-assisted theranostic designs have been used for various diseases particularly in cancer [185, 192] and other diseases such as Alzheimer [193], Parkinson's disease [194], Ischemia [189], etc.

In such a recent study by Zhang T et al 2019, an ultrasound imaging-assisted theranostic hollow nanocarrier was developed for combined chemotherapy and

photothermal therapy. The mesoporous hollow nanoparticles were loaded with chemotherapeutic drug doxorubicin and contained Polydopamine (PDA). On NIR irradiation, these polydopamine layers convert them into thermal energy for photothermal effect on the tumors and pH-selective drug release. These ultrasound imaging-assisted hollow nanocarriers achieved high tumor accumulation at the target site and a pH-responsive drug release. This in combination with the photothermal effect resulted in complete tumor suppression in 4T1 cell-induced breast cancer in mice [192].

One of the major uses of ultrasound particularly Focused Ultrasound (FUS) has been enhanced delivery through BBB. FUS has been approved by FDA safe for noninvasive and reversible disruption of BBB. Several theranostic designs have been developed using this approach. In such a recent study by Bai L et al 2019, exosomes have been derived from macrophages and used as a natural nanocarrier for brain cancer. These exosomes were then loaded with doxorubicin, and their transport to the brain has been enhanced by FUS. This delivery system was assisted by NIR fluorescence and ultrasound imaging. They achieved 4.45fold higher uptake in brain due to use of exosomes and FUS and efficient glioma suppression in GL261 cell-induced mice [195].

14.3 Conclusions

The development of multifunctional nanoparticles has greatly expanded the application of nanomedicines for various health care ailments with better diagnosis and therapy. The combination of suitable imaging modalities with chemotherapy and ligand-guided or external stimuli-guided site-specific delivery holds great potential for safer and efficient therapy. Most of these theranostic designs are based on NIR and MRI imaging or a combination of both. However, other imaging modalities like SPECT and PET have also been used. Among the stimuli-responsive designs, pH- and temperature-responsive designs have been most widely explored successfully and mostly in combination. Various such nanocarrier systems have been designed such as the polymeric conjugates, dendrimers, micelles, gold nanostructures, Iron oxide nanoparticles, Quantum dots, carbon dots, and stimuli-responsive systems. Some of these systems are shown in Table 14.4. In recent years, these multifunctional theranostic nanocarriers have been further modified for combined photothermal, photoacoustic, and photodynamic effects along with the chemotherapeutic and other drugs. These systems, therefore, hold great promise for better therapy for cardiovascular problem, atherosclerosis, rheumatoid arthritis, Alzheimer, psychotic diseases, and inflammation and, most importantly, for the cure of various types of cancer. Several of these systems are currently under clinical trials. However, the successful translation of these nanoplatforms from bench to bed shall depend on the *in vivo* toxicity, metabolism of the nanocarriers, biocompatibility, and cost-effective scale-up techniques.

Table 14.4 Some examples of nanocarrier systems in the Clinical stage of development

Nanocarrier/ type	Imaging Modality/ drug/therapy	Disease	Phase/ClinicalTrials. gov identifier	Reference
Gold NP	NU-0129, Spherical Nucleic acid	Glioblastoma	Early Phase I NCT03020017	[196, 199]
Polymeric NP	PET, Docetaxel	Solid Tumor	Phase I NCT03712423	[196, 199]
Gold NP	Photothermal, AuroLase(TM)	Head and Neck Cancer	NA NCT00848042	[197, 199]
SPIONs	MRI, Bevacizumab	Glioma	Phase I NCT00769093	[197, 199]
Liposome	FUS, Erlotinib	Lung Cancer	Phase IV NCT01455389	[198, 199]
Iron NP	Magnetic Thermal ablation	Prostate Cancer	Phase 0 NCT02033447	[198, 199]
Lipid NP	Doxorubicin, FUS	Liver Cancer	Phase I NCT02181075	[196, 199]

Note: The information in the Compiled Table has been obtained from ClinicalTrials.gov, and the other sources have been cited accordingly in reference

Acknowledgement The authors are grateful to the Chief Editors for providing the opportunity to write a book chapter for the esteemed book. They also acknowledge the HUJI Library for providing access for literature review.

Conflict of Interest The authors declare no conflict of interest.

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Ligand Nanoparticle Conjugation Approach for Targeted Cancer Chemotherapy

15

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Abstract

Cancer is the most devastating disease currently prevailing worldwide. For the treatment of cancer, various approaches like surgery, chemotherapy, radiotherapy, and hormonal therapy are utilized have been utilized. However, the nonspecific targeting approach has made the treatment ineffective in the majority of cases. The nonspecific targeting also leads to an inadequate supply of drugs to the desired tumor site, while cancer treatment requires a high dose of drugs with a high frequency of drug dosing. Despite the advancement in cancer research, treatment strategies, and available numbers of potent anticancer drugs, the efficacy of treatment still is a matter of concern due to the lack of drug selectivity to the target cells, pharmacotoxicities, and very poor patient compliance. Therefore, novel strategies are utmost important for the effective delivery of the anticancer drugs strictly to the specific tumor site, which can minimize the systemic toxicities related to frequent and high drug doses. The active targeting approach provides selective and site-specific treatment rather than passive targeting. The active targeting technique works based on the molecular identification of biomarkers that are generally overexpressed on tumor cells, through conjugated targeting moieties over the nanodrug carrier. These targeting moieties signify the biodistribution and affinity toward the target site of the drug carrier.

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M. K. Das, Y. V Pathak (eds.), *Nano Medicine and Nano Safety*,
https://doi.org/10.1007/978-981-15-6255-6_15

Keywords

Active targeting · Drug delivery · Nanocarriers · Receptor · Tumor

15.1 Introduction

Cancer is considered to be a bunch of diseases with abnormal and unconstrained growth and development of cells having a possibility of spread throughout the body. There are above ten million patients diagnosed with cancer, and it is expected to be increased to 27 million by 2030 [1]. The common three strategies of cancer treatment prevailing are chemotherapy, radiotherapy, and surgery. These treatment strategies show a nonselectivity, which affects the surrounding healthy tissues. The cytotoxic drugs, which are available today, are very potent and efficient in killing cells, but unable to differentiate between cancer cells and healthy cells. Due to this deficiency in selectivity, patients suffer from some undesirable toxic effects. Despite the challenge, it is the call of the time to improve the selectivity and specificity of anticancer drug delivery system toward tumor cells only, for the effective treatment of cancer. Many distinguishing features are found in tumor cells and tissues through molecular, biochemical, and physiological from the healthy cells and tissues like alteration in redox status, pH, receptor expressions, and increased permeability of tumor tissues and vasculature. Thus, exploiting these differences, cancer possibly benefits from drug targeting strategies [2]. Various molecular biomarkers of cancer comprise the varying protein expressions in the cytoplasm, cell organelles, and membrane surface. The differential proteins or receptors are precisely expressed or overexpressed in tumor cells from the healthy normal cells. This overexpression of receptors paved the way for exploitation by the active targeting of cytotoxic drugs to tumor cells. Targeting of nanocomposites to a specific tissue or organ via the blood or lymphatic circulation is termed as primary or first-order targeting, while the accumulation all-around a tumor cell is termed as secondary or second-order targeting and engineered the nanocomposites to uptake by cells and cell organelles, which is known as tertiary or third order targeting [3]. Nanotechnology has emerged as a powerful tool for the development of novel strategies to address unfulfilled clinical challenges, right from the treatment of deadly diseases like cancer or neuronal disorders to the prompt detection of diseases, which might eliminate the disease as early as the symptom appears. Nanoparticles interact very efficiently with cells, bacteria, and viruses as they are smaller in size than these biological agents [4]. This intimate interaction has been utilized to achieve the desired effect such as selective transport directly to the specific cells or tissues [5]. The precise detection of distinctive biomarkers specific for particular pathology appears in complex environments (e.g., urine, blood, and saliva) [6] or the development of clever nanorobots capable of performing surgery precisely inside the body [7]. Nanotherapeutics has shown great potential to act with these strategies through the engineered nanoparticles capable of delivering therapeutic agents directly to cancer cells bypassing the normal cells. Furthermore, the surface of nanocarriers can

be architected with versatile targeting moieties or ligands, which coupled specifically to the target proteins (receptors) expressed over the tumoral cells to facilitate the uptake of particles. This may improve the cancer treatment with reduced side effects created by the cytotoxic drugs and also lowers the resistance developed due to the high doses of drugs in conventional treatments [8]. Moreover, additional targeting ligands can be loaded on the surface of the particle that does not interact with the surface receptors but recognizes the specific internal cellular organelles. It has been widely explored for the delivery of cytotoxic drugs and genetic materials (i.e., silencing RNA).

15.2 Characteristics of Cancer Cells

The tumor tissues are poorly distensible having a haphazard array of blood vessels, endothelial cell-cell synapse, and larger basement membrane fenestration. Angiogenesis is not only the primary requirement for the growth of cancer cell from a smaller to a dormant bunch of cancer cells resulting in a solid tumor but also equally necessary for the metastasis [9]. The growth, development, multiplication, migration, and invasion of endothelial cells take place with the help of vascular endothelial grown factor (VEGF), which interacts with tyrosine kinase receptor present over the vascular endothelium. VEGF also increases the permeability of blood vessels and favors the rapid extravasation of plasma protein in tissue [10]. The tumor angiogenesis is characterized by its variability in shape, high density, and heterogeneity, with altered oxygenation, perfusion, pH, and metabolic conditions. The leaky vasculature exerts a major impact on the EPR (Enhanced Permeability and Retention) effect [11]. The normal physiology of blood flow in the tumors along with the transportation in tumor vessels is due to the abnormalities in tumor blood vessels. The tumor microenvironment experienced high osmotic pressure [12]. The leaky tumor blood vessels and altered lymphatics consequences in unnecessary accumulation of vascular contents in the tumor result in interstitial hypertension [12]. However, the pH of the intracellular region is similar in both healthy and cancer cells, while the pH of the extracellular region of the tumor is lower (pH 6.0–7.0) than that of the normal healthy tissues (pH 7.4). The tumor pH varies as per the tumor area [13]. Thus, the cancer cell possesses some variation in physiology from healthy cells such as altered vasculature, interstitial pressure, oxygenation, pH, metabolic conditions, and abnormal lymphatics. Utilizing these properties, encapsulated drugs can be delivered to the tumor site either by passive or active targeting approaches.

15.3 Physiological Hindrance of Cancer Cell Targeting

As drug and drug carriers are eternal agents for the human body, the body tries to prevent their entry inside the cells in a variety of ways. The obstacles faced by the targeted DDS are nothing but the anatomy and physiology of the human body. One such physiological barrier is the mucus layer present in a different region of the

body. Other hurdles are the biochemical barrier to recognition of targets and pharmaceutical hurdles to designing suitable techniques for ligand nanoparticle conjugation. The mucus layers, which protect the delicate tissues from the detrimental environment throughout the body, also restrict the entry to therapeutic agents. These mucosal layers trap and remove pathogenic agents, xenobiotics, and drugs to various body parts, for example, lungs, eyes, GIT, and the female reproductive system. Some viruses are hydrophilic and possess a net neutral electric charge, which makes it possible to invade the human mucosal barrier. The RES and MPS of blood are also a major hurdle for the distribution of nanocarriers to the desired site. The larger size and hydrophobic particles are easily detected by this defense system and eliminated out from the body. So, by making nanoparticle water-soluble, these systems can be bypassed. Polyethylene glycol (PEG), a nontoxic hydrophilic material, is extensively used for this purpose. PEG-coated nanocarriers show long circulatory time and better targeting capabilities. Blood-Brain Barrier (BBB) is another obstacle for brain tumor-targeted DDS. BBB is a semipermeable blood capillary membrane made up of single-layered endothelial cells present in the brain, which allows some specific materials to pass. Larger and low lipid-soluble agents cannot penetrate inside the brain through this BBB. Oxygen, CO_2, and glucose can pass through BBB, but hydrogen ions cannot. High electrically charged molecules are retarded its entry through BBB.

15.4 Strategies of Cancer Cell Targeting

Therapeutic agents can be transported either on their own or through a drug delivery vehicle to a particular organ or specifically to the tumor cell surface. The necessary items required for the targeted drug delivery system include the existence of specific targets/receptors on the cell surface and targeting moieties/ligands for the cell targets [14]. Targeted drug delivery approach utilizes the advantages of the pathophysiological condition of tumor tissue, or it can actively target the cancer cells using some cell-specific ligands.

Passive Targeting Passive drug targeting strategy utilizes the microenvironment, pH, and EPR effect of tumor cells. The cancer cells grow and proliferate more rapidly in comparison to healthy cells due to increased metabolism, which results in more requirement of nutrients and oxygen as well. In the competition for nutrients, the healthy cells become degraded and displaced by cancer cells [15]. The nanocarriers are accumulated in the cancerous tissue through the EPR effect [16]. As the vasculature are leaky in cancer cells, the passive targeting technique takes the benefit of it, assists drugs to bypass the systemic metabolism, and helps to enter directly into the tumor microenvironment [17]. So, the EPR effect has set the basis of targeted drug delivery approach, although the EPR effect comes across some difficulties. NPs and macromolecules can penetrate the cancer cells if RES and renal clearance are bypassed. A drug can get inside the neoplastic tissue through the EPR effect if it can persist for a minimum 6 hours duration in systemic circulation

[18]. Because of the size restrictions of the tumor cells, it was reported that the EPR effect is unable to sustain even the circulation of nanocarriers in the blood. It is a challenging task to develop nanocarriers for therapeutic use with enhanced retention time because the morphology of blood vasculature of the clinical tumor is significantly different from the mice tumor model used for preclinical trials. This could limit the intratumoral distribution of NPs [19]. Even though a practical clinical setup is required to take the advantages of the nanotherapeutic treatment, till now, FDA has approved several nanotherapeutics that work via the EPR effect, for example, Doxil (1995), Feridex (1996), Mylotarg (2000), Zevalin (2002), Abraxane (2005, 2013), Oncospar (2006), and Ontac (2008) [20].

Surface Engineering of Nanocarriers with PEG The hydrophobic nature of nanocarriers is responsible for the increased aggregation of NPs and opsonization by blood components [21]. Hydrophilic polymers like PEG, poloxamers, and poloxamines are mostly used to provide stealth property to the NPs by increasing the hydrophilicity of NPs [22]. PEG is mostly used polymer to protect NPs by protecting them from opsonization and phagocytosis [23]. PEG is unique due to its solubility in organic as well as aqueous solvents. Hence, it can provide active functional groups at both the terminal ends for various functionalities. The functional group selection of molecules solely depends on the affinity toward the hydroxyl groups of PEG. With the modification of the hydroxyl group of one end, different molecules, protein, peptides, drugs, liposomes, etc. can be linked with the heterobifunctional PEG. However, due to the formation of diol in high molecular weight PEGs, these heterobifunctional PEGs are limited [24]. The increased retention time of therapeutic nanocarriers in the lymph is a major factor to calculate their accumulation amplitude in the tumor microenvironment. The retention time of PLGA nanocarriers can be improved by PEGylation, which is achieved due to the stabilization of NPs by preventing their recognition by MPS, which results in increased tumor accumulation and lower accumulation in other healthy cells [25]. PEG is approved by FDA for clinical use. The surface hydrophilicity of NPs is given by the chain length, shape, and density of PEG, and it is also a determinant factor for their cell uptake [26]. It is well established that the NP size increases with the increase in the length of the PEG chain, but the copolymer of PEG and their blend give reverse effect due to its amphiphilic nature [25]. Although PEGylation increases the NP retention in blood, it alters the release behavior of drug or covers the functional groups of NPs at the tumor site.

Active Targeting The main objective of active targeting is to deliver the cargo directly into the infected cells, which can be achieved by conjugating the targeting ligand with the drug-loaded NPs. The envelope of NPs is designed to have the functional groups, which are necessary to interact with the surface receptor of cancer cells. The main mechanism of action in active targeting is ligand-receptor binding or antibody-antigen interaction [27]. Various materials like metals, polymers, lipids, and ceramics have been utilized to prepare nanocarriers for drug targeting applications. Currently, biodegradable polymers and lipids of natural and synthetic

origins are highly used in drug delivery [28]. At the molecular level, the tumor cells could be actively targeted by cellular recognition through aptamers, ligand-receptor interaction, and antibody-antigen recognition. Active targeting of the drug can be achieved by linking drugs or NPs with the chemical moiety called ligands, which is specific to the targeted cell. These ligands have a specific affinity toward the antigens/receptors expressed over the targeted cell surface, and they can distinguish between the normal and tumor cells based on types and expression levels of receptor/antigen [29]. In the active targeting approach, it facilitates cellular internalization of nanocarriers, whereas in a passive targeting approach, it enhances accumulation around the tumor cells. On this basis, the delivery systems are designed to target nanocomposites to endocytosis-prone surface receptors [30].

15.4.1 Cancer Cell Targets and Targeting Ligands

In cancer, some receptors are specifically expressed or overexpressed on cancer cells of various tissues, which set the basis of study physiology of cancer and its treatment. In a strategy of cancer treatment, overexpressed receptors are targeted by targeting ligands such as antibodies or other small molecules that specifically bind to these receptors and inhibit their functions. Thus, blocking the undesired stimulus for uncontrolled cell division leads to the destruction of the cancer cells and their propagation. Targeted cancer therapy intentionally does not interfere normal function of the receptor, but utilizes the overexpression of receptor for the effective delivery of cytotoxic drugs that are usually nonselective toward cancer and healthy cells. These drugs can be transported in a carrier coupling with targeting ligands against such overexpressed receptors. The advancement of nanotechnology offers a platform to escort and deliver cytotoxic drugs into tumor cells (Table 15.1).

15.4.2 G protein-Coupled Receptors

Most of the cancer cells overexpress G protein-coupled receptors (GPCRs) on the surface in comparison with their native form. The scientist has started to utilize this differentiation for the development of targeted chemotherapeutics, radiodiagnostics, and radiotherapeutics [31].

15.4.2.1 Bombesin (Bn) Receptors

Bombesin receptors (BnRs) belong to the GPCR superfamily. They are also termed as gastrin-releasing peptide (GRP) receptors, which are overexpressed in cancer cell lines of lungs, prostate, breast, pancreas, head/neck, colon, uterus, ovary, renal, glioblastomas, neuroblastomas, gastrointestinal carcinoids, intestinal carcinoids, and bronchial carcinoids. Several numbers of Bn-conjugated NPs were constructed incorporating cytotoxic drugs such as Doxorubicin, camptothecin, and Paclitexol, which have shown the promising result of better selectivity and specificity in preclinical trials [32]. Recently, some experiments employed nanotechnology to

Table 15.1 Studies on antitumor activity of ligand-nanoparticle conjugates

NPs	Outcome	Cell lines	Ref
Tf-coupled NPs			
Tf -DOX-liposome	The Tf-conjugated NPs showed greater efficacy than free doxorubicin in tumor cell lines, in vitro	HL60, Hep2, and L292	[97]
Tf–PEG-PLA micelles	Tf-PEG-PLA micelles showed significantly more uptake than nontargeted micelles, in vitro and in vivo	Intra-cranial rat tumor model of C6 glioma	[98]
Tf -Core-shell NPs (containing sorafenib in albumin shell and DOX in PVA core)	NP uptake and cytotoxic effect increase with Tf conjugation in tumor cells in both 2D and 3D cultures, in vitro	Hepatocellular carcinoma (HCC)	[99]
Tf-DOX-lipid-coated PLGA	Tf-functionalized NPs prevent tumor growth in the lung cancer-bearing nude mice, in vivo	A549 tumor-bearing mice	[100]
Tf-plasmid DNA-DAB G3 dendrimer	Increased DNA transfection by Tf-tagged NPs, in vivo	A431 tumor-bearing immunodeficient BALB/c mice	[101]
CPP-conjugated NPs			
TAT-conjugated liposome (TAT-LIP)	TAT-LIP showed high brain drug delivery due to its high delivery ability to cross the BBB	Brain capillary endothelial cells (BCECs) of rats	[102]
Dox-conjugated TAT-Au NPs	With pH-sensitive dox-conjugated TAT-Au NPs showed significant survival benefit as compared to the free dox	U87 mouse model	[103]
TAT-SPION	TAT-conjugated carriers are internalized into hematopoietic and neural progenitor cells in immunodeficient mice	C17.2 and mouse splenocytes and immunodeficient NOD/SCID mice	[104]
Penetratin-deferasirox-micelle	CPP-drug conjugates for use as nanocarriers for hydrophobic drugs, in vitro	RBE4	[105]
TAT-DOX-chitosan	TAT-targeted NPs could effectively reduce tumor volume, in vivo	BALB/c mice bearing subcutaneous tumors	[106]
LDL-coupled NPs			
Hematoporphyrin-DOX-NPs	The phototoxicity was enhanced using hematoporphyrin coupled NPs, in vivo	HepG2 tumor-bearing mice	[72]

(continued)

Table 15.1 (continued)

NPs	Outcome	Cell lines	Ref
LDL-DOX/siRNA-N-Succinyl-chitosan	LDL-functionalized NPs were significantly accumulated in the tumor site, in vivo	In vitro (HepG2, L-02, and HepG2/ADM) in vivo	[107]
LDL-DOX-PEG-liposome	LDL-conjugated nanocarriers across the BBB greater than free DOX in cells accessed to statins, in vitro	hCMEC/D3, U87-MG, SJKNP cells, MDA-MB-231, and A549 cells	[75]
Apolipoprotein E-dalargin/loperamide polysorbate 80- PBCA	NPs tagged with polysorbate 80 or apolipoprotein E induced an antinociceptive effect, in vivo	ICR and C57BL/6 J mice	[108]
LDL-DHA NPs	LDL-DHA NPs showed enhanced physical and oxidative stabilities compared to native LDL and free DHA, in vitro	TIB-73, BNL CL.2, TIB-75, and BNL 1ME A.7R.1	[109]
Integrin-targeted NPs			
cRGDyk-cisplatin-liposome	cRGDyk-tagged liposomes exhibited higher cellular uptake and higher cytotoxicity, in vitro and in vivo	RM-1 cells and RM-1 tumor-bearing C57BL/6 mice	[110]
RGD-albumin nanoparticle	RGD-tagged NPs increase cell uptake by pancreatic cancer cells, in vitro and in vivo	In vitro (BxPC3, SW1990, PANC-1and CFPAC-1), and in vivo BALB/C-nu/nu mice with pancreatic cancer xenografts	[111]
cRGD-platinum-polymeric micelle	cRGD-tagged micelles (cRGD/m) accumulated in the tumor, in vivo	Mouse model of U87MG (human glioblastoma cell line)	[112]
cRGDfK-gold nanorods	Targeted NPs showed better uptake in vitro, but not in vivo due to clearance of the NPs from the blood	In vitro (DU145 and HUVEC) and in vivo (prostate tumor-bearing athymic mice)	[113]
PR-b-(Polymersomes) [poly(1,2-butadiene)-b-poly (ethylene oxide)] encapsulating siRNA	PR-b-anchored vesicles induced high cytotoxicity in cancer cells, in vitro	T47D and MCF10A	[114]
FR-targeted NPs			
FA-5-FU-PNVCL-b-PEG micelles	FA-tagged micelles showed a cytotoxic effect in tumor cells, in vitro	EA.hy 926 and 4 T1	[54]
FA-2-ME-BSA NPs	FA-tagged NPs entered into the cells through the folic acid-mediated endocytosis, leading to higher antitumor efficacy, in vitro	SMMC-7721	[115]

(continued)

Table 15.1 (continued)

NPs	Outcome	Cell lines	Ref
FA-dextran-DOX – retinoic acid magnetic NPs	FA-functionalized magnetic micelles exhibit IC of the targeted drug to about 10 times slower than the free drug, in vitro	MCF-7 and MDA-MB-468	[116]
FA-SPION-DOX-PEG-lipid shell	FA-functionalized core-shell NPs codelivered drugs and SPIONs to the same cells, in vitro	HeLa cells	[61]
FA-DOX-magnetic NPs	FA-tagged DOX-NPs enhanced apoptosis of cancer cells, in vitro	C30 and CP70	[117]
EGFR-targeted NPs			
Biotinylated EGF-NeutrAvidin(FITC)-gelatin NPs	EGF-conjugated NPs were mainly transported in cancerous lungs	A549 tumor-bearing nude mice	[118]
EGF-PAMAM-QD-vimentin/yellow fluorescent protein siRNA	EGF-modified NPs can be internalized in EGFR expressed cells, in vitro	HN12, NIH3T3, and NIH3T3/EGFR	[80]
EGF-magnetic NPs	EGF-decorated NPs facilitated the MR imaging contrast at the cancer site	Murine tumor models of melanoma and hepatoma	[119]
EEEEpYFELV (EV)-PEG-liposome-protamine-heparin NPs	EGFR-targeted nanocarriers were localized in tumor cells in the cytoplasm, in vitro	NCI-H460	[120]
GE11-PEG-DSPE-PTX micelle	GE11-conjugated micelle significantly restricted the tumor cell growth, in vitro	U-937 and Hep-2	[82]

take advantage of overexpressed BnR to specifically deliver cytotoxic drugs loaded in NPs tagged with Bn or Bn analogs. Accardo A et al. have efficiently delivered Doxorubicin-loaded liposomes coupled with BnR ligand (7–14) peptide fragment called BN (7–14) into PC-3 tumor cells [33].

15.4.2.2 Somatostatin Receptors

Somatostatin receptors (SSTRs) belong to the GPCR superfamily and are overexpressed in cancer cell lines of lungs [34], neuroendocrine [35], prostate [36], breast [37], colorectal [38], gastric [39], and liver [40]. These receptors mediate the signal of basic processes involved in secretion, cell division, propagation, and apoptosis. Huang C.M. et al. [41] documented the utilization of SSTRs to target taxol into the cancer cells by tagging SSTR-specific ligand octreotide (OCT). OCT-taxol conjugates target SSTR-expressed human breast carcinoma (MCF-7) cells and showed triggered apoptosis [42]. Doxorubicin-loaded sterically stabilized liposomes (SSLs) conjugated with OCT using polyethylene glycol led to enhanced

selectivity and delivery of the drug in SSTR2 expressed cells via receptor-mediated endocytosis [43].

15.4.2.3 Endothelin Receptors

Endothelin (ET) is composed of three polypeptide molecules such as ET-1, ET-2, and ET-3, each of which is made up of 21 amino acids that can bind with GCPRs, endothelin receptor A (ETRA), and endothelin receptor B (ETRB or EDNRB). All three ETs can bind to ETRB with equal affinity, whereas ET-1 and ET-2 bind to ETRA with double affinity compared to that of ET-3 [44]. In a preclinical study drug, ligand conjugate showed promising efficacy in a low expressed ETRB model [45]. The ETRB pulls the attention as a target for ligand–drug conjugate due to its low level of expression on healthy tissue, its localization in the cell surface, and its fast endocytosis.

15.4.3 Integrin Receptors

Integrins are the receptors present over the cell surface, which facilitate cell-extracellular matrix (ECM) adhesion. Among various integrin receptors, αv (or αvβn) integrin receptors are highly expressed in activated endothelial cells and tumor cells, while in resting endothelial cells and normal cells, their expression is minimum. Thus, integrin receptors can serve as potential targets for targeted anti-cancer therapeutics [46].

15.4.3.1 Integrin αvβ3

Studies on DOX-loaded NPs showed that the targeted delivery to the integrin αvβ3 expressed cancer vasculature restricted the growth and proliferation of tumor cells, avoiding the toxicity and weight loss side effect of this drug [47]. Chen et al. developed integrin αvβ3-targeted PEGylated amphiphilic triblock copolymer-coated iron oxide NPs. The native NPs are conjugated with near-infrared fluorescent (NIRF) dye, i.e., IRDye800, and a cyclic Arginine-Glycine-Aspartic acid (RGD) peptide, i.e., c(RGDyK) for selective binding to αvβ3 receptor possessing U87MG glioblastoma cells as indicated by in vitro binding assay [48].

15.4.3.2 Integrin α-3

α-3 integrin receptors are overexpressed in breast cancer, ovarian cancer, and melanoma [49]. So, these α-3 integrin receptors have been utilized as a target for targeted drug delivery systems in the treatment of ovarian cancer [50]. Conjugation of OA02 peptide with micellar NPs comprising polyethylene glycol (PEG) block dendritic cholic acid (CA) copolymers (PEG5k-CA8 NPs) significantly increased the efficiency of cell uptake in α-3 expressed SKOV-3 and ES-2 ovarian cancer cells, but the opposite effect was observed with α-3 integrin-deficient K562 leukemia cells.

15.4.4 Folate Receptors

Folic acid (FA) or folate or vitamin B9 is utilized by living cells for the synthesis of purines and pyrimidine. Living cells get FA only after internalization through the folate receptors (FRs) [51]. As FRs are less expressed in normal cells and highly overexpressed on cancer cells such as the tumors of the brain, lungs, breast, colon, myeloid cells, kidney, and ovary, therefore, FA can be utilized as a targeting ligand for active delivery of therapeutic agents to the tumor cells [52]. Due to the smaller size and high affinity for binding of FA, it acts as targeting ligand. FA-conjugated nanocarriers with pH-sensitive spacer molecule potentiate rapid drug release inside the cancer cells at pH 5.0 [53]. Fa-conjugated micelles also facilitate specific drug targeting to the cancer cells [54]. Albumin-based NPs, when combined with hyperthermia, can effectively show the cytotoxic effect of antitumor drugs [55]. FA-conjugated Gold NPs also exhibit higher efficacy in specific drug delivery [56]. In a study where FA-conjugated berberine hydrochloride (BHC)-loaded gold NPs showed marked delivery of their cargo to FR-expressing HeLa cells [57], FA-conjugated gold NPs are also employed for the delivery of therapeutic agents such as DOX [58], siRNA [59], and PDT [60]. FA-functionalized magnetic NPs are also investigated for the delivery of DOX, PTX, methotrexate, and mitoxantrone to tumor cells [61].

15.4.5 Transferrin Receptors

The efficient transferrin (Tf) uptake by cancer cells that overexpress transferrin receptors (TfRs) makes it effective to deliver cytotoxic drugs, proteins, and genes [62]. TfRs are categorized into two types, TfR1 or CD71 and TfR2. TfR1 is prevalent at low levels in the majority of cells, whereas TfR2 specifically expresses in hepatocytes [63]. Although the expression of TfR1 is low in normal cells, it increases in the case of a tumor, indicative of malignancy [64]. For instance, in the case of several cancers of breast, ovary, and brain, the expression of TfR1 is found to be increased [65]. Jiang et al. [66] successfully developed a contrast agent for targeted magnetic resonance imaging (MRI) by conjugating transferrin with superparamagnetic iron oxide NPs (Tf-SPIONs) for the diagnosis of brain glioma in rats [67].

15.4.6 LDL Receptor

Based on densities, lipoproteins are classified into five categories such as chylomicrons, very-low-density lipoproteins (VLDL), low-density, lipoproteins (LDL), intermediate-density lipoproteins (IDL), and high-density lipoproteins (HDL). Lipoproteins are the transporter of lipids and cholesterol in the blood. In the LDL apolipoprotein, B-100 occupies around one half of the entire LDL surface, which helps in targeting the LDL toward LDL receptor (LDLR)-expressed tissues,

like liver, adrenal glands, and ovaries [68]. The body's immune system considers lipoproteins as an endogenous component, and therefore, the phagocytic system is unable to detect them. In many cancer presenting cells in acute myelogenous leukemia, adrenal adenoma, colon cancer, pancreatic cancer, lung cancer, brain cancer, and prostate cancers, LDLRs are overexpressed. Therefore, LDLs are suitable to be used as targeting ligand in targeted cancer chemotherapy [69]. In a study, more accumulation of DOX was observed in the liver in comparison to free DOX after administration of LDL-DOX conjugate into a mice model [70]. Hematoporphyrin (HP) can also bind the LDLRs on the membrane of the tumor cell [71]. HP-functionalized DOX-loaded NPs improve the effect of photodynamic therapy (PDT) in liver cancer treatment [72]. Osthole-loaded chitosan NPs conjugated with LDL improved the targetability of nanocomposites to liver cancer cells both in vitro and in vivo [73]. The BBB is also found to be overexpressed with LDLRs, and therefore, LDL can be employed for effective drug delivery into the CNS [74]. Moreover, simultaneous administration of statins with DOX-loaded NPs conjugated with LDL increases the drug delivery and induced tumor death in the CNS [75]. Also, apolipoprotein E (ApoE)-functionalized NPs can be endocytosed better in the CNS across the BBB through LDLRs [76].

15.4.7 Epidermal Growth Factor Receptors

Epidermal growth factor (EGF) belongs to the family of tyrosine kinase (RTK) receptors. Alternatively, it is also called ErbB or HER [77]. It has a significant relationship with cancer propagation [78]. EGFRs are significantly overexpressed in lung cancer, breast cancer, bladder cancer, and ovarian cancer. PTX-loaded EGF-functionalized polymeric lipid NPs showed a significant reduction in cell proliferation in vivo in tumor-bearing mice model [79]. Quantum dot-labeled EGF-conjugated polyamidoamine (PAMAM) Generation 4 dendrimers are also explored for selective delivery of genetic materials and imaging agents [80]. Also, EGFR possessing HepG2 cells could significantly take up EGF-tagged PAMAM/DNA NPs in vitro. However, significant accumulation of these NPs was observed in the cancer site in vivo [81]. PTX-loaded PEG-di-stearoyl phosphatidylethanolamine (PEG-DSPE) micelles functionalized with GE11 were effectively internalized by EGFR positive Hep-2 and EGFR negative U-937 tumor cell lines as well [82]. Aptamers are also a novel tool for selective targeting of tumor cells using targeted nanodrug therapy. Scientists have formulated 80-residue aptamer, J18-tagged gold NPs, which specifically targeted EGFR expressed A431 cancer cells [83].

15.4.8 Fibroblast Growth Factor Receptors

Fibroblast growth factor receptors (FGFRs) are overexpressed in breast cancer, prostate cancer, bladder cancer, and gastric cancer and responsible for tumor growth

and proliferation [84]. Overexpression of FGFR1, FGFR2, and FGFR4 is observed in breast and prostate cancer cells, whereas in gastric cancers, only FGFR2 is overexpressed. In papillary thyroid carcinoma, only FGFR1 and FGFR3 have been observed [85]. Xiao et al. developed a truncated human basic fibroblast growth factor (tbFGF) peptide-conjugated DOX-loaded cationic liposomal NPs. tbFGF is a modified peptide possessing active sites for the FGF2 receptor and part of heparin. This tbFGF could specifically bind to FGFR2 and results in cell lysis observed in mouse Lewis lung carcinoma (LLC) cells by the synergistic activity of DOX and plasmid loaded in the common cationic liposome. The one-third concentration of DOX in this system shows 50% cell lysis as compared to the concentration of free DOX [86]. It was observed that C57BL/6 J mice bearing B16 melanoma cells showed better accumulation of PTX-loaded PEGylated liposomes in mononuclear phagocyte system containing organs such as liver and spleen, whereas less accumulation was observed in other organs such as heart, lungs, and kidney as compared to PTX-loaded PEGylated-liposome and free PTX [87].

15.4.9 Sigma Receptors

Scientists are excited to explore sigma receptor-ligand interaction for cancer cell targeting. Sigma receptors are overexpressed in nonsmall cell lung carcinoma (NSCLC), breast, prostate, and melanoma [88]. Initially, the sigma receptor was considered a member of the opioid receptor family [89]. The sigma-1 receptor (S1R) is a widely studied protein, whereas the sigma-2 receptor (S2R) is elusive now [90]. The novel sigma-2 receptor-specific targeting ligand SW43 showed promising targeting efficacy and cell lysis by loaded gemcitabine pancreatic cancer [91]. S1R is generally found in the cerebral cortex, hippocampus, and cerebellar Purkinje cells [92], and therefore, it can be used as the target for the DDS in the treatment of CNS-related diseases such as Alzheimer's disease, schizophrenia, depression, stroke, amnesia, pain, and addiction [93]. S1R is generally expressed in mitochondria-associated ER membranes [94, 95]. S2R-specific ligands are still in the research and structural characteristics of S2R are yet to be known, while it is found to be overexpressed in a variety of breast cancer, pancreatic cancer, neuroblastoma, bladder cancer, and lung cancer. SV119-decorated DOX-loaded liposomes showed better cell uptake in human breast cancer (MCF-7), human prostate cancer (PC-3, DU-145), and human lung cancer (A549, 201 T) as compared to normal human bronchial (Beas-2B) cancer cells. Similarly, SV119-functionalized liposome showed a higher cytotoxic effect in DU-145 cells as compared to nonfunctionalized liposomal DOX [96].

15.5 Conjugation Strategies to Functionalize Nanocarriers

The drug delivery systems prepared for active targeting to tumor cells mostly comprises five basic components, viz., a drug carrier system, a corona of hydrophilic polymer to shield from opsonization and to increase retention time, a targeting ligand that specifically binds to a particular receptor of the cell at the disease site, a coupling agent or functional group, which links the carrier with the ligand, and a drug or imaging agent encapsulated or bound chemically with the carrier.

The anticancer drugs or drug carriers can be coupled with the targeting ligand by a suitable method of chemical conjugation. The ligand can be conjugated with the drug or drug carrier directly or by a spacer/linker (Fig. 15.1). The chemistry behind the conjugation must be chosen in such a way that it does not show any adverse effect on the selectivity of the targeting agent and drug activity [121]. The linker used to bind the drug and the targeting ligand helps in the reduction of steric hindrance and results in the increase in flexibility of the ligands, leading to enhanced efficiency of coupling with the receptors. The spacers employed may be constructed in such a way that they can possess additional control on drug release from the carrier inside the cell. They assist in targeting and efficient cell internalization of nanocarriers [122]. The conjugation between the targeting ligand and drug or its carrier is done by holding some chemical reactions involving the functional groups of drug or carrier and targeting ligand, which generally do not take part in biological function. It is well established that simply conjugating drugs to a carrier does not show specific targeting. However, functionalized nanocarriers composed of a biodegradable polymer, drug, and a targeting ligand are mandatory for effective targeted drug delivery system [17]. Different methods of functionalization of the NP surface are described in the following section (Table 15.2).

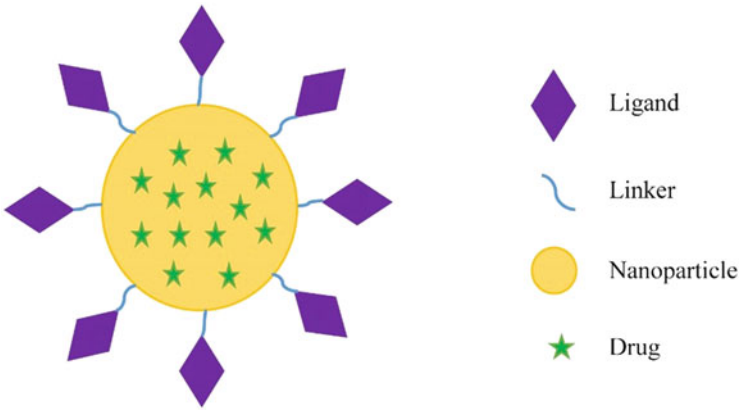

Fig. 15.1 Schematic diagram of ligand nanoparticle conjugation

Table 15.2 Pros and cons of different conjugation methods

Types of Conjugation	Pros	Cons
Physical adsorption (takes place via ionic, electrostatic, and Van-der-Waals forces)	• Easy and simple method • No modification of ligand and nanoparticle is required	• Denaturation of the ligand can take place due to reversible hydrophobic interaction • The weak interaction between ligand and nanoparticles • Serum protein can cause displacement of ligand
Covalent Conjugation (takes place via the linker Moiety)	• Stable and reproducible method • Linker moiety can overcome unfavorable reaction conditions	• Unfavorable reaction conditions may hamper the ligand • Linker moiety have a significant effect on function

15.5.1 Covalent Method of Conjugation

Antibody-NP Covalent Conjugation: Various methods of covalent conjugation are being used to link monoclonal or polyclonal antibodies with NPs using crosslinking agents. The importance of spacer moiety in conjugation chemistry is more than the binding of mAb with NPs, since the mAb could be polymerized itself and unable to identify the binding site of antibody on the NPs [123]. Depending upon the reactive functional groups present at both the end of the spacer molecule, they can be categorized into homobifunctional and heterobifunctional. Homobifunctional spacers possess the same reactive functional group in both terminals, whereas heterobifunctional spacers possess different chemical groups. To eliminate the chance of steric hindrance between drug and mAb, spacer groups of different lengths should be selected [124]. Sulfhydryl group-containing amino acids such as cysteine or cystamine could be covalently coupled with NPs to form thiolated NPs having free thiol groups available for antibodies or drugs. Alternatively, for the formulation of thiolated NPs, chemical compounds such as 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC/EDAC), tris-carboxyethyl phosphine hydrochloride (TCEP), and dithiol-DL-threitol (DTT) have also been utilized [125]. Some commonly used methods of chemical conjugation using various spacer molecules are mentioned below.

(i) Conjugation using Carbodiimide Chemistry: Following the carbodiimide chemistry, the covalent conjugation method is utilized to form an amide bond between the carboxylic and amine group of NPs and the antibody, respectively [126]. Briefly, EDC interacts with the carboxylic acid groups of PLGA, which activates the carbonyl moieties to bind with the amino group of antibodies. To improve the binding efficiency, NHS or its water-soluble analog (sulfo-NHS) is incorporated by EDC coupling methods [127]. Cirstoiu-Hapca et al. develop a double-step carbodiimide reaction for prepare thiolated PLA NPs by thiolation of the carboxylic groups of PLA. Then, the Paclitaxel-loaded PLA NPs were exposed to link the thiol group with anti-HER2 mAb using m-maleimidobenzoyl-N-hydroxysulfosuccinimide ester (sulfo-MBS) as a spacer [128]. Preattached PLGA

NPs with biotin or PEG are activated by the Carbodiimide conjugation method before EDC/NHS treatment. Biotin binding spacers such as Avidin, NeutrAvidin, and Streptavidin can be attached to the biotin-tagged PLGA NP. It was observed that NeutrAvidin showed a better affinity toward the protein in comparison to Avidin and Streptavidin. Each spacer has four binding sites for biotin to tag the biotinylated NPs, leading to NP aggregation. Therefore, optimized amount for biotinylation is necessary to restrict the aggregation [129]. Also, palmitic acid (PA) activated by NHS could react with cationic Avidin. For the quantification of the surface density of Avidin on NPs, a number of PA-Avidin complex were incorporated in PLGA NPs. More amounts of PA-Avidin on the NP surface increase the surface roughness [130].

(ii) Conjugation using Maleimide Chemistry: The interaction between the maleimide group and sulfhydryl groups leads to a highly selective and precise conjugation. Here, the conjugation between sulfhydryl reactive groups and amine-reactive groups via a maleimide heterobifunctional spacer imparts superior flexibility in antibody-NP hookup [131]. Using the carbodiimide chemistry, thiolated nanocomposites were formulated where carboxylic group-activated NPs could be coupled with cystamine. Sulfo-MBS was employed as a bifunctional spacer to link the thiolated NPs and NeutrAvidin, which produced functionalized NPs. In these modified NPs, NeutrAvidin showed greater affinity to bind than plain NPs. It is well proven and confirmed that NeutrAvidin-biotin binding has no impact on its activity during the tagging processes with the NPs [132]. In a study, on PLA NP, thiol group was covalently inserted. The PLS NPs were prepared using the salting-out method with around 25,000 thiol groups per NP. Over these NPs, anti-HER2 and anti-CD20 antibody were tagged covalently taking sulfo-MBS as a spacer. The functionalized NPs were investigated for their targeting efficiency against target cancer cell lines. The results showed that the type of antibody and its configuration signify a lot rather than its quantity on NP surface for cell internalization as depicted in human ovarian carcinoma cells (SKOV-3). Anti-HER2-decorated NPs exhibit precise binding and internalization. Despite the more quantity of anti-CD20 on anti-CD20-functionalized NPs, it remained over the cell surface [133].

(iii) Conjugation using Click Chemistry: Click chemistry is the most efficient method of conjugation, which takes place in mildly reactive aqueous solutions. In this conjugation process, there is no production of any unwanted byproducts such as dicyclohexylurea, which occurred in carbodiimide reaction. The click reaction generally held between 1,3 dipolar cycloaddition of azides and terminal alkynes catalyzed by copper, known as CuAAC (copper-catalyzed azide-alkyne cycloaddition). These 1,3-triazoles are biocompatible, and therefore, their use is approved by the FDA for drug formulation. Herein, the PEG or propargyl-dPEGNHS spacer was prepared by the activity of EDC and sulfo-NHS at pH 6 to interact with the amine functional group of antibody [134]. This antibody-coupled azide group then reacts with the alkyne group possessing fluorescence-labeled PLGA. Recently, it was reported that the copper-free azide-alkyne cycloaddition can reduce in vivo cytotoxicity [135].

(iv) Conjugation using Only Spacer: Apart from the above-mentioned conjugation techniques, nanocomposites can also be functionalized using a linker avoiding

any kind of chemical modification. So, in this NP preparation technique, there is no formation of unnecessary intermediates as occurred in carbodiimide-amine and maleimide-thiol reactions. The noncovalent attachment of homobifunctional linker molecule Bissulfosuccinimidyl suberate (BS3) with PLGA NPs facilitates the formation of a covalent amide bond between the antibody and NPs. In this simple process, an amide bond was formed between the carboxylic group and amine group of BS3 and targeting ligand, respectively. The enhanced cell uptake of NPs was observed due to the covalent bond that exists between the ligand and BS3 [136].

Polysaccharide-NP Covalent Conjugation: The amine functional group of chitosan could form a covalent bond with the carboxyl functional group of PLGA. The carboxyl group of PLGA NPs could react with the amine group of chitosan after activation by EDC. These NPs could constantly release the cytotoxic drug mitoxantrone [137]. In a study, the thiol functional group of 2-iminothiolane was immobilized by covalent attachment with amino groups of chitosan NP surface. This covalent conjugation results in enhanced mucoadhesive properties, leading to increased residence time in the intestine than chitosan alone [138].

PEG-NP Covalent Conjugation: PEG can be attached by forming a covalent bond with the reactive groups of NP surface, which can additionally reduce the possibility of optimization in blood. PEG composites can interact with amino acids, glycosylated proteins, and thiols, which allows PEG to serve as a crosslinker between the NP and the ligand [139].

15.5.2 Physical Adsorption Methods

As an alternative to the covalent conjugation methods, antibodies could also be linked with the NP surface using simple adsorption techniques, e.g., Paclitaxel-loaded PLGA NPs are coated by simply adsorbing cationic SMFv-polylys, where positively charged polypeptide polylysine (polylys) was tied with SM5–1 scFv (SMFv), which was obtained from SM5–1 mAb. The positively charged SMFv-polylys was linked with negatively charged PLGA NPs using electrostatic interaction. This occurred due to the isoelectric point, electrostatic force, and negative charge of PLGA in neutral pH, which strongly facilitates the coupling of positively charged targeting proteins on the PLGA NP surface [140]. Similarly, transferrin and bovine serum albumin (BSA) at a ratio of 1/1 (w/w) were physically adsorbed with PLGA NPs by simply mixing with NPs (1 mg/ml concentration) by shaking at room temperature for 3 h [141]. Additionally, by employing different surfactants during formulation, the surface charge of PLGA NPs could be altered. For instance, cetyltrimethylammonium bromide (CTAB), which is a cationic surfactant, can be used to formulate positively charged NPs ideal for the adsorption of plasmid DNA [142]. In a study, hydrophobic PLGA was tagged with an antibody molecule using its hydrophobic part to target human invasive ductal breast carcinoma. To enhance the hydrophobic interaction, the phosphate-buffered saline (PBS) of pH 5 was replaced with the buffer of neutral pH [123]. The hydrophilic polymer PEG can also be tagged with hydrophobic NPs by the physical adsorption method

[139]. More than one layer over core PLGA NPs by physical adsorption could show better efficacy in targeting tumor cells. PLGA NPs could be coated with pluronic or in conjunction with heparin or chitosan through a urethane bond formed between the amine and hydroxyl group of heparin or chitosan and pluronic, respectively [143].

15.6 Cell Internalization of Nanocarriers

It is very difficult to detect cancer in the acute state, and it becomes disseminated in the advanced stage of cancer. Hence, the intravenous route is mostly considered for the delivery of cytotoxic agents via nanocarriers. The nanocomposites are mainly internalized in the target cell by the endocytosis mechanism. The endocytosis of NPs can take place by either phagocytosis or pinocytosis (Fig. 15.2). The phagocytic cells like macrophages, neutrophils, and dendritic cells engulf the foreign substances by phagocytosis, while the pinocytosis takes place in all other cells. The pinocytosis can be classified as micropinocytosis, clathrin- or caveolae-mediated endocytosis, and clathrin/caveolae-independent endocytosis [144]. The larger particles are generally taken up by the phagocytosis pathway. For phagocytosis, nanocarriers must be enveloped with the opsonins for their recognition by phagocytic cells via opsonin receptors like mannose and scavenger receptors. The binding of the ligand with a receptor causes the rearrangement of actin and forms the phagosome, which leads to the creation of a cup-like shape, with some outgrowth around the substrate resulting in the internalization of the NPs [145]. Pinocytosis pathway is generally suitable for fluids and suspensions possessing small particles in them. Based on the type of proteins engaged, pinocytosis can be categorized into micropinocytosis, clathrin- and caveolae-dependent endocytosis, and clathrin- and caveolae-independent endocytosis. All the mammalian cells exhibit clathrin-dependent endocytosis for the uptake of nutrients like cholesterol (LDL) using the LDL receptor and iron using the Tf receptor [146]. This ligated receptors coupled with the cytoplasmic adaptor proteins and forms a clathrin lattice [147]. The vesicles are detached from the membrane via GTPase activity and generate clathrin-coated vesicles [148]. Caveolae are present in the cholesterol-rich area of the membrane possessing caveolin-1. This mechanism of internalization bypasses the lysosomes, and therefore, several pathogens utilize this transport mechanism to get entered into target cells [149]. The caveosome has a neutral pH and takes the help of actin to move within the cell [150]. The nanocarriers, which utilize this pathway, can bypass the lysosomal degradation and enhance the drug delivery efficacy to endoplasmic reticulum or nucleus. It is reported that the anionic nanocarriers use the caveolae-dependent endocytosis mechanism [151].

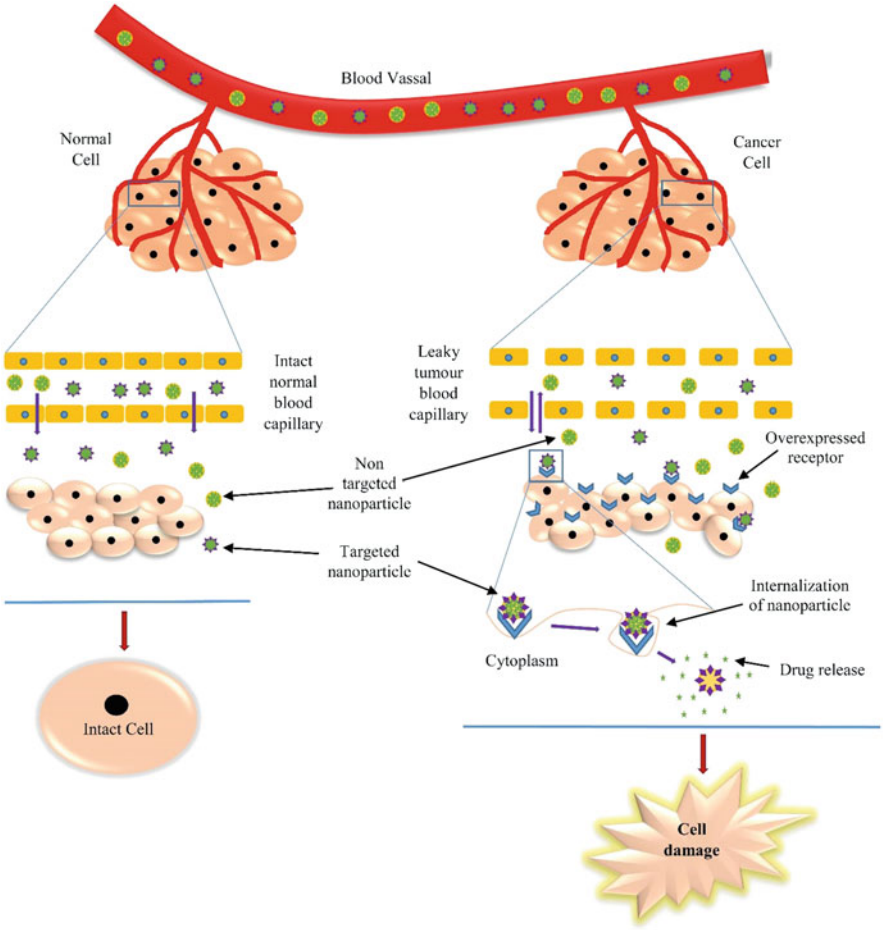

Fig. 15.2 Biofate of ligand nanoparticle conjugate

15.7 Conclusion and Prospects

With the advancement of nanotherapeutics, rigorous research has been continuously done on targeted nanodrug carriers. Some of the key factors responsible for efficient drug targeting have been described in this chapter. But to get optimal clinical success in humans still a challenge, due to many unknown conditions. Most importantly, the behavior of the targeted DDS inside the cell is not fully known. However, many kinds of research demonstrate the intracellular behavior of nanocarriers, but till now, any generalized conclusion for every NP and biomarker does not persist. Mostly, intracellular trafficking is also influenced by the phenotype of the tumor cell. This may have a great impact on the clinical practice of the treatment since the patient

might possess a versatile disease phenotype. However, the proper understanding of the intracellular behavior and biological fate of the nanocarriers might have a huge impact on the clinical outcomes. Proper knowledge might help in the smart designing of cell organelle-specific nanocarriers. Indeed, it is a very challenging task to develop a targeted DDS for cancer treatment.

Acknowledgments The authors are grateful to Dibrugarh University, India, and Department of Science and Technology (DST), Government of India, for providing facility and research fellowship to carry out this work.

Conflict of Interest The author declares that there is no conflict of interest. All the tables and figures are self-made and original.

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Tunable Biopolymeric Drug Carrier Nanovehicles and Their Safety

16

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Abstract

Biopolymers are types of biomolecules encompassing a wide range of macromolecules such as polysaccharides, proteins, DNA, RNA, enzymes, and polyphenols that are abundant in nature. The most striking properties of these biopolymers are that they are renewable, eco-friendly, nontoxic, biodegradable, and natural, and most of the time, they are relatively inexpensive materials. The capability to design novel material through nanotechnology and related developments have widened and enabled the development of superior-controlled drug delivery systems. Because of the unique properties of biopolymers together with nanotechnology capabilities, the use of biopolymeric nanomaterials as a drug nanocarrier was inevitable. This chapter covers the literature in the field of biopolymeric nanovehicle design for tailor-made devices such as drug carriers. The advantages and disadvantages of loading techniques such as physical, chemical, and encapsulation methods in the preparation of biopolymeric drug carrier vehicles are discussed. Finally, the potential application area of these biopolymeric carriers from ocular delivery to targeted cancer therapy and, especially, their safety will be outlined with clinical evidence.

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M. K. Das, Y. V Pathak (eds.), *Nano Medicine and Nano Safety*,
https://doi.org/10.1007/978-981-15-6255-6_16

Keywords

Biopolymers · Nanoparticles · Nanocarriers · Drug delivery · Gene and cancer therapy

16.1 Introduction

For many years, therapeutic agents and drug molecules have been directly used in the treatment of many diseases and novel drug molecules have been designed, synthesized, or discovered to eliminate health problems. The usage areas of these therapeutic agents are limited because of their unstable chemical structure, low solubility, and poor bioavailability and they can undergo oxidative degradation and have inappropriate concentration levels or therapeutic levels [1]. Nowadays, there is growing interest in the design of drug carrier systems to avoid these challenges. Drug-carrying nanovehicles derived from different types of biological sources including carbohydrates [2], proteins [3], and polyphenolics [4] are generally designed to regulate the pharmacokinetic and pharmacodynamic ability of active molecules [5].

Since the 1980s, the term nanotechnology has attracted great interest leading to many advances in fields including electronics, mechanics, aerospace engineering, and biomedicine [6]. Nanomaterials, nanosurfaces, and nanoparticles (NPs) represent important aspects of nanotechnology; especially, nanoparticles ranging in size from 1–100 nm are cornerstones for the design of superior material properties [7]. Recently, many research groups have shown interest in the use of nanomaterials and made significant progress in biomedical fields, e.g., controlled drug release [8], gene therapy [9], tissue engineering [10, 11], imaging of specific sites, and probing of DNA structure, and so on [12, 13]. However, instead of synthetic polymers, NPs derived from biopolymer-based structures have attracted substantial attention especially for drug delivery use due to their appealing properties, abundance in nature, biocompatibility and biodegradability, and renewable, nontoxic, and inexpensive properties [14, 15]. A general classification of biopolymers is shown in Fig. 16.1.

These materials afford many advantages when used as delivery systems due to their exceptional properties such as improving drug bioavailability, reduction of drug side effects, increasing solubility, protecting drug molecules, enabling higher drug efficacy, increasing cell membrane crossing, enhancing drug diffusion to the cell surface, enhancing the residence time of drug at the specific site, and promoting drug targeting and therapeutic biodistribution.

The evolution of controlled drug release systems from the middle of the nineteenth century is summarized in Table 16.1. Accordingly, controlled drug delivery technology has made considerable progress over the last 70 years. This progress in the last 70 years can be divided into two generations. Accordingly, the progress in drug delivery began in 1950 with the introduction of the first continuous release formulation [16–19], and then, the first generation drug delivery systems

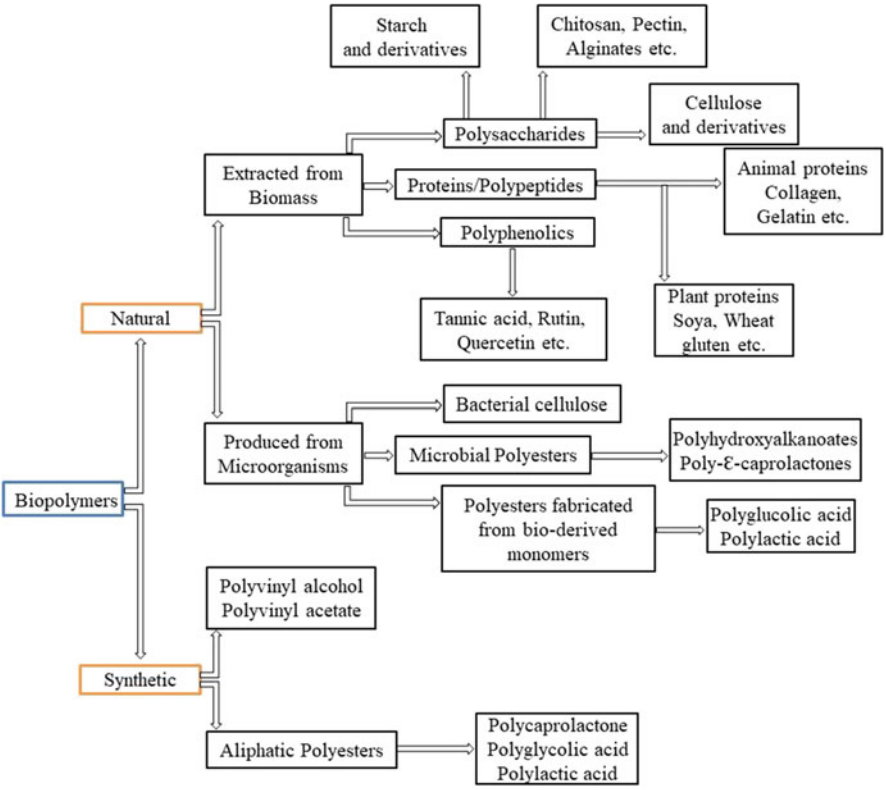

Fig. 16.1 Basic classification of biopolymers

Table 16.1 Evolution of drug delivery systems through the years

Years	Controlled drug delivery systems
1950–1980 basics of controlled release	Oral delivery (twice- or once- a day)
	Transdermal delivery (once- a day or week)
	Drug release mechanism (dissolution, diffusion, osmosis, or ion-exchange
1980–2020 smart delivery systems	Zero order release (zero- vs first- order release)
	Smart polymers and hydrogels (environmental sensitive, self-regulated release)
	Peptide and protein release (biodegradable depot)
	Nanomaterials (gene delivery, targeted delivery)
2020–2050 modulated delivery systems	Switchable release (on-off release, sensitive release)
	Targeted delivery (anticancer drags, siRNA)
	Long-term delivery systems (>6–12 months with the minimal initial burst effect)
	- in vitro - in vivo correlation (prediction of pharmacokinetic profiles from in vitro release study)

(1950–1980) focused on the development of oral and transdermal sustained release systems and established a few controlled drug delivery mechanisms.

The second generation drug release systems involving current technology between 1980 and 2020 began with the development of zero-order release systems, self-release systems, and long-term formulations where drugs can be stored and nanotechnology-based delivery systems. During the second generation, systems, polymers, and hydrogels, originally called "smart" materials, were developed to make delivery systems that can be triggered by changes in the environment of the carrier. Stimuli such as pH, temperature, magnetic field, or light can be used as triggers to release, control, and modulate the drug release performance. Biodegradable NPs, solid implants, and in situ gel-forming implants have been used to maintain the release of peptides and proteins [20]. In the last decade, the second generation drug release systems have continued to be developed mostly as nanotechnology-based drug delivery systems [16]. In this review, the methods used for NP synthesis from the most prominent natural and synthetic biopolymers, their size distribution, and their use in drug delivery systems are discussed. The biopolymers, used in the synthesis of NPs, are chosen as polysaccharides, proteins, polyphenols, and a few synthetic biopolymers.

16.2 Design of Biopolymeric Nanovehicles

Biopolymer-based NPs can be prepared in various forms and morphology, and their use in drug delivery has received great attention [21–23]. Biopolymeric NPs were developed primarily for drug delivery systems as alternatives to liposome technology to overcome problems associated with the stability of these materials in biological fluids and during storage [24]. The selection of biopolymer can directly affect (1) the size and morphology of NPs, (2) the surface charge and permeability of the prepared NPs, (3) the biodegradability and the degrees of biocompatibility and cytotoxicity, and (4) drug loading/release performances. Therefore, these parameters are considered in the preparation of nanovehicles to attain the required drug release performances [25, 26].

Despite the difficulties in size adjustment during the synthesis of NPs from biopolymers, the innate properties of biopolymers, as well as higher bioactive material loading capacity compared to clay, metal, and other inorganic-based NPs, have attracted great interest from many researchers [27, 28]. In addition, bipolymeric NPs can be considered bioactive material [29], can readily transport the cargo materials into tissues and cells [27, 28], and are innately biocompatible and biodegradable with low immunogenicity. Moreover, biopolymeric NPs can increase the efficacy of drugs, reduce the unwanted toxic effects of free drugs, protect the drug from harsh environments, increase cellular uptake, and can transport many active agents to different regions in the body [30–33].

16.2.1 Polysaccharide-Derived Nanocarriers

Polysaccharide-derived NPs and nanostructured surfaces help to improve the biocompatibility of materials that are toxic to cells, providing new approaches for immobilization of active agents. NPs prepared from natural polysaccharides can be readily designed to deliver peptides, proteins, and nucleic acids where needed [34–36]. The most commonly used polysaccharides in NP synthesis are chitosan, hyaluronic acid, cyclodextrin, pectin, alginic acid, dextran, and carrageenan and their composites [30, 37].

It was reported that cross-linked κ-carrageenan NPs using a polysaccharide with an average size of less than 100 nm could be synthesized and display thermo-responsive behavior by undergoing reversible volume change in a temperature range acceptable for living cells, e.g., 37–45 °C [38]. These kinds of material or nanogel are considered intelligent therapeutics as they can unload the cargo materials with temperature-controlled release rate as thermo-sensitive drug carriers. In another study, different polysaccharides of chitosan and carrageenan were used in NP preparation via electrostatic interactions [39]. Chitosan-carrageenan NPs can have many potential applications not only in drug delivery but also in tissue engineering and regenerative medicine because of their innate properties [39, 40]. Furthermore, it was reported in the literature that chitosan-carrageenan NPs show noncytotoxic behavior during in vitro tests against L929 fibroblasts and provide controlled release of ovalbumin, used as model protein, for up to 3 weeks [41]. Although curcumin has many pharmacological effects, it is difficult to use as a beneficial drug because of the difficulty in transition to metabolites and its short half-life. Therefore, β-cyclodextrin-curcumin nanoparticle complexes were synthesized, and in turn, β-cyclodextrin-curcumin nanoparticle complexes increased the dissolution rate of curcumin by ten-fold and curcumin permeability increased from β-cyclodextrin-curcumin nanoparticle complexes by tenfold compared to free curcumin [42].

NP drug delivery systems encounter many difficulties in the treatment process due to drug resistance. NPs prepared from hyaluronic acid (HA), a polysaccharide, were modified to target the loaded drug to the relevant site [43]. As a result, in vitro and in vivo studies have revealed that HA NPs were highly biocompatible and capable of targeting and effectively surmounting drug resistance and also have great potential in cancer therapy [43]. HA NPs with sizes varying between 237 and 424 nm were modified so that the drug could reach the target site and be visualized at the site of action. The systemic administration of modified-HA NPs to a tumor-bearing mouse was monitored as a function of time using noninvasive near infrared fluorescence imaging, enabling the visualization of biological distribution. Regardless of particle size, significant amounts of HA NPs were found to circulate in the bloodstream for two days and were selectively collected at the tumor site. It was confirmed that smaller-sized HA NPs can reach the tumor site more effectively and faster than larger HA NPs. However, the concentration of HA NPs in the tumor site decreased dramatically when the mice were pretreated with an excess of free HA. This shows that HA NPs can accumulate in the tumor site by a combination

of passive and active targeting mechanisms [44]. Another modification was performed to circumvent the preferential deposition in the liver after systemic administration, as this is the main drawback of HA-based drug conjugates in cancer treatment. In another study, poly(ethylene glycol) was conjugated with HA in NP formulation and resulted in 1.6 times more effective use in tumor tissue than bare HA NPs, showing that p(ethylene glycol)-conjugated HA NPs may be useful tools in cancer treatment [45]. Our group reported the porous and biodegradable HA and HA:sucrose NPs with the size range of 50–200 nm [46]. These porous, biodegradable HA, and HA:sucrose NPs with rough surface and irregular shapes were shown to be suitable for chemical modification to be used for drug conjugation and/or adsorption. These HA and HA:sucrose NPs were reported to be promising materials as drug carriers, including for hydrophobic cancer drugs, with adjustable and sustained release capabilities [46].

Studies with pectin polysaccharides generally include NP synthesis for encapsulation of proteins as pectin-encapsulated proteins retain their stability in the presence of many variables. It was shown that drug release systems made with pectin NPs via encapsulating ensure the ability of bioactive substances over longer storage times [47, 48]. In a study conducted by Izadi et al., whey protein was encapsulated within pectin NPs and pectin-whey protein NPs showed good stability at low pH and high resistance to gastric protease. Additionally, pectin-whey protein NPs were synthesized with the potential to carry drugs for the treatment of colon cancer chemotherapy treatment but were not found to be very effective because of the ability to bind hydrophobic ligands. It was further reported that these NPs have the potential to serve as new and effective tools in oral drug delivery applications [49]. Moreover, the pectin NPs serve as a ligand for galectin-3 receptors in colorectal cancer cells, and therefore, the particles were loaded with cancer drugs and the drug-loaded pectin NPs were coated with Eudragit S100. The in vivo studies showed that drug-loaded NPs coated with Eudragit S100 successfully protected the drug until it reached the colon site and showed stability for long periods of time. The drug delivery system was found to be 1.5 times more effective than the free drug [50].

In order to produce more effective drug delivery systems employing NP, various methods such as chemical modification, coating, and preparing them in porous formulation with different sizes and morphologies were found to be effective for sustained and controlled drug delivery applications [45–50].

16.2.2 Proteins/Polypeptide-Based Nanocarriers

Proteins and polypeptides are high molecular weight natural polymers that can be used in their natural state, as well as in chemically, physically, or enzymatically modified forms to alter their functional properties [51, 52]. It is possible to generate new functions and new performance of proteins for specific applications. For example, in drug delivery systems, protein-based materials are expected to provide superior performance as most proteins are biocompatible and are easily digested in the human digestive tract [53, 54]. Additionally, some proteins exhibit antioxidant

properties that may be beneficial to protect chemically unstable active components [55]. Proteins can be crosslinked for NP production via chemical, physical, or enzymatic curing processes used to stabilize particle structures [56]. However, protein NPs are generally very sensitive to changes in pH, ionic strength, and/or temperature, which leads to changes in surface charges and hydrophilicity/hydrophobicity. Proteins that are used to produce biopolymeric NPs can be classified as having animal and vegetable origin. The most commonly used animal proteins in NP synthesis are albumin, casein, gelatin, collagen, fibroin, whey protein, and so on, whereas the plant-derived proteins used for synthesis of NPs are lectin, zein, gliadin, soy protein, etc. [3, 37, 57].

By using biochemical and chemical methods, α-lactalbumin NPs of varying sizes between 2 and 5 nm were synthesized [58]. In the study, metal ions were loaded into the synthesized NPs to release specific drugs to organs and tissues within the body and to use them as a controlled drug delivery system. It was also shown that such NPs may have many applications in the food and pharmaceutical industry, e.g., in formulating foods with high nutritional value in the oral and digestive tracts. Human serum albumin (HSA) was used in NPs and modified with PEG [59]. The major advantage of PEG modification of HSA NPs is that it helps to achieve excellent cellular targeting with minimal monoclonal antibody, making the binding of monoclonal antibodies more economically viable. In addition, the physical and chemical activities of NPs were stable for a period of 12 weeks. This shows that the NPs can be used as a targeted nanoparticle delivery system to successfully deliver various drugs or nucleotides in targeted immunotherapy and gene therapy applications [59]. Enzymatic degradation of protein-based nanoparticle drug delivery vehicles is an important factor affecting the route of administration as well as the site-specific distribution of the drugs. Thus, synthesized bovine serum albumin NPs were coated with polylysine polymers and their stability in proteolytic medium increased. siRNA used as a model drug could be targeted and released under control by polylysine-coated bovine serum albumin (BSA) NPs [60].

Recently, different studies were conducted to demonstrate the successful delivery of bioactive substances using casein protein. Model hydrophobic chemotherapeutic drugs such as mitoxantrone, vinblastine, irinotecan, docetaxel, and paclitaxel were successfully retained in casein-based NPs and nanomaterials [61, 62]. Zhen et al. showed that cisplatin drug-loaded casein NPs had the ability to penetrate cell membranes, target tumors, and prevent tumor growth in hepatic tumor-bearing mice [63]. In another study, curcumin as a model drug was encapsulated in casein nanomicelles and the solubility of curcumin increased by at least 2500-fold, and cytotoxicity of curcumin to human leukemia cell line increased in the presence of casein nano micelles [64]. In another study, flutamide-loaded casein NPs were reported to have effective anticancer activity in rats with prostate cancer [65]. It was shown that drug-loaded casein NPs could release the drug slowly for up to 4 days and showed higher antitumor activity than the free drug as assessed by their ability to reduce tumor growth and prostate-specific antigen levels [65]. Moreover, the gastric digestibility of casein enabled the use of casein NPs as targetable drug system for gastric cancers. In this context, paclitaxel was entrapped within NPs of

casein and its cytotoxicity on gastric carcinoma cells was studied [66]. In the study, it was observed that casein NPs with entrapped paclitaxel were found to be approximately 1.5 times more effective than the free drug. In addition, casein-paclitaxel NPs were found to show no undesirable toxicity in buccal and esophageal epithelium [66].

In one study, collagen NPs were reported to be biodegradable and thermally stable and shown to be easily sterilized [67]. Moreover, when used as a drug delivery system, it may provide greater uptake of collagen-based NPs as a systemic delivery carrier to a number of cells, particularly macrophages, which may be an additional advantage [68]. Due to high adsorption capacity and water dispersibility, collagen-based NPs were used as sustained release systems for antimicrobial agents or steroids [69]. In addition, collagen NPs were used to increase the dermal delivery of certain drugs and afforded better and faster transport [67, 70]. Gelatin protein obtained by acidic and basic hydrolysis of collagen was also used for nanoparticle synthesis followed by use in anticancer, antiHIV, antimalarial, antimicrobial, analgesics, etc., release systems [3].

Silk fibroin is generally defined as protein polymers spun into fibers by some lepidoptera larvae, such as silkworms, spiders, scorpions, mites, and flies [71]. Silk proteins were reported to be promising materials for drug delivery and tissue engineering due to biocompatibility, slow biodegradability, self-assembling ability, excellent mechanical properties, and controllable structure and morphology [71]. Active amino groups and tyrosine residues on silk fibroin particles provided favorable conditions for vascular biological conjugation of endothelial growth factor and sustained endothelial growth factor release for three weeks [72]. Similarly, curcumin was encapsulated into silk fibroin and chitosan NPs, and pure silk fibroin curcumin NPs showed higher drug release and intracellular uptake and were more effective against breast cancer cells compared to silk fibroin-chitosan curcumin NPs [73]. In another study, silk sericin was successfully prepared in self-assembled micelle nanostructures by mixing with pluronics, and it was observed that the nanomicelles were capable of transporting both hydrophilic and hydrophobic drugs and were used as controlled drug delivery systems [74].

NPs prepared from plant proteins are more convenient than animal proteins in drug delivery systems due to their cheaper and more hydrophobic nature, and these NPs can be made without the use of crosslinkers and have many functional groups that can be easily used to adsorb or covalently bind molecules to alter their properties [75]. It was also reported that plant proteins may have properties such as being capable of inhibiting the spread of certain diseases, acting as direct drugs [76]. Zhong et al. used high-hydrophobic zein NPs to encapsulate oil-soluble compounds and showed their controlled release [77]. In addition, zein NPs were also used for controlled release of hydrophilic, drugs and controlled drug release systems were established with release occurring for up to 20 days [78]. In another study, controlled release of water-soluble lysozyme from zein nanocapsules at neutral pH was successfully performed [79]. In addition, the potential targeting of zein NPs to the liver was demonstrated, and drug release in the targeted region was observed for at least 24 h [76]. In another study, doxorubicin-loaded zein NPs were

reported to show controlled release of drugs over 4 days and increased cytotoxicity in doxorubicin-resistant breast cancer cells [80]. In a study by Duclairoir et al., gliadin NPs were shown for use as hydrophobic and amphiphilic drug delivery systems with their controlled delivery ability [81]. Furthermore, due to the bioadhesive properties of gliadin NPs and the ability of neutral amino acids to make hydrogen bonds with the mucosa, mucoadhesive gliadin NPs containing amoxicillin were developed for the destruction of *Helicobacter pylori* in the stomach [82].

16.2.3 Polyphenol-Based Nanocarriers

Since ancient times, compounds of plant origin have been known to be very useful in the treatment of many diseases. Studies have also reported that diets containing fruits and vegetables reduce the occurrence of chronic diseases such as cardiovascular diseases [83], diabetes [84], and cancer [85]. Among the compounds of plant origin, the consumption of polyphenols was reported to have positive effects on human health [86]. Tannic acid from black tea, quercetin abundant in red onion and cabbage, epigallocatechin-3-gallate from green tea, resveratrol from grapes, and curcumin from turmeric are just examples of many polyphenols of plant origin [87–91]. Polyphenols were reported to have prophylactic properties against many diseases due to their antioxidant, anti-inflammatory, anticarcinoma, antimicrobial, antiviral, and cardioprotective properties [87–92]. Therefore, controlled intake of polyphenols as drug active substances was extensively studied in the literature [93–99].

In recent years, NPs of many polyphenols were synthesized in studies conducted by Sahiner et al. and investigated as potential natural active agents/drugs by means of controlled release from directly produced polyphenolic particles [88, 100–107]. For example, poly(tannic acid) (p(TA)) particles were synthesized and their controlled degradation provided linear and continuous tannic acid release for up to 12 days [107]. In addition, these p(TA) particles were shown to be antibacterial, blood compatible, and biocompatible [107]. Furthermore, micro- and nano-p(TA) particles were prepared using many different biocompatible crosslinkers, and controlled tannic acid release was achieved successfully for up to 30 days [105]. It was reported that porous p(TA) particles possess greater antioxidant properties in comparison to nonporous particles [104]. In another study, donut-like tannic acid-iron nanocomplexes with magnetic and conductive properties were reported and their antioxidant properties were investigated [106]. P(TA) NPs were synthesized and their anti-inflammatory effects were investigated by Perelshtein et al. [108]. Other phenolic compounds of poly(rutin) and poly(quercetin) micro- and NPs were reported to have fluorescence, antioxidant, and antibacterial properties, and their potential use in the biomedical field as drug delivery systems was proven [88, 103]. The biocompatible, blood-compatible, and antioxidant properties of poly(rutin) and poly(quercetin) particles were reported with their controlled degradation by showing linear and continuous release of rutin for up to 24 h and quercetin

for up to 120 h [102]. Another polyphenolic NP derived from rosmarinic acid was synthesized, and their enzyme inhibitory effect, antioxidant properties, and rosmarinic acid release with degradation of particles were illustrated [101]. Poly (naringin) particles from polyphenol naringin were also reported to be low toxicity, antioxidant, and biodegradable material for biomedical applications [100].

16.2.4 Others Nanocarriers Derived from Small Biological Molecules

Some biopolymers can be prepared from natural monomers and used as drug delivery systems. For example, micro- and nanoparticles can be synthesized from amino acid, e.g., poly(L-Lysine) micro−/nanoparticles with tunable surface charge were reported to have blood compatible and biocompatible properties [109] and possess great potential for biomedical purposes. In another study, ε-Poly-L-Lysine/plasmid DNA nanoplexes were successfully synthesized for in vivo gene delivery [110]. Moreover, carbon dots from amino acid precursors, such as arginine, lysine, histidine, cysteine, and methionine, were demonstrated to be blood compatible and highly antimicrobial with excellent fluorescence properties, which enabled visualization during in vivo studies [111]. Other biomolecules, such as dopamine that is a neurotransmitter, were used in the corresponding nanoparticle preparation with porous morphology and chemically modifiable forms [112]. P(dopamine) particles were also shown to be blood compatible, biocompatible, and biodegradable, releasing dopamine molecules up to a few weeks. Also, sugar molecules such as sucrose, lactose, and maltitol were treated as monomers in the synthesis of their corresponding micro−/nanoparticles [113–115]. For example, the synthesized poly(sucrose) particles were found to be biocompatible and were used as drug delivery systems [113]. Can et al. also report micro−/nanoparticles from lactose molecules as poly(lactose) particles with chemically modifiable structure and tunable surface charges for potential drug delivery systems [114]. Moreover, poly (maltitol) particles were synthesized from maltitol and also shown to be modifiable with blood and biocompatible properties. Also, the prepared poly(maltitol) particles were used as drug delivery material, and increased amounts of drug can be loaded after modification [115].

16.3 Drug Loading and Release Studies for Biopolymeric Nanovehicles

Different drug loading techniques are used and have been improved for the efficiency of loading and release, as well as for the release time. Loading efficiency generally depends on the loading method employed and the physicochemical properties of the drug, as well as the natural properties of the nanovehicles. Three basic drug-loading techniques of physical loading by adsorption of drug from solution, chemical loading by covalent binding of the drug, and encapsulation/

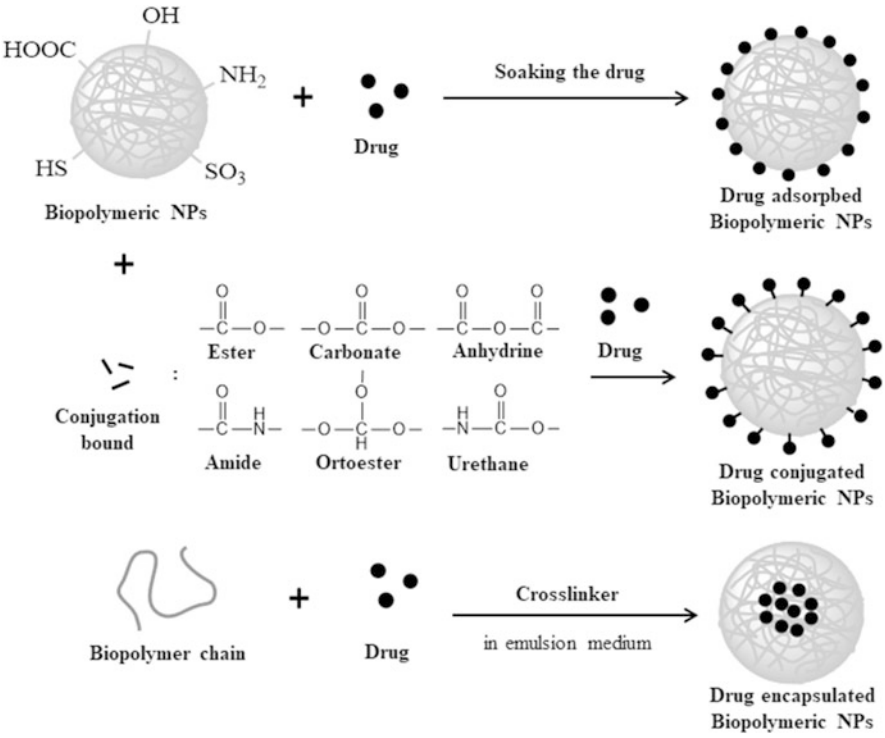

Fig. 16.2 Schematic representation of drug loading processes using drug adsorption, drug conjugation, and encapsulation methods for biopolymeric nanoparticles (NPs)

entrapment of the drug into nanovehicles are the most widely used methods presented in Fig. 16.2.

16.3.1 Physical Drug Loading and Release

The loading of the drug into vehicles physically is considered to be the most simple process and is generally employed for micro- and nanocarriers; however, it has several drawbacks, e.g., limitation of drug loading, lack of appropriate solvents for both drug and carriers, rapid release kinetics, and so on [116]. In this process, the active agent physically interacts with the carrier vehicles via physical adsorption from the corresponding drug solution as shown in Fig. 16.2. The physical interaction between the active agent and nanovehicle basically depends on the functional groups in the drug molecule and nanovehicle. Therefore, the shape, size, charge, and especially functionality play significant roles in the carriers, e.g., on polymeric matrices. Therefore, loading efficiency can be controlled by modification of the functional groups on the polymers by rendering different appropriate groups that can interact with the drug of interest. Among the carrier vehicles, hydrogel-based

nanocarriers are among the convenient materials for these modification reactions as they have –OH, –COOH, –SH, $–SO_3$, and -NH functional groups that can be readily converted into the desired ones. Also, among the biopolymers, carbohydrate-based particles are commonly used as drug carrying vehicles in many biomedical applications because of their renewable, biocompatible, nontoxic, and biodegradable properties and modifiable structure. For instance, Sahiner et al. (2017) reported that carrageenan particles could readily react with cationic modifying agents such as diethylenetriamine (DETA) and employed them in the adsorption of rosmarinic acid (RA) as a therapeutic agent. The amount of therapeutic agent, RA, loaded into the amine-modified forms of carrageenan particles increased tremendously, e.g., ~240 fold that is to 43.7 mg/g RA from 0.18 mg/g RA via physical adsorption process due to the high interaction affinity of e amine groups on the modified particles for RA molecules. Hence, the modified carrageenan particles resulted in sustainable and long-term drug release capability over 20 h. It was shown that a polymeric carrier can be used effectively in drug loading/releasing studies by adsorption processes by adapting chemical modification methods [117]. In another study, poly(dopamine) particles were prepared by self-oxidation polymerization of dopamine and new functionalities with negative and positive charges ($–SO_3^-$ and $–^+N(CH_3)_3$ groups) were utilized as the responsible functional groups on the carrier materials for drug delivery purposes [112]. The model drug acyclovir was loaded into bare and modified poly(dopamine) particles, and the loading and release capacities of modified poly(dopamine) particles (with chlorosulfonic acid) were increased nearly two-fold in comparison to the unmodified form of the particles because of the interaction of sulfonic acid groups on the modifying agents with amine groups of drug [112]. In addition to chemical modification of carriers, surface area, pore size, distribution, [118] and porosity [119, 120] of the carriers are important parameters to be considered to increase loading efficiency and release kinetics of the drugs. Thus, nanosized porous materials are anticipated to interact more with the drugs in comparison to the nonporous bigger size forms during physical adsorption processes of drugs. In the design of advanced drug delivery devices, chemical and morphological tunability of carriers is the crux for the physical loading process.

16.3.2 Chemical Drug Loading and Release

The design of polymer-drug conjugates was first reported by Helmut Ringsdorf in 1975 [121, 122]. The Ringsdorf model depends on chemical linking between drug and macromolecules through a labile bond like a hydrolyzable or biodegradable bond. The most commonly exploited biodegradable bonds are reported as carbonate, anhydride, ester, amide, orthoester, and urethane as demonstrated in Fig. 16.2. These reactive groups can be used to link to the end groups of the polymer chain or can be pendant groups on the side chain [123]. With time, the technology has gradually improved and is implemented for different types of nanocarrier systems. Among all techniques, the drug conjugation by chemical linkage method is the most widely preferred and useful technique because of superior properties such as high stability,

high carrier capacity, prolonged half-life, nontoxicity, nonimmunogenicity, and antigenicity. Additionally, the conjugation of drug to nanocarrier also improves the solubility, lowers the toxicity, protects from enzymatic degradation, and prevents or reduces aggregation of the drug [124, 125]. Chemical drug loading also allows controlled release kinetics from the carrier vehicles. Our group (2019) successfully demonstrated the usability of porous and degradable hyaluronic acid (HA) nanoparticles and copolymeric forms with sucrose (Suc), HA:Suc nanoparticles, as drug delivery systems with controllable antibiotic [126] or cancer drug [127] release capabilities. For this purpose, coumarin-derived cancer drugs or vancomycin as antibiotics was loaded into HA-based particles by absorption and conjugation processes. In the absorption process, the drugs dissolved in DMSO directly interacted with the carrier, whereas in the conjugation process, N,N´--carbonyldiimidazole (CDI) was used as coupling agent between carrier and drugs to attain covalent drug binding onto the carrier. As mentioned, generally a coupling agent, e.g., CDI, is used and reacts with the carboxylic acid groups on HA particles by the esterification reaction in DMSO at 25 °C for 1 h in the first step. Then, these HA particles were activated and reacted with hydroxyl groups on the drugs at 80 °C for 24 h. According to the study, vancomycin-conjugated HA particles were loaded with 349 ± 31 mg/g drug, whereas only 18 ± 3 mg/g of the same drug was loaded into HA particles via the physical absorption process. The association efficiency of the drug is notably enhanced to about 19-fold through drug conjugation to the polymeric network. In in vitro release of drug, approximately 11.4 ± 2.8 mg/g vancomycin antibiotic was quickly released over 8 h from the drug-absorbed HA particles, whereas the conjugated drugs showed longer and sustained release capabilities from the carrier matrix of almost 50.5 ± 4.2 mg/g vancomycin release within 168 h [126]. These results supported the view that drug loading capacity and release kinetics can be significantly improved by the conjugation process because of the chemical linkage of drug molecules into carrier systems. It was shown that sustainable drug release for a hydrophobic cancer drug was tenfold increased from degradable and porous HA-Suc nanoparticles by chemical conjugation compared to physical adsorption. The HA unit of the particles inherently has the ability to target the specific overexpressed receptors, i.e., CD44 receptors, on the cancer cells or cancer stem cells [128]. Alongside this, Suc sections of the particles have targeting effects against the tumor site because malignant cells tend to consume more energy because of their rapid proliferation, and this requirement can be met by Suc moieties within the carrier network. Furthermore, some studies reported that saccharides have affinity against binding proteins such as lectins contained in many cancer cells and show targeting ability to the cancer site [2, 127, 129]. Therefore, targeting and releasing the payload to cancer cells were achieved by HA and Suc moieties of the copolymeric HA:Suc particles, affording prolonged drug delivery for a hydrophilic cancer drug with the drug conjugation process. In a study conducted by Yan et al., the insulin-silk fibroin nanoparticle bioconjugates improved the stability of insulin in vitro [130]. In another study, two different drugs, curcumin and doxorubicin cancer drugs, were loaded into chitosan-legumain peptide NPs by two different loading methods: physical loading of curcumin and chemical loading of doxorubicin

to overcome multidrug resistance gene for cancer therapy. Legumain in the particle structure triggered codelivery of drugs to the cancer cells. This dual delivery nanocarrier system shows perfect inhibition effects on cancer cells due to long-term circulation time and targeting ability against the tumor site [131].

16.3.3 Loading by Encapsulation/Entrapment and Release

Various types of encapsulation techniques are reported in the literature such as emulsification, spray drying, extrusion, electrospinning, layer-by-layer deposition, and coacervation for nanosized encapsulated systems. Coaservation is the most suitable technique to prepare hydrogel-based nanocarriers via encapsulation, which is sometimes called entrapment [1]. In the encapsulation/entrapment or drug loading by coaservation process, the therapeutic agent can be embedded into the polymeric matrix during the production of the carrier vehicles, as illustrated in Fig. 16.2. To develop promising carrier materials via encapsulation methods, some important parameters for carrier vehicles should be considered beforehand, such as particle size and morphology, hydrophilicity, encapsulation efficiency, and the proposed application of the encapsulated compound [1]. Also, the stability of the drug with high self-life, bioavailability, and sustainable release kinetic could be provided through carrier systems. These types of techniques are generally preferred to carry hydrophobic drugs especially because of the higher and controlled loading capacity of the method. The entrapment efficiency of the drug basically depends on the solid-state drug solubility within the polymeric network, which is related to drug-nanovehicle interactions in the presence of functional groups such as carboxyl or ester [132]. Zhang et al. (2017) prepared dextrin nanogels targeting metastatic breast cancer as a cancer drug delivery material for cancer therapy. In this study, doxorubicin cancer drug was encapsulated in AMD3100-coated dextrin nanogels for targeting and releasing purposes. According to the in vitro and in vivo results, this drug-encapsulated AMD3100-coated dextrin nanogel system had high drug loading capacity and decreased the toxicity of the cancer drug by loading techniques with effective targeting capacity [133].

Most commonly, encapsulation methods are generally used in the delivery of biomacromolecules, such as DNA, nucleic acid, protein, and genes, as treatment agents for targeting cancer and gene therapy. In a study, carbohydrate derivate heparin NPs were prepared and modified with polyethyleneimine as protein and gene delivery in the growing of stem cells. Fibroblast growth factor (FGF) as protein was loaded into heparin NPs by encapsulation methods. Then, the in vivo release of FGF and specific genes was introduced to human endothelial progenitor cells and shown to enhance nanovascularization in an animal model [134]. In another study, chitosan-carrageenan NPs were used to encapsulate recombinant human erythropoietin and prepared NPs had 50% continuous in vitro release profile over a 2-week period. It was reported that the surface loading of the prepared NPs and the molecular weight of the chitosan reduced the rate of recombinant human erythropoietin release and, therefore, allowed longer release time [40]. To improve the drug release

profile of chitosan NPs used as drug delivery systems, chitosan, carrageenan, and alginate polysaccharides containing composite NPs were synthesized via electrostatic interactions and used for encapsulation of bovine serum albumin (BSA). The release profile of BSA encapsulated in composite NPs showed a first burst release in the first few hours of the experiment and subsequently a slow release profile over time [135].

The advantages of loading techniques for various biopolymeric nanocarriers and bioactive molecules are listed in Table 16.2.

These investigations support the fact that the drug loading techniques should depend on the types of nanoparticle systems and physicochemical properties of the therapeutic agent. To overcome the deficiencies of drug molecules such as unstable chemical structure, exposure to oxidative degradation, low solubility, fast release, and especially toxicity, the biopolymeric nanocarrier or NPs should be prepared by considering the most appropriate loading techniques such as adsorption, conjugation, and encapsulation.

16.4 Clinical Application and Safety of Therapeutic Carrier Nanovehicles

Therapeutic agent carrier nanovehicles are promising materials with superior pharmacokinetics and biodistribution compared to free drug molecules. Recently, researchers have focused on the development of biopolymeric nanocarriers for drug delivery applications because of their inherent biodegradable, biocompatible, nontoxic, high surface area, nonimmunogenic, and, especially, biomimetic properties [137].

Biopolymeric nanocarriers can enhance the residence time of the drug on the releasing surface, decrease drug loss, improve bioavailability, and improve cellular uptake. Nevertheless, many problems such as selective binding, targeted delivery, and, especially, toxicity need to be surmounted for use of these materials in the pharmacological industry [138]. For many years, many drug carriers composed of carbohydrates, proteins, polyphenols, and small biological molecules have been used for the design of novel nanovehicles and extensively studied for in vitro and in vivo drug delivery application to afford long-term, sustainable, and site-specific release capabilities and ensure higher safety for healthy cells and tissues [2, 26]. The advantages of using nanoparticles in therapeutic applications are listed in Table 16.3.

Chitosan is one of the most commonly employed carbohydrate-based biopolymers in drug delivery systems due to its exceptional properties of being nontoxic, nonimmunogenic, biocompatible, biodegradable, antimicrobial, on-site gelation, mucoadhesion, and permeability enhancement [118, 145]. Mitra et al. reported the effect of dextran-doxorubicin (DEX-Dox) conjugate, which was encapsulated with chitosan NPs as an antitumor and tumor-targeting carrier system against J774A.1 macrophage tumor cells in mice. Dox is a chemotherapy drug used to treat cancers, and one of the most serious side effects is cardiotoxicity. Conjugation of Dox with DEX minimizes the toxicity effect, and then encapsulation of the

Table 16.2 Advantages of loading techniques for various biopolymeric nanocarriers and bioactive molecules

Materials	Advantages	Loading process	Active agent and release time	Ref
Carrageenan particles	Modified with positively charged molecules to increase drug loading and release	Physical loading	Rosmarinic acid 20 h	[117]
Poly(dopamine) particles	Modified with anionic and cationic molecules to increase drug loading and release	Physical loading	Acyclovir 15 h	[112]
Degradable and porous hyaluronic acid particles	Enhancing the loading efficiency, sustainable and long-term releasing	Chemical loading	Vancomycin 168 h	[126]
Degradable and porous hyaluronic acid: Sucrose particles	Increasing the solubility of hydrophobic cancer drug and enhancing the release capacity, promoting of the drug targeting to the cancer site	Chemical loading	Curcumin derived cancer drug 150 h	[127]
Silk/fibroin particles	Improves the stability of drug	Chemical loading	Insulin	[130]
Chitosan-legumain particles	Legumain triggered dual delivery to the cancer cells by long-term circulation time with highest tumor targeting for multidrug resistance gene	Physical and chemical loading	Curcumin and doxorubicin 15–25 h	[131]
Dextrin particles	High drag loading, decreasing the cytotoxicity of drug	Encapsulation	Doxorubicin 10 h	[133]
Heparin particles	Modified with polyethleneimi to improve of gene loading capacity and encapsulated of fibroblast growth factor to improving of bioactivity	Encapsulation	Fibroblast growth factor 24 h	[134]
Chitosan/ carrageenan particles	Encapsulated human erythropoietin to allow longer release	Encapsulation	Human erythropoietin 330 h	[40]
Chitosan/ carrageenan/ alginat particles	Encapsulated the protein to more slowly release over time	Encapsulation	Bovine serum albumin	[135]
Human serum albumin particles	High loading efficiency., promote the encapsulation of photosensitizer IR780 iodide, linearly and long-term releasing to the specific cancer site	Encapsulation	Paclitaxel dimeric prodrug and IR780 dye 100 h	[136]

Table 16.3 The advantages of nanoparticle use in therapeutic applications

Materials	Advantage	Application	Ref
Dextran-dox conjugate encapsulated with chitosan NPs	Cardiotoxicity decreases and increases the therapeutic efficacy of cancer drug	Chemotherapy	[139]
HA coated pDNA based polyplexes	Good mobility in bovine vitrous humor, effectively taken up in vitro, improvies drug delivery efficiency, biocompatible for retinal delivery	Retinal target gene therapy	[140]
Insulin loaded Alginic acid NPs with nicotinamide	Mucoadhesive material, high insulin loading capacity, high pharmacological avability and bioavalibility and reduces of the glucose level	Insulin treatment in diabet	[141]
Timolol maleate loaded thiolated pectin NPs	Mucoadhesive effects, more corneal permeability through the excised goat cornea	Ocular delivery system	[142]
Paclitaxel dimeric drug and NIR dye loaded albumin NPs	Enhancestumor accumulation, reduces systemic toxicity and improvestherapeutic performances	Chemotherapy and photothermal therapy	[136]
Lectin conjugated gliadin NPs	Increases eradiction rates in vitro and higher cleaning efficiency in vivo *Helicobacter pylori* treatment	Drug delivery system	[143]
Poly(rutin) and poly (quercetin) NPs	Degradable nature and sustainable delivery, highest antioxidant capacity, good bioavalibility and inhibites of cancer cell lines	Therapeutic delivery	[102]
Transferin conjugated dextran-spermin NPs	Reduces drug loss and side effects, monitoring of drug, across the blood brain barrier, pH-triggered cellular uptake, targeting ability against brain tumor	Targeted drug transplantation along the blood-brain barrier	[144]
Dox loaded AMD3100 coated dextrin NPs	Directly taken up by the cancer cells by tumor targeting, inhibites of tumor metastasis and reduces cancer cell proliferation, greater cytotoxicity	Breast cancer targeting	[133]

conjugated drug-DEX molecules with chitosan NPs increases the efficacy of the drug. In this study, it was concluded that this antitumor carrier nanoparticle system enhanced survival time of the mice in comparison with drug conjugated and free drug forms, as well as improving the therapeutic efficiency of the drug *for* in vivo study [139]. The other important carbohydrate-based biopolymer is hyaluronic acid (HA), which is inherently nonthrombogenic, nonimmunogenic, bioactive, biodegradable, and also biocompatible in mammalian organisms. HA-based carrier materials have a wide range of applications encompassing ocular delivery systems and gene delivery systems for cancer therapy. HA has biological functions such as inflammatory responses to cancer metastasis and is specific to the CD44 receptor

[121, 146, 147]. There is growing interest in retinal gene therapy, which affects vision loss in many people, and the use of therapeutics in ocular delivery, which do not aggregate and remain mobile in the vitreous humor to reach the retina, necessitates specific carbohydrates such as HA. Martens et al. reported the intravitreal injection of therapeutic HA-coated nonviral gene complexes into the retina in the bovine eye for gene therapy. The results illustrated that this HA-coated nanocomplex system has good mobility in bovine vitreous humor, is effectively taken up in vitro, improves drug delivery efficiency, and is biocompatible for retinal delivery [140]. Devarajan developed bioadhesive insulin-loaded alginic acid NPs and forms containing nicotinamide to increase permeability of NPs for the treatment of diabetes. The results indicated that alginic acid NPs have the highest insulin loading capacity of 95%, and insulin-loaded alginic acid NPs with nicotinamide showed high pharmacological availability and bioavailability and importantly reduced the glucose level in a diabetic rat model [141]. In another study with pectin NPs, thiolated pectin NPs were synthesized using ionic crosslinks, e.g., magnesium chloride as ionic crosslinker, and timolol maleate was used as model drug for ocular delivery study. The thiolation of pectin was carried to improve the mucoadhesive properties of natural polymers. In the study, timolol maleate was loaded into the thiolated pectin NP system, providing significantly higher ex vivo corneal permeation of timolol maleate across the excised goat cornea than the conventional aqueous solution of timolol maleate, and it was concluded that thiolated pectin is a mucoadhesive polymer that can be used for ocular delivery of timolol maleate [142].

In a similar study, protein-derived carrier systems were promising candidates for drug and gene delivery in clinical applications related to their unique protein structure. These structures provide site-specific drug conjugation and targeting ability [3]. Patil et al. (2019) reported human serum albumin (HSA) nanoparticles that contained paclitaxel cancer drug and photosensitizer IR780 iodide as near infrared (NIR) dye in the core of the particles as seen in Fig. 16.3a**.** In the particle design, dimeric paclitaxel was prepared by using thioether bridges (PTX_2-S) to enhance the loading efficiency and stability of drugs and created tumor redox heterogeneity-triggered drug release as well as facilitated IR780 iodide to combine chemo−/photothermal therapy [136]. The results for in vivo antitumor efficacy and the biosafety of cancer drug and NIR dye-loaded albumin NPs (HSA(S-Cy) are demonstrated in Fig. 16.3b by the NIRF images of tumor-bearing mice after intravenous administration with HSA(S-Cy) and photos of excised tumors. As can be seen in the NIR fluorescence signal, HSA(S-Cy) NPs have good biodistribution and enhanced accumulation at the tumor site in 36 h continuing up to 96 h and showed significant in vivo photothermal activity. It was reported that these albumin-based carrier systems are promising materials with enhanced tumor accumulation, reduced systemic toxicity, improved therapeutic performances, and more safety for in vivo applications in chemo−/photothermal therapy with no significant damage to the heart, liver, spleen, lungs, and kidneys after treatment [136].

In a different study, a carrier system for eradication therapy of *Helicobacter Pylori* (*H. Pylori*) was developed and the method significantly reduced the effective dose of triple therapy with amoxicillin, clarithromycin, and omeprazole drugs and

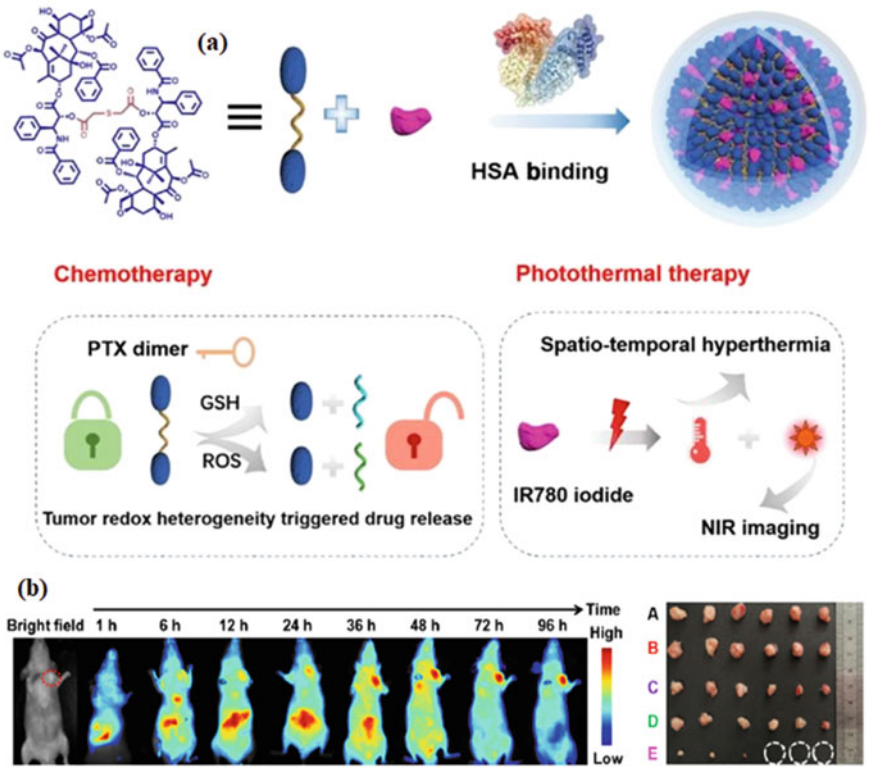

Fig. 16.3 (**a**) Schematic illustration of paclitaxel dimeric prodrug PTX2-S and IR780 loaded into albumin NPs (HSA(S-Cy)) for combined chemo−/photothermal therapy. (**b**) In vivo antitumor efficacy in the NIRF images of tumor-bearing mice after intravenous administration with HSA (S-Cy) and photos of excised tumors. From up to down: PBS, PBS under irradiation (L+), HSA(S), HSA(S-Cy), and HSA(S-Cy) under irradiation (L+), adopted from ref. [136]

prevented bacteria from gaining antibiotic resistance [143]. For this purpose, proteinic-based gliadin NPs and lectin-conjugated gliadin NPs were synthesized for triple drug treatment, and amoxicillin, clarithromycin, and omeprazole drugs were loaded by encapsulation. The reason for lectin conjugation is the binding ability of this protein against carbohydrates on the bacterial surface. In vitro antibacterial study of triple-therapy lectin-conjugated gliadin NPs showed higher eradication rates than triple-therapy gliadin NPs and bare triple therapy. Furthermore, targeted lectin-conjugated gliadin NP systems exhibited higher in vivo cleaning efficiency than nonconjugated gliadin NPs and bare drugs. These results clearly state that the targeted lectin-conjugated gliadin NP system with triple therapy causes maximum bacterial destruction from the intestine by selective release of these triple drugs. The mucoadhesive properties of the systems help keep them in contact with the mucosal layer, and the specific ligand (lectin) provides interaction with *H. Pylori*, which demonstrates the potential removal of bacteria in the intestine.

Therefore, it was stated that the targeted lectin-conjugated gliadin NPs with triple therapy lead to a higher drug concentration at the site of action due to their superior cellular uptake capacity, thereby maximizing the therapeutic index. Thus, injection with the targeted lectin-conjugated gliadin NPs with triple therapy can be more effective for the elimination of *H. Pylori* [143].

Rutin (RT) and quercetin (QC) are well-known phenolic biomolecules with various biological properties such as antimicrobial, anti-inflammatory, antiallergic, antioxidant, anticancer, antidepressant, and antidiabetic features. It was reported that crosslinked poly(rutin) and poly(quercetin) NPs can be readily prepared and used directly as therapeutic agent release material due to their degradability in physiological conditions, e.g., pH-triggered release can provide long-term RT or QC release [102, 103]. These nanoparticles formulated from phenolic materials are promising candidates in cancer therapy with excellent antioxidant ability and highest inhibition effects against A549 cancer cells, in addition to hemocompatible nature and noncytotoxic effects [102].

Targeting ability of the vehicles is very important as it provides target specific release of the active agents to the disease sites and avoids the site effects of the toxic drugs on healthy tissues. Polymeric nanoparticles could be decorated by targeting agents for specific delivery. To create targeted release systems, different agents such as antibodies, peptides, small molecules, aptamers, designed proteins, and nucleic acid can be attached to carrier materials by chemical bonding [118, 148, 149]. These targeting moieties are conjugated with polymeric nanoparticles by using chemical linkers to obtain bionanoconjugates for site-specific delivery. Poly(ethylene) glycol (PEG) is a well-known linker for the bioconjugation process for these molecules owing to various customizable end functional groups and tunable polymeric chain lengths. These types of bionanoconjugates have many advantages and disadvantages based on design techniques and materials used [148]. In one study, targeted drug carrier nanovehicles derived from dextran-spermine biopolymers were synthesized by conjugation with transferrin as a targeting agent and capecitabine as a cancer drug for treatment of brain tumors. This system is a promising material for the treatment of brain tumor with increased drug transport across the blood brain barrier and pH-triggered cellular uptake. Therefore, these types of biopolymeric nanocarrier systems can be used for in vivo diagnosis and tumor treatment applications because of outstanding properties including site-specific delivery to the brain with targeting ability and imaging and monitoring of therapeutic effect of drug due to magnetic properties. The use of this targeted drug delivery system in the treatment of cancerous cells can reduce drug loss and side effects [144]. In a study, AMD3100-coated dextrin nanogels were synthesized and loaded with Dox, a cancer drug, for targeted drug release in metastatic breast cancer. CXCR4 receptors and their cognate ligand SDF-1 are regulated at the metastasis site of breast cancer. The inhibition of the interaction of these molecules by receptor antagonists such as AMD3100 could inhibit tumor growth, induce apoptosis, and prevent metastatic spread. These studies revealed that the use of Dox-loaded AMD3100-coated dextrin nanogels could directly be taken up by the cancer cells due to tumor targeting with AMD3100

agents, leading to the inhibition of tumor metastasis and reduction of cancer cell proliferation with greater cytotoxicity for breast cancer treatments [133].

16.5 Concluding Remark

This chapter highlights the preparation of tunable biopolymeric drug carrier nanovehicles by utilizing different methods in the synthesis process and a variety of drug loading techniques with recent advantages of these nanocarriers for delivery applications with clinical evidence. Recently, biopolymers have gained tremendous interest for the development of nanocarrier systems because of their inherent features, e.g., mucoadhesive, nontoxic, nonimmunogenic, biocompatible, biodegradable, and biomimetic properties and more importantly tunable functionality for drug delivery in biological systems. For example, these carriers can enhance drug bioavailability, decrease drug side effects, improve solubility, protect the drug molecules from harsh environments, enable higher drug efficacy, increase the residence time of drugs, and so on. Thus, various studies on the design of biopolymeric carrier systems for the regulation of pharmacokinetic and pharmacodynamic abilities of therapeutic agents through tunable morphology and chemical structures will be continuously performed. Especially, due to nanometer size and modifiable surface characteristics, these carriers can directly cross the cell membrane and offer good biodistribution, also promoting drug targeting of specific sites. In order to produce effective and targeted drug delivery systems, numerous techniques such as modification, coating, marking, or conjugating with targeting agents could be performed. The therapeutic agent loading techniques using physical adsorption, chemical linkage, and encapsulation methods also affect the loading efficacy and release time, as well as stability, solubility, toxicity, and bioactivity of delivery systems. To summarize, the use of biopolymer-based nanoparticles as nanocarriers in controlled delivery systems is adaptable, safe, and feasible for therapeutic and clinical applications due to (1) control of particle size, e.g., nanometer range to facilitate cellular uptake and phagocyte system, (2) ability to modify and conjugate to increase the efficiency of the nanocarrier system, such as greater drug loading/release, and (3) targetability and on-demand release capability via smarter stimuli-responsive nanovehicles. These promising drug carrier biopolymeric nanovehicles are becoming indispensable materials for a wide range of biomedicine utilizations, e.g., for ocular delivery, gene therapy, diabetic treatment, and especially cancer treatments with chemotherapy.

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Nanomedicine for Challenging Solid Tumors: Recent Trends and Future Ahead

17

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Abstract

From the last two decades, many technological inventions and high-end equipment are available for cancer detection, diagnosis, and treatment modalities. However, the complete cure for cancer is not possible till date. Effectiveness of the treatment depends on the tumor size, lymph node involvement, and distant metastasis. Recent chemotherapeutic drugs have improved therapy but the problems of inability to reach tumor site, crossing of BBB at effective doses, toxicity, and poor pharmacokinetics still persists. The advanced-stage tumors are aggressive and existing chemotherapeutics are not effective, possess side effects, and recovery is not possible. The mode of management for metastatic tumors is fairly dependent on the available treatments; however, in solid tumors, they are much more dependent on the type of biomarkers and their expression pattern which can serve as diagnostic, predictive, and prognostic markers. The novel nano approaches used in treatment modalities for solid tumors are also discussed.

Almost all existing chemotherapeutic drugs are administered either in the adjuvant and neoadjuvant settings depending on the pathological staging and tumor burden as per the National Comprehensive Cancer Network (NCCN) guidelines. This chapter gives an insight into the pathophysiology, signaling pathway of solid tumors of lung, head & neck, and breast cancer, with the recent research utilizing nanoformulations to treat respective organ cancer is also discussed. Finally, the ways to overcome hurdles persisting in solid tumors and nanotechnology-based approach are discussed.

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M. K. Das, Y. V Pathak (eds.), *Nano Medicine and Nano Safety*,
https://doi.org/10.1007/978-981-15-6255-6_17

Keywords

Breast cancer · Nanotechnology · Nanodevices · Nanomaterials · Nanoformulations

17.1 Introduction

Cancer is a disorder of cell cycle regulation. The mechanics of the molecular pathways that control cell cycle are often mutated and disturbed in neoplasia. Cell cycle progression of mutated cell leading to uncontrolled cell proliferation is critically dependent on the cumulative mitogenic stimuli received from diverse sources. The changes at cellular, genetic, and epigenetic levels cause abnormal cell division leading to carcinogenesis. The programmed cell death, apoptosis, is disturbed when there are mutations in DNA and other epimutations leading to uncontrolled cell division. Only certain mutations are responsible for carcinogenesis. Apart from genetic and epigenetic causes, DNA damage is one of the primary causes of cancer. Factors causing DNA damage can be endogenous (reactive oxygen species) or exogenous like tobacco consumption, smoking, alcohol consumption, environmental chemicals, UV solar radiation, human papillomavirus, etc. Such agents that can alter the DNA or other related systems in the body to cause cancer are called initiators of cancer development. This is followed by the influence of promoters which help in cell proliferation. This further leads to progression wherein the benign tumor develops characteristics favorable for malignancy.

17.1.1 Stages of Tumor Development

Carcinoma is a multistage process with the following steps:

1. Hyperplasia: The mutated cell divides in an uncontrolled manner
2. Dysplasia: The genetic changes in the hyperplastic cells lead to abnormal growth and disorganization
3. Carcinoma in situ: Here. the altered cells become de-differentiated or anaplastic. At this stage, the altered cells remain confined to their initial location without invasion to other tissues
4. Malignant tumors: The cells invade to neighboring tissues, i.e., metastasize to healthy tissues.

This chapter gives a detailed insight into carcinogenesis of breast, lung. and oral and lip cavity including nanoparticles used to treat them. Finally, it gives an insight into the use of novel nano approaches to tackle various hurdles presented by tumors.

Solid tumor is an abnormal mass of tissue that usually does not contain cysts or liquid areas. Solid tumors may be benign (not cancer), or malignant (cancer). Different types of solid tumors are named for the type of cells that form them.

Uncontrolled growth of lung cells causes tumor that reduces a person's ability to breathe. Lung cancer is mostly categorized as Non-small cell lung cancer (NSCLC) and Small cell lung cancer (SCLC). NSCLC is the most common and is classified into lung adenocarcinomas, squamous cell carcinomas, and large cell carcinomas. SCLC is the second most common form and is termed based on the appearance of the cells under the microscope. Similarly, Head and neck cancer remains the sixth most commonly occurring cancer globally having high mortality rate especially in developing countries. Squamous cell carcinoma is the general form of head and neck cancer affecting oral cavity, larynx, paranasal sinuses, pharynx, and nasal cavity. On the other hand, Breast cancer is the most frequently occurring malignancy in women having an incline incident rate. Due to its highly heterogeneous characteristics, this cancer type has shown to have a few cases where there is slow growth with an excellent prognosis, and others just having aggressive tumors [1]. Breast cancer is classified mainly into three types based upon the presence or absence of different markers mainly in the breast cancer cells. *Hormone receptor-positive* breast cancer has either the estrogen receptor (ER) or progesterone receptor (PR) protein in the cancerous cells and this type of cancer makes up to 70% of all breast cancer. *ERBB2-positive* (also known as HER2-positive) breast cancer has high levels of ERBB2 protein and is reported to make up to 15% to 20% of breast cancer. *Triple-negative* breast cancer lacks all the three ER, PR, or ERBB2 protein and generally constitute to 15% of breast cancer cases [2].

17.1.2 Epidemiology

Briefly, epidemiology of different cancers is such that in India, considering both the sexes, widely prevalent cancer includes those of breast, lip and oral cavity, and lungs. The statistics of mortality are no different with the highest mortality observed in the order of breast, lip and oral cavity, and lungs (refer Table 17.1).

From the last century, lung carcinoma has progressed from a rare and obscure disease to the utmost common cancer in the world and the most prevailing cause of death from cancer. Lung cancer is a foremost reason of death worldwide; according to WHO, in 2018, 2.09 million new cases were estimated. In men, lung cancer is the most frequently occurring cancer, and in women, it is the third most frequently occurring cancer. 1,368,524 new cases of lung cancer in men were diagnosed and 725,352 in women were reported [American Institute for Cancer Research]. Risk of

Table 17.1 Statistics of mortality as per WHO, Globocan, 2018

Cancer	Rank	Incidence (%)	Mortality (%)
Breast	1	15.46	12.11
Lip and oral cavity	2	11.42	10.09
Lungs	4- Incidence 3- Mortality	6.45	8.82

evolving lung cancer in women is about 1 in 17 and for men, the chance is about 1 in 15.

Worldwide, head and neck cancer estimate for >650,000 cases and 330,000 deaths yearly. Head and neck cancer estimate for 3% of malignancy in the US, with almost 10,800 Americans dying and 53,000 emerging head and neck cancer every year from the disease. The occurrence of head and neck cancer is twofold less in females than males with ratio covering from 4:1 to 2:1. The prevalence rate of head and neck cancer in males is twenty per one lakh in regions of Hong Kong, Brazil, the Indian subcontinent, Eastern and Central Europe, Spain, France, and Italy, and among African Americans in the US. In India, tongue and mouth cancers are common; in Hong Kong, nasopharyngeal cancer is common and laryngeal/pharyngeal cancers are found more common in different populations. In 2018, about 120,000 new cases of OCC were observed in India, out of which 72,000 patients died.

Around 1 million cases have been reported across the globe every year signifying that breast cancer is rapidly growing and seems to be the most common malignancy. More than 14,000 deaths have been reported each year and it has been more specifically observed among women of age group of 50–64 [3]. Due to a high and increased incident rate as well as mortality rate, breast cancers have now become the most common malignancy of all cancer types. A huge increased trend of breast cancer cases has been observed in several Asian countries, parts of South America, and Africa [4]. It is predicted that the occurrence of this cancer type might reach up to approximately 3.2 million new cases over a period of 20–30 years.

17.1.3 Risk Factors

Genetic, environmental, and behavioral are the major risk factors for the development of lung cancer. For lung cancer, behavioral risk factors consist of smoking and tobacco. The solitary greatest risk factor in the growth of lung cancer is the use of tobacco cigarettes, with up to 90% of lung cancers attributed to smoking [5].

The foremost risk factor responsible for HNSCC comprises consumption of tobacco (both 'smokeless' and smoked), more consumption of any of the alcoholic beverage, the mastication of areca nut, HPV (human papillomavirus) (HPV-18 and HPV-16), and any human herpesviruses (HHVs) (HHV-4). Nowadays, it has been observed that the patients analyzed with oropharyngeal cancer are young and those who have never smoked or consumed any products like tobacco. One of the major risk factors for cancer of lip, specifically of the lower lip is ultraviolet radiation. Radon, asbestos, pollution, air quality, infection, and inflammation in the lungs are the environmental risk factors which may lead to lung cancer. Genetic risk factors are also responsible for the growth of lung cancer. GWAS (Genome-Wide Association Studies) related chromosome regions 15q25-26, 6q21, and 5p15 with augmented risk for lung cancer. Adenocarcinomas mutations like EGFR and EML4-ALK are related in non-smokers.

In recent years, the percentage of lung cancer in non-smoker (LCINS) has augmented, even after monitoring for race or ethnicity and gender. Worldwide, it is estimated that 25% of lung cancer patients are non-smokers. Environmental risk factors are stated to play a major role in LCINS, comprising smoke exposure, occupational exposures, environmental particulate matter, radon, and indoor air pollution. Consequent bacterial load in mouth and poor oral hygiene are emerging as noteworthy risk factors for oral cancer. Additionally, epidemiological studies showed that industrial employment along with occupational exposures to asbestos, wood dust, solvents or acid mists, and textiles and leather manufacturing are related with an amplified risk of HNSCC.

Various studies have been reported earlier stating that nearly 20–30% of breast cancer cases have been associated with the occurrence of various risk factors that contribute majorly to this disease. These risk factors have been categorized mainly into two: (1) Intrinsic Factors and (2) Extrinsic Factors. The intrinsic risk factors mainly involve age, sex, race, and genetic makeup of an individual. The second set of risk factors comprise mainly of the diet, lifestyle, and other environmental factors [6]. Moreover, it has also been reported that breast density also plays a major role in conferring the risk of this disease. Increase in the density contributes to a major risk as compared to patients having low breast density [7].

17.1.4 Pathophysiology

EGFR (Epidermal Growth Factor Receptor) is a prototypical member of a TKs receptor (tyrosine kinase) family, which consists of four receptors: EGFR (HER1, ERBB1), ERBB2 (Neu, HER2), ERBB3 (HER3), and ERBB4 (HER4). The epidermal growth factor and transforming growth factor are the distinctly recognized targets of EGF receptor [93]. EGFR generates a dimer with another EGFR once it binds with its ligands, and causes autophosphorylation. This autophosphorylation further activates intracellular signalling events comprising, Ras/Raf/mitogen activated protein kinase (MAPK), protein kinase C (PKC) pathways, signal transducer and activator of transcription (STAT), mammalian target of rapamycin (mTOR), Janus kinase (Jak), phosphatidylinositol-3kinase (PI3K), AKT [94] enabling cell growth, survival, angiogenesis and metastasis [95]. EGFR plays a vital role in squamous cells and transmitting the message via the Ras–MAPK, PI3K–PTEN–AKT and phospholipase C pathways. Intranuclear EGFR stimulates CCND1, connecting progression of cell cycle to mitogen stimulation.

EGFR downregulation has been observed in multiple tumor types, including lung cancer, breast cancer, and HNSCC. Signaling pathway of EGFR genes has been known to be mutated in HNSCC and lung cancer. It was evaluated that recurrent EGFR gene overexpression is reported in about 90% of HNSCC tumors, 62% in squamous cell of NSCLCs, and in subtypes of ADC.

Overexpression of EGFR is frequently related with adverse prognosis. On the basis of geographical location, KRAS and EGFR mutations have been found in ten to 30% of NSCLCs.

It has been found that the EGFR has been directly linked to phosphorylation of stearoylCoA desaturase-1 (SCD1), thereby in the upregulation of monounsaturated fatty acid production. Owing to its significant role in cancer, several EGFR-targeted therapies have also been developed. Further challenge that lies in the way is to understand more EGFR-targeted therapies and how it interplays membrane trafficking for cancer treatment. This may further help to increase the efficacy and overcome or delay the occurrence of resistance to such treatment.

RAS/RAF/MEK Pathway

In the RAS/RAF/MEK pathway, the *RAS* genes activating oncogenic mutations are common in various human cancers, along with lung cancer. Not only in SCLCs but in 10–15% of NSCLCs mainly in adenocarcinoma (20–30%), *RAS* mutations are found. The mutations arise at various hot spots in genes affecting 12, 13, and 61 codons which activate intrinsic GTPase action. In lung cancer, about 90% of the *RAS* mutations are found to be *KRAS* mutations. Multiple number of drugs have been developed that aim to target various aspects of RAS metabolism and function. Downstream effecter of the RAS pathway is a BRAF protein threonine/serine kinase. *BRAF* mutations arise rarely in lung cancers (3% of NSCLCs), but frequently in melanoma (70%) [8].

The mitogen-activated protein kinases (MAPK) pathway, involving a cascade of protein kinases composed of RAS, RAF, mitogen-activated protein/extracellular signal-regulated kinase (MEK), is one of the best-characterized signaling cascades that regulate a variety of normal cellular functions. Targeting the MAPK pathway has gained insight into the field of cancer therapy. ERK inhibitors have also been reported to become the optimal target to overcome acquired drug resistance in the RAF-MEK-ERK pathway [9]. Specifically, the MEK1 and MEK2 inhibitors, homologous in nature and because of their dual specificity, sharing ERK as their only known catalytic substrate, makes MEK a promising target for cancer drug development in future [10]. The identification of relevant biomarkers specifically of MEK inhibitor responses remains a major challenge, and also seems to be a promising approach for tailoring individualized therapies.

ALK fusion (Anaplastic lymphoma kinase fusion proteins) may play a role in stimulating RAS. Thus, it is undesirably related with the existence of EGFR or KRAS mutations, and might favor histology of ADC and non-smoker status. Seven percent of NSCLCs results in the initiation of an effective ALK fusion protein. Augmentation of any of the *MYC* family member occurs in SCLCs of 18–31% and in NSCLCs of 8–20%. Amplification of MYC occurs in both NSCLC and SCLC; however, amplifications of *MYCL* and *MYCN* always arise in SCLC [11].

The p53 Pathway

p53 functions include DNA damage, as a sensor of numerous stress signals, hypoxia, and activation of oncogene. This transcription factor has downstream target genes involving cell cycle arrests (G1 and G2), DNA repair or apoptosis, and upstream regulatory genes, including p14 and Mdm2. In lung cancer, p53 is the utmost commonly mutated gene. In DNA-binding domain, deactivating mutations are exhibited in 90% of SCLC and 50% of NSCLC. Amplification of Mdm2 is infrequent (6% of NSCLC), though at the level of protein and mRNA overexpression is recurrent, occurring in 30% of both NSCLC and SCLC [12]. Over 80% of HPV-ve HNSCCs have mutations of p53 which results in the loss of function. Overexpression or amplification of any other family member of p53, TP63, is evaluated in about 80% of HNSCCs [13]. The p53 downstream pathway comprises of p53 transcription target genes, which play vital roles in the death receptor pathway and the mitochondrial apoptotic pathway: Bcl-2 the anti-apoptotic gene and Bax the pro-apoptotic gene are upregulated and downregulated by p53, respectively; TRAIL-death receptor 5 (tumor necrosis factor receptor-like apoptosis inducing ligand receptor DR5) is included in the family of tumor necrosis factor receptor. In lung cancer, these 4 factors are mainly downregulated, which results in outcomes of robust resistance in mitochondrial and death receptor-induced apoptosis.

Currently, there have been least six different approaches reported to treat these cancer cells with mutant or wild-type form of the tumor suppressor gene. Immunotherapy and virus-gene-mediated therapy have gained much insight into the current scenario [14]. Loss of p53 suppressor gene can also facilitate switching mechanism with regard to resistance during any anti-androgen therapy in prostate cancer too. Thus, this tumor suppressor gene p53 pathway considered as the bridging point between the apoptotic mechanisms itself confers a novel therapeutic approach in treating cancer.

PI-3K/AKT Pathway

The phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) signaling pathway is known to be involved in the regulation of various multiple cellular physiological processes. One of the important approaches in recent times for the treatment of tumors is to rationally design a specific drug using molecular targets in the PI3K/AKT signaling pathway [15]. In the current status for the treatment for the PI3K/AKT signaling pathway, various mTOR blockers have been studied extensively. Activation of such PI3K/mTOR proves to be a common mediator of resistance to various anticancer agents that includes conventional chemotherapy. The recent development of CRISPR/Cas9 technology has also been shown to play a role for the regulation of PI-3K/AKT pathway. In spite of its role in cancer biology, PI3Ks have also been found to play a major role in the regulation of cellular metabolism and in immune system functions [16]. PI3K inhibitors also have gained their importance with CDK4/6 inhibitors in breast cancer in recent times.

PI 6INK4/Cyclin Pathway

Preclinical and clinical data suggest that CDK 4/6 inhibitors have significant potential in breast cancer treatment. CDK 4/6 inhibitors have shown to have antitumor activity, and ongoing studies are exploring the combination of these agents with existing endocrine treatments and with inhibitors of various upstream and downstream signaling molecules. Reports claim that combining PI3K inhibitor with an antiestrogen resulted in much enhanced, robust tumor regression during the preclinical trial. Although endocrine therapy is primarily being focused as one of the main treatments for ER+ breast cancer, there is an increased requirement for novel treatment approaches due to the de novo and acquired resistance that mainly occurs in many patients with the progression of this disease [17]. However, identifying the optimal treatment combinations and refining the suitable biomarkers that will be beneficiary to the patient response for the treatment needs to be explored and still remain the major challenge.

In p16INK4/cyclin D1/Rb pathway, the first tumor suppressor gene to be known is the Rb gene, and is the downstream effector of G1 arrest mediated by p53 through initiation of the CDK inhibitor p21 (cyclin-dependent kinase). *Rb protein loss arises in 90% and 70% of high-grade neuroendocrine SCLC and LCNEC tumors, and in 15% of NSCLC. Rb inactivation functions by NSCLC phosphorylation is mainly attained by the p16 CDK expression loss or overexpression of cyclins E and D1. RB is mainly lost in SCLC, but cyclin D1 or p16INK4 alterations are infrequent [12].

MYC

The *MYC* gene family encodes three nuclear phosphoproteins (MYC, MYCN, and MYCL), which heterodimerize with MAX proteins and function as transcription factors for genes in a variety of cellular processes, including cell growth, cell proliferation, and apoptosis. Myc has been considered as the most common deregulated oncoproteins in all cancer types [8]. Certain drug-eluting stents, which released Myc inhibitors continuously for short distances, seem to be promising mean by which high-local compound concentrations could be achieved, thus overcoming some of the pharmacologic drug-related shortcomings [18]. Understanding how Myc reprograms itself into various metabolic pathways seems to be a promising approach to understand the mechanism of altered oncoproteins. Deregulating MYC in cancer has also a great potential as a therapeutic approach for studying the inhibition of such potent oncogene in cancer [19]. Also, it has been reported that the MYC protein expression interplays with the B-cells and B-cell lymphoma, thereby providing evidence that the MYC-responsive machinery might have significance with relation to the modifications in B-cell lymphoma [20]. Amplification of one member of the *MYC* family occurs in 18% to 31% of SCLCs and in 8–20% of NSCLCs. *MYC* amplification occurs in both SCLC and NSCLC, whereas *MYCN* and *MYCL* amplifications nearly always occur in SCLC.

TGFβ (Transforming Growth Factor-β) Pathway
TGFβ (transforming growth factor-β) pathway is a significant and inhibitory growth factor pathway associated with lung cancer and HNSCC. TGFβ1 functions through the TGFβ receptors, and the signal is transduced by SMAD3 and SMAD2 phosphorylation, which, together with SMAD4, monitors the target genes transcription. Mutations with SMAD2, SMAD4, and SMAD3 have also been reported in HNSCC and NSCLC [21].

Notch Signaling Pathway
The Notch signaling pathway is associated with the regulation of cell cycle exit, survival, and self-renewal capacity. In mammals, this signaling is comprised of 4 receptor isoform (Notch1, Notch2, Notch3, Notch4) and five ligands: DLL1 (Delta-like 1), DLL3 (Delta-like 3), DLL4 (Delta-like 4), Jagged 1 and 2. The pathway is initiated when any cell expressing a notch receptor interacts with the appropriate ligand expressing on another cell. The Notch signaling pathway is activated in lung cancer and HNSCC [22]. Inhibition of this pathway is a potential therapeutic method of lung cancer and HNSCC. In HPV +ve, HPV-ve HNSCCs and lung cancer, abnormal regulation of STAT (the signal transducer and activator of transcription) family has been stated. STAT3, STAT5, and STAT6 are overexpressed in NSCLC and upregulation of STAT3 and its gene targets contribute to the malignancy of HNSCCs, resistance to chemotherapy, radiotherapy, and EGFR-targeted therapy.

17.2 Treatment

The standard common treatments available for treating cancer are surgery, radiation, and chemotherapy. For better efficacy, different combinations of treatments are used depending on the stage of cancer and its primary site. Classic open surgery or minimally invasive procedures like transoral robotic surgery (TORS) or laser surgery are employed depending on the anatomy and tumor characteristics. All of these treatments are associated with toxicity leading to some degree of late organ dysfunction that may be substantial whether a surgical or nonsurgical approach is taken. Immunotherapy is used as novel therapy for treating cancer and also called as biologic therapy intended to boost the natural defense of body to fight cancer. Adoptive cell therapy, vaccines, immune checkpoint inhibitors, and monoclonal antibodies are included in immunotherapy [23].

Immune Checkpoint Inhibitors Programmed Cell Death protein-1 (PD-1) is an immunoreceptor and a negative regulator of the immune response. Interaction between PD-1 and its ligands, PD-L1 and PD-L2, on tumor cells leads to downregulation of T-cell response in the tumor microenvironment. Cytotoxic T lymphocyte-associated protein 4 (CTLA-4) is expressed mainly on T cells (CD4+, helper and CD8+, killer T cells) with some expression in other immune cells including B lymphocytes and fibroblasts. There is an overexpression of PD1/PD-

L1 and CTLA-4 in lung and head and neck cancer. Nivolumab and Pembrolizumab are two FDA-approved PD-1 blocking antibodies for lung cancer and head and neck cancer. Some of the FDA-approved PD-L1 blocking antibodies are Atezolizumab, Durvalumab, and Avelumab. Ipilimumab and Tremelimumab are two most well-known CTLA-4 blocking antibodies. Clinical trials for CTLA-4 inhibitors, Ipilimumab and Tremelimumab, are still ongoing because of their promising effect on NSCLC [23].

Targeted Antibodies

Anti-Epidermal Growth Factor Receptor (EGFR) Antibodies

Cetuximab inhibits the phosphorylation of EGFR and transmission of signals to the cell, due to it preventing the attachment of other ligands via its direct binding to the receptor.

It is noteworthy that despite the high EGFR expression in tumor cells of HNSCC, the response rate of cetuximab monotherapy ranges between 10 and 15% in the treatment of recurrent or metastatic stage of the disease.

Erlotinib, Gefitinib, and Afatinib are approved as first-line treatments for targetable EGFR alterations.

Panitumumab is a completely human EGFR monoclonal antibody. Other human EGFR antibodies include zalutumumab and nimotuzumab. Nimotuzumab has a promising effect in patients with advanced HNSCC.

Some monoclonal antibodies, such as Nimotuzumab and Ficlatuzumab, have shown efficacy in combination with chemotherapy and radiotherapy. Nimotuzumab (h-R3) is a humanized monoclonal antibody to EGFR, which binds to this receptor and inhibits binding of EGFR to cancer cells. It is in clinical trials for NSCLC. Ficlatuzumab is a monoclonal antibody (IgG1) humanized and directed to hepatocyte mesenchymal intraepithelial (HGF) and is currently under study for NSCLC and its combination with Nimutuzumab has revealed signficant efficacy together with chemotherapy and radiotherapy [24].

Anti-Vascular Endothelial Growth Factor (VEGF) Antibodies

The most common molecules used in targeted therapies are Bevacizumab, Sorafenib, Sunitinib, and Vandetanib. Bevacizumab is a humanized monoclonal antibody that targets VEGF-A. The antitumor therapy uses its ability to inhibit angiogenesis and to increase the delivery of chemotherapeutic agents to tumor cells by reducing microvascular permeability and reducing the pressure inside the tumor. Bevacizumab was approved by the FDA for treatment of advanced cancer types, including colon cancer, kidney cancer, cervical cancer, and brain cancer. Preclinical trials reported that Bevacizumab has the ability to increase the sensitivity of HNSCC to radiotherapy.

A biomarker study indicates that bevacizumab also improved tumor vasculature and blood perfusion in NSCLC patients.

Other VEGF inhibitors that are evaluated in clinical trials for the treatment of HNSCC include Pazopanib, Axitinib, Nilotinib, and Linifanib [24].

ALK Inhibitors

Crizotinib is a multi-targeted TKI that is active against ALK, ROS1, and MET. It has been approved as a first-line treatment for ALK+ or ROS1+ NSCLC. Ceritinib and Alectinib are second-generation ALK TKIs approved for Crizotinib-resistant or intolerant cases.

Brigatinib was given to 222 patients with Crizotinib refractory, ALK+ NSCLC under a phase II study (ALTA, NCT02094573).

Lorlatinib (PF-06463922) was tested in phase I/II study (NCT01970865) of ALK +/ROS+ NSCLC. A majority of the participants had prior treatment with $\geq$2 ALK TKIs.

Ensartinib (X-396) is a novel ALK inhibitor with additional activity against ROS1, MET, SLK, Axl, LTK, ABL, and EPHA2. A phase 3 study (NCT02767804) comparing Ensartinib and Crizotinib in a front-line setting is currently recruiting patients.

MET Inhibitors

Crizotinib has shown some activity in selected MET-amplified and exon 14-skipping mutant NSCLC (NCT00585195). Cabozantinib, a multi-targeted MET inhibitor, was given to five patients with exon 14 mutations and had a stable disease for 5 months. Capmatinib (INC280) is a selective MET inhibitor. In a phase I study (NCT01324479), relapsed NSCLC patients with high cMET expression were given Capmatinib.

RET Inhibitors

Vandetanib, Sorafenib, Sunitinib, Lenvatinib, Ponatinib, and Cabozantinib are multi-targeted TKIs with RET-blocking activity. They are currently approved for other malignancies. In a phase 2 study with Cabozantinib, 38% PR was seen among 16 evaluable patients, and there was a median PFS of 7 months. Vandetanib in advanced RET-rearranged NSCLC showed an ORR 53%, a DCR 88%, and a median PFS of 4.7 months in 17 eligible patients. In a phase 1 study (NCT01582191), Vandetanib was combined with Everolimus (mTOR (mammalian target of rapamycin) inhibitor) to prevent resistance development based on in-vitro studies.

PI3K Inhibitors

PQR309 is a pan-PI3K, mTOR inhibitor. Its safety and maximum tolerated dose have been recently established in a phase 1 study (NCT02483858 (completed)) with advance solid cancers. No response data are available for NSCLC cohort.

BRAF/MEK Inhibitors

Vemurafenib and Dabrafenib are currently approved for BRAF V600E-positive malignant melanomas, but single-agent activity in BRAF V600E-positive NSCLC is limited. Dabrafenib had an ORR of 33% in platinum refractory cases with a median duration of response of 9.6 months in a single study (NCT01336634).

mTOR Inhibitors

The first-generation inhibitors are derived from rapamycin, a macrolide antibiotic that is produced by *Streptomyces hygroscopicus* bacteria. Rapamycin forms a complex with the cytoplasmic protein peptidyl-prolyl cis-trans isomerase tacrolimus binding protein, which connects to mTOR. There are rapamycin analogues, which are used in humans, including Temsirolimus and Everolimus. The second-generation mTOR inhibitors are ATP-competitive and include Torin1, PP242, and PP30. Temsirolimus is an intravenous drug that was approved by the FDA for the treatment of kidney cancer. The results of several trials performed in vitro on cell lines and in vivo on models of xenograft demonstrated that Temsirolimus inhibits proliferation of HNC. Researchers are currently waiting for the results of phase I/II trial, which used Temsirolimus in combination with the weekly administration of chemotherapy with paclitaxel and carboplatin in recurrent or metastatic HNSCC (NCT01016769). Everolimus is another mTOR inhibitor, which is used as an immunosuppressant to prevent organ transplant rejection and for the treatment of kidney cancer and other cancer types. There are several trials that demonstrated antitumor effect of Everolimus for the treatment of HNSCC. Currently, Everolimus is being evaluated in several clinical trials. The randomized phase II trial compares Everolimus to placebo in the adjuvant treatment of patients with locally advanced HNSCC (NCT01111058).

Fibroblast Growth Factor Receptor (FGFR) Inhibitors

BGJ398 is a potent, selective pan-FGFR (Fibroblast Growth Factor Receptor). FGFR1 amplification is found in around 21% of squamous NSCLC cases. In a single phase 2 trial, 26 evaluable patients showed a PR of 15% and an SD if 35% in dose $>$100 mg. Dovitinib is another FGFR inhibitor tested in squamous NSCLC. Among 26 patients, the ORR was 11.5%, the DCR was 50%, and the median PFS was 2.9 months.

17.2.1 Other Potential Targeted Therapies

Dalantercept (ACE-041) is a novel anti-angiogenic agent, which inhibits ALK1 signaling. In contrast to other anti-angiogenic agents, ACE-041 does not block the proliferative phase of angiogenesis but it modulates the maturation phase of angiogenesis.

Bortezomib is the first therapeutic proteasome inhibitor to be tested in humans and has demonstrated 50% disease control rates in patients with recurrent and metastatic HNSCC. Notably, recent studies demonstrated that the combination of bortezomib with docetaxel, or with cetuximab and radiotherapy, may result in reduced progression-free survival (PFS) or overall survival (OS).

G-Protein Coupled Receptors (GPCRs) are the largest family of cell-surface molecules involved in signal transmission and their improved understanding may provide promising opportunities for drug discovery in cancer prevention and treatment. The Notch signaling pathway is associated with multiple biologic functions,

including regulation of self-renewal capacity, differentiation, cell-cycle exit, and survival. The Notch pathway may be a potential therapeutic target in the treatment of different types of cancer. NOTCH1 mutations have been reported to occur in 10–15% of HNSCC. Increased activity of Notch has been observed in a number of cancer types. Notch inhibition can be conducted by inhibiting four receptors using γ-secretase inhibitors; however, they do not inhibit Notch activation but reduce the activity of further γ-secretase substrates. The tumor suppressor role of Notch signaling requires to be evaluated in further studies.

Both CCND1 and CDKN2A alterations can lead to an increase in downstream CDK4/6 signaling. The CDK4/6 inhibitor Palbociclib has recently been approved for breast cancer. In head and neck cancer, Palbociclib has been tested in combination with cetuximab in a phase I trial and was well tolerated. Some preliminary efficacy was observed, and a phase II study is currently enrolling patients (NCT02499120).

17.3 Current Challenges

For lung cancer, the standard treatment includes surgery, chemotherapy, radiation therapy, and immunotherapy. Radiation therapy, as well as surgery, cannot be used to treat widely spread lung cancer. A combination of two or more chemo drugs is used for better efficacy. However, chemotherapy may also damage healthy cells in the body, including blood cells, skin cells, and nerve cells. Moreover, chemotherapy may lead to relapse and development of resistance leading to lethality and mortality. Thus, because of several disadvantages of chemotherapy and recent advantages being offered by immunotherapy, it is used as novel approach for lung cancer. Immunotherapy includes adoptive cell therapy, monoclonal antibodies, vaccines, and immune checkpoint inhibitors. But primary and adaptive resistance to immunotherapy might occur which limits the efficacy of treatment by following patients under immunotherapy. Immunotherapy may lead to immune-related adverse events (irAEs) in a significant figure because of the initiation of over immune reactivity stimulation, else due to the induction of absolute autoimmune phenomena. This calls for concepts of nano-drug delivery and nanotechnology in medicine [23].

17.3.1 Tumor Challenges and Nanoformulations to Overcome It

The tumor itself plays a resistive role in allowing drugs to show their effect. Solid tumors of breast and lung cancer and malignancies of different organs present a very challenging scenario to target and cause a hindrance to drug to act on them. The physiological properties of these tumors demand advanced and novel formulations that can permeate these complex structures and show their effects. Apart from this, a major challenge is faced by formulators to design a formulation that reduces the off-target effects of these cytotoxic anti-cancer drugs. These off-target effects are the major reason for side effects [25]. Tumors present complexities like tumor interstitial

fluid pressure (TIFP) [26], tumor microenvironment (TME) hypoxia [27], complex extracellular matrix with increased fibroblast causing a rise in TIFP, solid tumors having high cell packing and density [28]. Most tumors lack lymphatic vessels but have extensive blood vessel development (angiogenesis) [29] which are tortuous and varied in spatial distribution that act as a hindrance for drug to penetrate. Novel drug delivery system focuses on overcoming these barriers and improving efficacy [30] of anticancer drugs. Formulation strategies focus on passive tumor targeting, where it exploits the enhanced permeability and retention effect (EPR) and [31], wherein the leaky tumor vasculature allows nanoparticles enter the tumor. Active tumor targeting focuses on attaching ligands like folic acid that specifically attach to folate receptors expressed in certain cancers [32]. The vast array of barriers presented by tumor demands the utilization of novel drug delivery systems, smart drug delivery systems like pH responsive [33], thermoresponsive [34], etc., systems in treating cancer. The following section covers the formulation and drug delivery strategies adopted to overcome barriers in treating solid tumors and other malignancies.

Nanoparticles have emerged as potent drug carriers which on account of their small size and large surface area to volume ratio have proven to be more efficacious and decrease toxicity [35]. Nanoparticles can further dictate the release pattern of drugs**.** Nanoparticles with a large surface area to volume ratio offer a platform for a wide variety of surface modification which can help in targeting specific diseases like cancer but also to organs like bones [36]. Surface modification with hydrophilic systems can also help in decreasing uptake by mononuclear phagocytes which can in turn prolong the circulation time in blood [37].

The following section covers the issues expressed by solid tumors of different organs and the formulation strategies employed in recent years to overcome it.

17.3.1.1 Tumor Interstitial Fluid Pressure (TIFP)

Hypoxia (lack of oxygen) in tumor is responsible for increasing interstitial fluid pressure. It depends on the size of the tumor to the nourishing blood vessels. If the size of tumor volume exceeds 1–2 mm^3 more than the nourishing blood vessels, the tumor becomes devoid of oxygen and nutrients supplied to it. In such cases, to compensate for the lack of oxygen and nutrients, the transcription factors, there is rise in the number of growth factors like vascular endothelial growth factor-A (VEGF-A) and platelet-derived growth factors (PDGF) that trigger angiogenesis [38]. The VEGF is responsible for increases in the permeability of tumor vasculature and increases the fenestrations [39]. Thus, angiogenesis leads to the formation of new vessels and hence a rise in interstitial fluid [40]. The interstitial fluid pressure (IFP) in normal tissues is 0–3 mmHg as compared to tumor tissues which are around 5–40 mmHg [41]. Factors responsible for increasing tumor interstitial fluid include:

1. Permeability of blood vessels
2. Poor lymphatic drainage
3. Dense extracellular matrix (ECM)
4. Dense collagen matrix
5. Rapid proliferation of cancer cells

The abnormal tumor environment provides a window for improvement of formulation and use of novel drug delivery systems to better target cancer.

Lipid Nanoparticles To Overcome TIFP

1. *Nanostructured lipid carriers*: Gao et al. developed modified cationic lipid nanoparticles for decreasing IFP and hinder pulmonary metastasis of breast cancer. The cationic nanostructured lipid carriers (NLCs) were modified with low molecular weight gelatin. NLCs help in penetrating the lipid bilayer of cells and increase the permeability. The NLC consisted of two anticancer drugs which were docetaxel (DTX) and quercitin. This drug containing NLC core was modified with low molecular weight gelatin owing to the fact that gelatin is biocompatible, biodegradable, and low cost. The gelatin coat consisted of Imatinib (IMA), a tyrosine kinase inhibitor (TKI). IMA has the potential to stop the expression of Bcr-Abl gene and PDGF receptors as well [42]. It also has the ability to inhibit excessively produced ECM by inhibiting PDGFR-β and disrupting the "CAFs—ECM interaction [43]. This leads to decreased IFP and better delivery of drugs to the target tumor. The concept was to use gelatin loaded with IMA to decrease TIFP and enhance the uptake of NLCs loaded with DTX and quercitin. NLCs were prepared by solvent injection, followed by solvent evaporation. Briefly, the lipids glyceryl monostearate (GMS) and Caprylic/Capric triglyceride (GTCC) were dissolved in methanol at 60 °C. To this, DTX and quercitin were added to form organic phase I. Egg phosphatidylcholine (EPC) and Tween 80 were added to ethanol and dissolved under ultrasonic waves. Both the organic phases were mixed and then this was injected into aqueous phase having water and cetrimonium bromide (CTAB). This was stirred at 60 °C at 600 rpm till the concentrated NLC was obtained. Preparation of GNPs (gelatin-coated nanoparticles) was done by adding NLC solution drop by drop into 0.1% gelatin aqueous solution (35 mL) containing 2 mg IMA in the water bath at 60 °C, 600 rpm for 30 min, and solidified for another 30 min in ice bath. In-vitro cytotoxicity studies revealed that most decrease in cell viability was observed with GNP-DTX/Qu/IMA which was with lowest IC_{50} value. This was due to better interaction between cationic lipid and negatively charged cell membrane. Apoptosis studies were such that highest to lowest apoptosis were of the order: GNP-DTX/Qu/IMA > NLC-DTX/Qu > DTX + Qu > DTX. This clearly shows that IMA in gelatin coat has additive effect to a large extent. The gelatin was degraded by matrix metalloproteinases (MMP) to release IMA. This further exposed the positively charged lipid which better interacted with negatively charged cell membrane and released the cytotoxic cargo, DTX, and quercitin to efficiently induce apoptosis. The decrease in IFP was also estimated by GNP-DTX/Qu/IMA in comparison with saline. In-vivo studies on 4T1 tumor-bearing mice found that the saline-administered mice had IFP of 29.8 mmHg and NLC-administered formulation had IFP of 17.2 mmHg. In similar comparison for reduction in tumor volume with saline, it was found that saline had a tumor volume of (1622.4 ± 272.6) mm^3 and NLC had a tumor volume of (568.4 ± 73.6) mm^3 [44].

2. *Liposomes*: The hindrance of TIFP to drug penetration was overcome by a liposomal formulation prepared by Fan et al. in which sterically stabilized liposomal formulation (SSL) loaded with IMA and doxorubicin (Dox) was individually and in combination was tested for decrease in TIFP and tumor volume. Liposome was prepared from 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000] (DSPE-PEG_{2000}), egg phosphatidyl choline (EPC), and cholesterol in ratios of (62.0: 31.8: 6.2, m/m/m) for SSL-Dox and SSL-IMA (57.1: 37.9: 5.0, m/m/m). The liposomes were prepared by thin film hydration technique in which DSPE-PEG_{2000}, EPC, and cholesterol were dissolved in chloroform and evaporated at 37 °C under reduced pressure. The obtained dry lipid film was then hydrated with 123 mM or 300 mM ammonium sulfate for SSL-DOX or SSL-IMA, respectively. Following this, a gradient developed by ammonium sulphate was utilized to elute the blank liposomes from Sephadex G-50 column equilibrated with PBS (pH 7.4). Then, after optimization of appropriate quantities of Dox and IMA, they were passively loaded into blank liposomes and eluted from Sephadex G-50 column to remove unentrapped drug. The tumor IFP was measured in C57BL/6 mice by inoculating B16 cells to develop armpit melanoma model. The dose of SSL-IMA administered i.v. was 20 mg/kg and that of free IMA was 100 mg/kg administered by oral gavage. The tumor IFP reduced by 42.7% relative to the initial tumor IFP. Single dose administered at 20 mg/mg of IMA also significantly reduced the tumor IFP which lasted for 50 h. Probable mechanism could be that IMA blocks PDGF-beta which in turn inhibits fibroblast, which are responsible for contractile property contributing to high tumor IFP by rising matrix tension. IMA for this reason also reduces the tumor vasculature. The decrease in IFP gave way to administer SSL-Dox which showed antitumor effects at concentration as low as 1 mg/kg when administered with SSL-IMA [45].

17.3.1.2 Tumor Microenvironment Challenges

Low Tumor pH

Tumor microenvironment (TME) is often acidic and often hypoxic at many times. Acidic TME is often due to metabolism happening at the cancer site. This low pH makes the permeation dependent on ionizing property of drug. Weakly basic drug often gets ionized at the acidic pH of the tumor and fail to permeate.

pH-sensitive Liposomes Masarweh et al. prepared sodium bicarbonate liposomal nanoparticles. Sodium bicarbonate acted to raise the pH of the TME so that doxorubicin uptake could be increased. Doxorubicin is a weakly basic drug whose absorption was found to be affected by the low pH of TME. Liposome formulation was prepared from 50 mM hydrogenated soyabean phosphatidylcholine (55%), DSPE-PEG_{2000} (5%), and cholesterol (40%), by dissolving in ethanol and warming it. This was added to dextrose solution (5% w/v) containing sodium bicarbonate 0.5M, warmed to the same temperature (65 °C). The multilamellar vesicles (MLV) formed were then subjected to lipid extrusion through different sizes of polycarbonate

membrane (400 nm, 200 nm, 100 nm, and 80 nm) to get uniform sized liposomes. Doxorubicin was loaded on to liposome by active loading technique using ammonium sulphate gradient. Effects of uptake of Dox in the presence of sodium bicarbonate was evaluated on triple-negative (4T1) breast cancer cell lines where it was found that cells treated with doxorubicin had 48% viability compared to 12% viability in cells treated with doxorubicin plus bicarbonate at an initial media pH of 6.5. At pH 7.4, the effect of bicarbonate was minor (22 vs. 5% viability). In-vivo studies on female BALB/c mice showed the potency of using liposomal dox in combination with liposomal bicarbonate which manifested in best therapeutic outcome to mice. It was also found that the liposomal dox with liposomal bicarbonate mainly accumulated in extracellular matrix of tumor [46].

Long-circulating pH-Sensitive Liposomes Monteiro et al. developed long-circulating pH-sensitive liposome formulation loaded with paclitaxel (PTX) for targeting breast cancer. Lipids in this liposomal formulation is composed by dioleylphosphatidyl-ethanolamine(DOPE), cholesteryl-emisuccinate (CHEMS), distearoyl phosphatidyl-ethanolamine-polyethylene-glycol$_{2000}$ (DSPE-PEG$_{2000}$), and distearoyl phosphatidyl-ethanolamine-polyethyleneglycol$_{2000}$-folate (DSPE-PEG$_{2000}$-folate) in the molar ratio of 5.7:3.8:0.45:0.05, respectively. CHEMS has the ability to protonate in lower pH of tumor thus converting itself to hexagonal phase which assist in release of the drug. The method employed was thin film hydration. To prepare the liposomes, PTX and lipids were dissolved in aliquots of chloroform in a round bottom flask. It was then evaporated under reduced pressure to obtain thin film. Hydration was done with NaCl (0.9% w/v), followed by vigorous shaking. The formed vesicles were then sonicated at 20% amplitude in an ice bath for 5 min using ultrasonic high intensity processor. Folate was coated on to synthesized liposomes by using 0.05% of DSPE-PEG$_{2000}$-folate and adding it to the lipid film. Biodistribution was done on BALB/c female nude mice and it was found that maximum distribution in liver, spleen, and kidney. It was also found that percentage release was higher as pH decreased, up to 30% in pH 6.8 and up to 70% in pH 5.0 [47].

pH-sensitive Silica Nanoparticles Bhavsar et al. developed pH-responsive mesoporous silica nanoparticles (MSNs) for targeting breast cancer. The prepared MSNs were further modified using succinic anhydride to produce carboxylic acid functionalized MSN-COOH. The functionalized MSN-COOH were briefly prepared as follows: a solution of 3.5 ml NaOH (2M) in 480 ml of deionized water to which 1 g surfactant CTAB and 5 ml co-surfactant (isopropyl alcohol) were added and heated up to 80 °C to form homogenous solution. To this, 5 ml of tetraortho silicate (TEOS) was added to form a slurry. It was then calcified in the air at elevated temperatures up to 550 °C. To prepare COOH functionalized MSN, the prepared MSN were first functionalized with amino group (-NH_2), with the help of (3-aminopropyl)triethoxysilane (APTES). The prepared MSNs-NH_2 then treated with succinic anhydride in the presence of DMSO (dimethyl sulfoxide) to get MSN-COOH. This prepared MSN was then loaded with the drug Anastrazole and

its entrapment efficiency and loading capacity was estimated. The drug-loaded MSN-COOH was then conjugated with chitosan-folic acid (CH-FA) conjugate (prepared by EDC-NHS chemistry) to get CH_FA modified drug-loaded MSN. Characterization for pH dependent in-vitro release was done in dialysis bag at pH 7.4 and pH 5.5 and it was found that almost 96% of ATZ was released at pH 5.5 which was almost 1.39 times more release of ATZ than at pH 7.4. Chitosan had the ability to swell in low pH environment, thus allowing for higher permeability at pH 5.5. Thus, there was stimuli-responsive sustained release at low pH indicating the potential to exploit the low pH of tumor for site-specific release. The in-vivo anticancer activity of MSN-ATZ-CH-FA against free ATZ, MSN-ATZ was studied against Ehrlich Ascites Carcinoma (EAC) induced breast cancer in Balb C mice. It was found that MSN-ATZ-CH-FA best controlled the metastasis highest efficacy in treating the induced tumor [48].

Tumor Hypoxia

Rapidly growing tumors often get deprived of oxygen as they fall short of the blood supplied. Such hypoxic regions are mostly those regions, when oxygen available is consumed within 70–150 μm of tumor vasculature by rapidly proliferating tumor cells which decreases the amount of oxygen diffusing to the interior tumor tissues. Hypoxia often results increased levels of glycolysis increased production of hypoxia-inducible factor (HIF-1). Hypoxia also results in increased chances of metastasis to distant organs, upregulation of factors that are responsible for angiogenesis, etc. From formulation and novel nano drug delivery perspective, hypoxia-responsive systems can greatly aid in abating tumor progression. Smart drug systems mostly are (a) hypoxia-responsive prodrugs (HRPs) and (b) hypoxia-responsive linkers (HRLs) [49]**.** Hypoxia-responsive nanocarriers have been of great focus for better penetration into the tumor.

Dendrimers Xie et al. *developed stimuli*-responsive nano carrier system that shrunk in low oxygen environment. Both Doxorubicin (Dox) and siRNA were delivered with the purpose to increase penetration and improve the chemosensitization to doxorubicin at comparatively decreased concentration. Briefly, the preparation of shrinkable nanocarrier was as follows: Polyamidoamine (PAMAM) dendrimer was conjugated to polyethylene glycol (PEG)-2000 using hypoxia-sensitive linker which was azobenzene (AZO) to form PAMAM-AZO-PEG. This was followed by incorporation of Doxorubicin (DOX) in the hydrophobic core of PAMAM, and then the hypoxia-inducible factor 1a (HIF-1a) siRNA (si-HIF) was bound to the surface of PAMAM through electrostatic forces between anionic siRNA and cationic primary amine groups at the periphery of the PAMAM (i.e., PAP+DOX+si-HIF). The effect of PEG conjugation was such that it assisted in enhanced EPR effect due to longer circulation time. After entering the tumor, due to low oxygen content the hypoxia sensitive linkage broke off giving way to dissociation of PEG from the complex structure of small sized and charged PAMAM

containing DOX and siHIF. Hypoxia-based release was established in the presence of reducing agent $Na_2S_2O_4$. Cleaving of hypoxic AZO bond was confirmed by UV-Vis spectroscopy and it was found that as long as AZO bond remained, the PAP solution was yellow colored and there was characteristic absorption peak at 445 nm and after AZO bond cleavage by reducing agent, the peak at 445 nm was lost. After separating, TLC was performed for the presence of any PEG-2000 spots, but the hypoxic condition was reduced PEG and no spots were observed. TEM analysis of reduced PAP solution confirmed the reduction of PEG in hypoxic condition as the size of PAP was decreased to 5.4 nm. In-vitro studies on MCF-7 cell line showed that though the release of DOX from PAP was slower as compared to DOX solution, the release was higher of PAP-DOX as compared to free DOX in hypoxic (1.6-folds) and normoxic (1.3-folds) conditions. In-vivo studies on MCF-7 tumor bearing mice also demonstrated higher penetration of PAP into the tumor [50].

Block Copolymers Another problem presented by hypoxic tumor environment is the resistance to radiation therapy. Radiation therapy kills the cells by the ionizing radiation used in it which interact with biomolecules like DNA. Oxygen plays a vital role fixing the DNA damage, but in hypoxic tumor it hinders this process and results in radio-resistance. Therefore, much attention has been given to develop formulation to deliver radiosensitizers so that effectiveness of radiotherapy can be improved. Yin et al. synthesized block copolymers of amphiphilic biodegradable poly(ethylene glycol)-block-poly(L-glutamic acid) (PEG-*b*-PLA) introduced with metronidazole (MN) moieties to form PEG-b-P(LG-g-MN), which behaved as radiosensitizer. This then was loaded with Dox to improve chemosensitivity and further improve the radio-sensitivity. PEG-b-P(LG-g-MN) were prepared by condensation reaction between MN and PEG-*b*-PLA in the presence of suitable catalyst. DOX was encapsulated into the micelles of PEG-b-P(LG-g-MN) by nanoprecipitation method where in the DOX and micelles were added in organic solvent DMSO and quickly injected into aqueous PBS. To estimate the release of DOX in hypoxic condition, NADPH was used. The release in normoxic condition was up to 30% which rose to 80% in hypoxic condition. In-vitro cytotoxicity on 4T1 showed that DOX-hypoxia responsive micelles (DOX@HMs) had IC_{50} values of 0.45 ug/ml in hypoxic condition and 0.55 ug/ml in normoxic condition, which confirms enhanced release of DOX in hypoxic condition. Hypoxic micelles without DOX (HMs) had sensitized enhanced ratio up to 2.18 at a dose of 180 ug/ml and with DOX-loaded HMs (DOX@HMs) had similar SERs at lower concentration of 150 ug/ml indicating the synergistic effect of anticancer drug. In-vivo studies on BALB/c female mice were done and bulky tumors (4T1) having volume of 500 mm^3 were treated with DOX@HMs. Without radiation the decrease in tumor volume was up to 28% and along with radiation at a dose of 4 Gy, majority of tumor was reduced indicating potent agent for tumor reduction. Thus, DOX@HMs showed better chemosensitivity and anticancer effects [51].

17.3.1.3 Angiogenesis and Vascular Disrupting Agent (VDA)

Vascular disrupting agents act on developing or developed blood vessels unlike anti-angiogenic drugs that stop new developing blood vessels and thus act as a preventive measure. VDAs act in a way such that the blood vessels of tumor are disrupted causing the developed tumor to undergo necrosis or ischemia [52]. VTAs also behave in similar fashion to VDA and there exits ambiguity in distinguishing it from VDA. VTA like VDAs inhibit the existing developed vessels by inducing necrosis and ischemia. VTAs are generally of two types small molecule based which destabilize microtubulin and ligand type like peptide, proteins, antibodies, etc. [53]. VDAs include some of the newer class of anticancer agents as well as novel techniques like hyperthermia, photothermal treatments, photodynamic treatments, etc.

a. Hyperthermia-Induced Vascular Disruption (VD)

1. *Direct Hyperthermia-mediated VD*: The effects of hyperthermia on vascular injury to tumors was studied and established by Badylak et al. The effects of microwave-assisted hyperthermia which produced a temperature of about 43 °C to 45 °C for 20 min in Walker 256 tumor rats and transmissible venereal tumors (TVT) of dogs were studied in ameliorating the tumor. It was found that there was significant tumor vascular endothelial damage at both the temperatures and there was significant effect of hyperthermia on both the tumors [54].
2. *Photothermal-based hyperthermia for VD*: Diagaradjane et al. prepared gold nanoshell having silica core for modulating tumor vascular changes for better radiation response on tumor. Gold nanoshells were activated optically and the hyperthermia produced was analyzed by magnetic resonance temperature imaging. Briefly, the gold nanoshells were prepared as follows: initially, gold colloids were synthesized which had a colloidal size of 1–3 nm. This was then added to amine-modified silica nanoparticles. The gold colloids adsorbed on the amine groups that behaved as nucleating sites for $HAuCl_4$ to reduce in the presence of formaldehyde to form gold shell. The reduced gold further provides a platform for remaining colloids to reduce on the surface of gold shell and core of silica. The silica core had a size of 120 nm and the shell was of the size of 12–15 nm thick. It was then coated with thiolated PEG (SH-PEG). The prepared nanoshells were evaluated for enhancement in radiation effects by hyperthermia. Mice bearing tumors of 7–8 mm diameter were treated with 20 min of near infrared laser illumination for hyperthermia and 10 Gy radiation after 3–5 min after hyperthermia. It was found that there was a decrease in tumor volume and the time taken for tumor volume to double for hyperthermia-radiotherapy combination as compared to nanoshells alone, or with hyperthermia only or with radiation therapy only. Hyperthermia-radiation combination also reduced the tumor microvessels' density to the greatest extent as compared with single modality treatment. Hyperthermia caused by gold nanoshells increased perfusion of tumor vessels by distorting the vascular patterns. The possible mechanism proposed was that hyperthermia initially increased the perfusion and decreased the hypoxic cells and subsequently

the radiation effects assisted in vascular distortion and its collapse ultimately causing necrosis of tumor cells [55].

Thus, gold nanoparticle-mediated hyperthermia proved to be a potential agent in treating tumors by vascular disruption and showed considerable promise in alleviating the disease state.

3. *Metal-assisted radiosensitization for VD*: Kunjachan et al. developed targeted gold nanoparticles and combined it with image-guided irradiation to improve the radiotherapy. To establish the effects of gold nanoparticles and radiation on vascular disruptions, they were tested on pancreatic tumor model. The gold nanoparticles were decorated with polyethylene glycol and further functionalized with targeting moiety Arg-Gly-Asp (-RGD), a tumor neovascular targeting ligand. Au dose injected in mice bearing pancreatic tumor xenografts was 1.2 mg/g. The prepared gold nanoparticles were coated with PEG followed by EDC-NHS reaction coupling reaction to form RGD functionalized gold nanoparticles. To assess in vitro effects of functionalized gold nanoparticles and irradiation (+RGD:AuNP/+IR), studies were performed on human umbilical vein endothelial cells (HUVEC) that overexpressed $\alpha_v\beta_3$ integrins. This was compared against control in which no functionalized AuNP and radiation was provided (-RGD:AuNP/-IR). The radiation dose was tested in the range of 10 Gy, 5 Gy, and 0 Gy. HUVEC survival was observed for the +RGD:AuNP/+IR (10 Gy) sampled vs. the unirradiated controls and was found to be (58% vs. 98%). It was also found that there were morphological changes and endothelial cell rupture for (+RGD:AuNP/+IR) as compared to control. Similarly, survival rate was just 33% for (+RGD:AuNP/+IR). Further in-vivo on tumor vascular damage in Panc-1 tumor xenografts at 24 h post-IR showed that there was high degree of vascular damage, with segregated endothelial cells at the damaged site with (+RGD:AuNP/+IR). To study the effects of irradiation in increasing the effectiveness of nanoparticle, it was administered with functionalized gold nanoparticle. The DNA damage that occurred due to radiation was assessed in combination with nanoparticle. The groups (+RGD:AuNP/+IR), (-RGD:AuNP/+IR), (+RGD:AuNP/-IR), and (-RGD:AuNP/-IR) had shown a DNA damage up to 57%, 19%, 6%, and 6% respectively. This showed gold nanoparticles assisted to damage DNA of cancer cells synergistically in combination with radiation [56].

17.3.1.4 Active and Passive Tumor Targeting

Tumor vasculature often exhibits leaky vasculature which provides a window of opportunity for nanoparticles to enter and reside in the tumor environment. This is called the enhanced permeation and retention (EPR) effect. The nanoparticles' size and their surface modification to enhance their circulation time play a vital role in EPR effect and together this leads to passive tumor targeting. This strategy is often combined with active targeting wherein targeting moieties are attached which increases the selectivity of drugs in targeting tumors and thus reducing the side effects [57].

1. *Micelles*: Lu et al. prepared spherical micelles of pluronic F127 and chondroitin sulphate (PF127-ChS) for passive and active targeting of solid tumors. Initially, micelles of PF127 and ChS were prepared and then loaded with Doxorubicin (DOX). The PF127 was oxidized to get aldehyde modified pluronic (PF127-CHO). The amine-terminated ChS was prepared by functionalizing ChS with adipic acid dihydrazide by EDC NHS chemistry. The micelles of pluronic and modified ChS were prepared by Schiff base reaction where the aldehyde and amine reacted with each other. The DOX was then loaded onto the prepared micelles by stirring vigorously for 24 h. The prepared micelles had a size of 155–241 nm and showed enhanced release at acidic environment. In-vitro release at pH of 7.4, pH 6.5, and pH 5.5 revealed that there was damage to the micelles as the pH decreased which was indicated by increasing size. At pH 7.4 the size was 240 nm, at pH 6.5 the size was 410 nm, and at pH 5.5 the size was around 650 nm which established breaking of Schiff base bond as pH decreased. Cytotoxicity analysis on human lung cancer cell line, A549 cells, showed that cell viability was above 90% at concentration of 100 ug/L indicating biocompatibility of PF127 and ChS. Confocal microscopy and flow cytometry analysis proved that the micelles were effectively taken up by A549 cells. Thus, PF127-ChS micelles showed that it had potential for passive targeting and active targeting at the same time [58].
2. *Modified liposomes*: Li et al. prepared dual targeting octreotide (Oct) modified magnetic oleanolic (OA) loaded liposomes. To prepare this liposome, initially, soya lecithin (SPC), cholesterol, and OA (weight as 50, 6, and 5 mg, respectively) were dissolved completely in 3 ml absolute ethanol. Five milligrams L-glutamic acid (Glu) and 10 μl Tween-80 were dissolved in 10 ml phosphate-buffered saline (PBS, pH = 6.5). The ethanol solution of lipid was added dropwise to the 45 °C PBS solution with a constant drop rate. The absolute ethanol was evaporated to obtain Glu-OA-liposomes. Then, the OA-Olips were obtained by mixing the prepared Glu-OA-liposomes and Oct solution (1 mg/ml) in a 1:1 ratio under shaking for 4 h. The resulting solution was purified by dialyzing against 10 wt% sucrose solutions using a 14 kDa membrane. Fe3O4 MNPs were prepared by co-precipitation method shown in the Supplementary Material. To prepare OA-OMlips, aqueous suspension of MNPs (0.2 mg/ml) was mixed with the prepared OA-Olips (1:2, v/v) at room temperature. After that, the solution was stored at 4 °C overnight to precipitate the aggregated MNPs. The supernatant was centrifuged at 90 × g for 10 min to further remove nonadsorbed MNPs. Finally, the OA-OMlips were successfully prepared. In-vitro antitumor effects showed that there was more than 86% bioavailability for HeLa and A549 cells. To study in-vivo antitumor effects, the S180 tumor-bearing mice were used as animal model and the tumor were grown up to a volume of 100–150 mm^3 and were injected with saline, free OA, OA-lips, OA-Olips, OA-OMlips, OA-OMlips with magnet and cyclophosphamide (CTX). CTX served as a positive control group in the experiment, which is a conventional medication used as chemotherapy. There was significant reduction in the tumor volume of modified liposome formulation as compared to naked liposomes. The tumor volume was 53% less in case of

OA-Olips with magnet as compared to without magnet, thus establishing enhanced targeting and accumulation with help of magnet. Thus, the size of modified liposomes was obtained in the range of 163.20 nm which was suitable to target tumor passively by EPR effect and octreotide and magnetic targeting acted as active targeting agent [59].

17.3.1.5 Multidrug-Resistance

Most solid tumors like those in breast cancer exhibit drug resistance due to increased efflux of drug. This leads to decreased efficacy of drug and rise in multidrug resistance. This increased efflux is mediated by Pgp-1 protein that expels the drugs that enter the cellular lipid bilayer. Many strategies have been utilized like administering PgP-1 inhibitors like verapamil to overcome this issue. From formulation point of view, it has been projected that the nanoparticles encapsulating or carrying the drug will have the ability to limit the drug from interacting with PgP proteins after entering the lipid bilayer of cell membrane. This was established recently by researchers working on improving the effects of paclitaxel (PTX) by incorporating it in solid lipid nanoparticles (SLN).

Solid Lipid Nanoparticles

Xu et al. prepared PTX-SLN and compared its uptake with PTX in Cremophor-EL and PTX-DMSO vehicles in MCF-7/ADR breast cancer cell line which overexpressed PgP protein. SLN-loaded PTX was prepared by dissolving PTX in chloroform solution containing egg phosphatidylcholine (PC) and DSPE-mPEG2,000. The chloroform solution was dried completely using nitrogen following which Trimyristin (Tm) was added to dried phospholipid and maintained at 65 °C such that PTX dissolved in Tm and phospholipid. This oily hot solution was then added to prewarmed water and sonicated to get milky white dispersion which was dipped in liquid nitrogen to freeze the solution. This was then thawed at room temperature to get SLN. Further Rhodamine (RHO)-loaded SLN was prepared by sonication as previously described and further subjected to 5 cycles of high-pressure homogenization. Increased cytotoxicity was observed in MCF7/ADR cell line for PTX-SLN as compared to DMSO and Cremophor-EL vehicles used. Also, there was significant rise in uptake of SLN-PTX as compared to both vehicles in MCF-7/ADR cell line. It was concluded that SLN-PTX followed different routes of uptake endocytosis other than the clathrin and caveola. It had the ability Pgp efflux pumps in MDR cells [60].

17.3.2 Nanoformulations to Treat Different Cancers

Recent years have focused on using nanoformulations to treat different cancers. Tables 17.2, 17.3 and 17.4 below present an overview of different nanoformulations to treat cancers of breast, head and neck, and lung cancer.

Table 17.2 Nanoformulations to treat breast cancer

Nanosystem prepared	Cell line/in vivo test	Outcome	Reference
Iron nanoparticle embedded poly(*dl*-lactide-co-glycolide) (PLGA) nanoparticles loaded with doxorubicin and conjugated with herceptin	FACs analysis on 1. MDA-MB-231 2. SK-BR3 3. NIH3T6	Diagnosis using magnetic nanoparticles and targeted doxorubicin delivery using Herceptin conjugation	[61]
Tamoxifen-poly(ethylene glycol)-thiol gold nanoparticle conjugates	1. ERR(−) MDAMB-231 2. ERR(+) MCF-7 breast cancer cells	Selective targeting of estrogen receptor-α with enhanced potency and future potential for enhanced laser photothermal therapy	[62]
Curcumin-albumin nanoparticles	MDA-MB-231 (breast adenocarcinoma)	1. Albumin nanoparticles increased bioavailability of delivered curcumin 2. Better antiproliferative effects on cell lines of curcumin-albumin nanoparticles	[63]
Superparamagnetic iron oxide NP loaded with DOX	1. In-vitro: MDA-MB-231 2. MDA-MB-231 tumor bearing female athymic nude mice	1. Increased cytotoxicity of SPION-DOX with hyperthermia 2. Better tumor growth inhibition	[64]
Mesoporous silica nanoparticles (MSN) loaded with Dox and Bcl-2 interfering siRNA	1. MDA-MB-231	1. pH and redox responsive system 2. Co-delivery of Dox and Bcl-2 siRNA resulted in better cell apoptosis of mDA-MB-231	[65]
Rapamycin (RPM) and piperine (PIP) loaded PLGA nanoparticles	1. MDA-MB-231	1. Better cytotoxicity of RPM loaded PLGA NP 2. Improved absorption of RPM due to inhibitory effects on Pgp by PIP	[66]
Hyaluronan modified SPIONs	1. CD44 HA receptor-overexpressing MDA-MB-231 cells 2. MDA-MB-231 tumor-bearing mice	1. Decreased cell viability with laser-irradiated HA-SPIONs 2. Effective tumor destruction by NIR irradiation on HA-SPIONs	[67]

(continued)

Table 17.2 (continued)

Nanosystem prepared	Cell line/in vivo test	Outcome	Reference
Core-shell system of poly-l-histidine nanocores loaded with immune regulator R848 bound to HA-Dox	1. Human breast cancer cell line: MCF-7 and MDA-MB-231 2. Mouse breast cancer cell line: 4T1 and luciferase-labeled 4T1 (4T1-Luc) 3. Sprague Dawley rats	1. Better cell internalization and inhibit their proliferation 2. Tumor growth regulation and their killing	[68]
Estrone-modified glycol chitosan nanoparticles (GCNP-ES) loaded with paclitaxel (PTX)	1. In-vitro on MCF-7 cell line 2. Mice with MCF-7 breast cancer xenograft	1. More than fivefold internalization if GCNP-ES as compared to GCNP alone 2. More than 81% tumor accumulation in mice xenograft model of GCNP-ES	[69]
Lipid nanoparticles encapsulating siRNA and coated with Fab' antibody targeted against heparin-binding EGF-like growth factor	1. MDA-MB-231 cell line over-expressing heparin binding EGF like growth factor 2. MDA-MB-231 carcinoma-bearing mice	1. Effective suppression of polo-like kinase 1 2. Tumor growth suppression	[70]
Light activated core-shell nanoparticles with Chlorin e6-, docetaxel-, and anti-Twist siRNA-containing polymeric nanoparticle (CDTN)	1. mouse TNBC cell line 4T1	1. Spatio-temporal release when irradiated by laser light 2. down-regulation of twist-protein expression and tumor growth reduction	[71]

17.4 Conclusion

The use of targeted therapy has increased with revelation and understandings of newer different pathways. The disease has become more dynamic with cancer cells adopting different pathways when certain pathway is blocked thus developing resistance. Also, the PFS and OS have failed to improve over the years. The complex situation requires newer targeting agents and strategies that have been addressed by nanomedicines. Recent years have hence seen a surge in the research and development of nanoformulations that consider the tumor microenvironment and respond in such a way that the drug is delivered at the site of action. Considerable focus has been on improving the efficacy of drugs and decreasing the toxicity due to anticancer agents. Trend and future scope of nanomedicine have been toward developing

Table 17.3 Nanoformulations to treat head and neck cancer

Nanosystem prepared	In-vitro cell line/in-vivo animal model	Outcome	Reference
Fucoidan-based nanoparticles containing BYL719 (FiBYL719)	1. Cal-33 tumor-bearing mice 2. H22 PDX model	Tumor growth inhibition and radiosensitization	[72]
Cetuximab targeted gold nanoparticles (GNPs)	1. A431 cells injected in nude mice	1. Enhanced radiation effects and tumor growth inhibition 2. Probable ROS generation and DNA damage	[73]
Lipid–calcium–phosphate nanoparticles (LCP NPs) decorated with siRNA	Human HNSCC SCC4 and SAS xenograft models	1. Silencing of VEGF-A and anti-angiogenic effect with decreased tumor proliferation 2. Significant tumor volume decrease (70–120%)	[74]
Cisplatin-glucose loaded gold nanoparticles	1. A431 cells 2. A431 injected in nude mice	1. Better internalization of glucose conjugated gold nanoparticles in vitro 2. Better tumor growth inhibition on combining with radiotherapy 3. Potential as CT contrast agent	[75]
Hafnium oxide (HfO_2) nanoparticles	–	Preclinical trials have shown increased cancer cell death when combined with radiotherapy	[76]
α-Tocopheryl succinate-based polymeric nanoparticles	1. Human aortic endothelial cells (HAEC) 2. FaDu cells	1. Decrease in angiogenesis by increasing ROS production 2. Decrease in matrix metalloproteases 2 and 9 and inhibit cell migration	[77]
Dendritic mPEG-BMA4 nanoparticles loaded with saracatinib	HNSCC cell lines HN6, HN8, and HN12	Decreased invasion and metastasis of HNSCC	[78]
Albumin-sericin nanoparticles (Alb-Ser NPs), modified with poly-L-lysine (PLL) decorated with hyaluronic acid	Laryngeal cancer cell line, Hep-2	Significant inhibition of cell growth and induction of apoptosis in cells	[79]
Peptide hydrogel loaded with doxorubicin and curcumin	1. HSC-3 cell 2. HSC-3 xenograft mouse model	1. Significant in-vitro apoptotic response on HSC-3 cell 2. Significant antitumor effect on HSC-3 tumor bearing SCID mice	[80]
PEGylated prodrug (RPTD) of Dox and cRGD peptide modified nanoparticles encapsulating hematoporphyrin	1. Human OTSCC cell line, CAL-27 2. Human oral epithelial cell line (HOEC) 2. CAL-27 cells injected female BALB/c nude mice	1. Laser irradiation resulting in ROS production and release of DOX 2. PDT-based ROS generation 3. Synergistic effects of laser in increasing cytotoxicity 4. Tumor targeting and complete suppression of tumor growth	[81]

Table 17.4 Nanoformulations to treat lung cancer

Nanosystem used	In-vitro cell line/in-vivo animal model	Outcome	Reference
G3139 anti-sense nucleotide loaded in lipid nanoparticles	1. A549 lung cancer cells 2. A549 murine xenograft tumor model in male BALB/c mice	1. Downregulation of Bcl2 expression in-vitro 2. Inhibition of tumor growth	[82]
Dox conjugated Gold nanoparticles (AuNP) with polyvinylpyrrolidone (PVP)	A549, H460, and H520 lung cancer cells	1. Decrease in the proliferation and induction of apoptosis of lung cancer cells 2. Upregulation of tumor suppressor genes	[83]
Microparticles Erlotinib (ETB) loaded solid lipid nanoparticles (SLN) to prepare dry powder inhalation (DPI)	A549 cells	Potential lymphatic uptake and anticancer effect on lung cancer cells	[84]
SP5-52 peptide conjugated and gemcitabine-loaded silk fibroin nanoparticles	1. Lewis lung carcinoma cells (LL/2 cell line) and BEAS-2B cells 2. Lung tumor induced in Balb/c by injecting LL/2 carcinoma cells	Higher therapeutic efficacy of targeted drug loaded silk fibroin nanoparticles	[85]
Folate conjugated and radiosensitizing agent loaded poly (N-isopropylacrylamide)-chitosan shell and PLGA core nanoparticles	1. A549 and H460 lung cancer cells 2. H460 tumor bearing mice	1. pH and temperature responsive system developed 2. Reduction in tumor growth rate 3. Safe simultaneous radiotherapy and chemotherapy possible	[86]
Folic acid (FA)-conjugated poly-amidoamine dendrimer (Den)-based nanoparticle (NP) for delivery of siRNA and cis-diamine platinum (CDDP)	H1299 lung cancer cells	1. DNA damage and apoptotic cell death. 2. Decreased toxicity to normal cell	[87]
Disulfiram (DSF) loaded PLGA nanoparticles	A549 lung cancer cells	Influence of various formulation parameters on cytotoxicity of DSF loaded PLGA NP	[88]
PTX loaded hyaluronic acid-disulfide-vitamin E succinate (HA-SS-VES, HSV) conjugate nanocarrier system	1. A549 cell line 2. Mice bearing A549 tumors	1. Enhanced cytotoxicity and apoptosis 2. Decrease in tumor growth rate 3. Stimuli triggered release	[89]

(continued)

Table 17.4 (continued)

Nanosystem used	In-vitro cell line/in-vivo animal model	Outcome	Reference
Paclitaxel (Pt)-chitosan core and gemcitabine (GEM)-hyaluronic (HA) shell nanoparticles	1. NCl-H460 cells 2. Mice bearing NCl-H460 cells xenografts	Enhances in-vitro cytotoxicity and in-vivo antitumor effect	[90]
Curcumin and Dox encapsulated albumin nanoparticles	1. B16F10 melanoma cells 2. C57BL/6 mouse model induced by B16F10 lung metastasis	1. Synergistic antitumor effects along with targetability to lung tumor sites 2. Decrease in metastatic melanoma mass	[91]
Dox and Cisplatin loaded Methoxy poly(ethylene glycol)-poly(ethylenimine)-poly(l-glutamate) (mPEG-OEI-PLG) nanocarriers	1. B16F10 cell line 2. B16F10 tumor-bearing mice models	1. Enhanced cyto-toxicity of combination drugs 2. Enhanced retention and accumulation of drugs leading to significant tumor growth inhibition	[92]

multimodal theranostic systems that diagnose, image, and deliver therapeutic moiety simultaneously. Also, careful consideration has to be given to the regulatory and toxicity aspects of nanomedicine.

Acknowledgement Authors would like to thank the Institute of Pharmacy, Nirma University, for providing necessary support to publish the chapter.

Conflict of interest Authors declare no conflict of interest. The tables are created by authors from the literature sources.

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Part III

Regulatory, Safety and Marketing Aspects of Nano Medicine

Recent Trends for Nanomedicine Safety

18

Biswajit Mukherjee, Debasmita Dutta, Prasanta Ghosh, Brahamacharry Paul, Ramkrishna Sen, and Samrat Chakraborty

Abstract

Nanomedicine has gained tremendous attention in medical professionals owing to its smart strategies of treatment and huge applications in healthcare. Nanomedicine is mainly used for drug delivery with special attention to drug targeting, their excellent properties of high drug loading, sustained drug release, surface tunability, surface modification possibilities, and unique surface properties. A major portion of nanomedicine has been occupied by nanocarrier systems. For diagnosis and therapeutic purposes, nanoparticles, nanoliposomes, dendrimers, and other nanoparticulate devices have been reported for treatment/diagnosis of diseases. Several nanomedicines with emerging therapeutic efficacy have been already commercially available and many more are in pipeline. Despite their impressive therapeutic benefits, some critical challenges are associated with their transition from laboratory to clinical usage. Toxicity caused by nanoformulated drug is the most adverse barrier for its broad range of application. In this chapter, we have reported different nanocarriers available for their diversified applications in the management of several diseases, with a special emphasis on recently published reports, clinical evidence, and, their toxicity and safety concerns.

Keywords

Nanotoxicity · Nanomedicine · Biosafety · Clinical trial · FDA-approved nanoformulations

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M. K. Das, Y. V Pathak (eds.), *Nano Medicine and Nano Safety*,
https://doi.org/10.1007/978-981-15-6255-6_18

18.1 Introduction

Nanomedicine is a branch of medical science, which deals with an emerging field of knowledge, applications, and novel tools of nanotechnology for diagnostic and therapeutic smart approaches to manage human healthcare system. According to the European Science Foundation, nanomedicine is defined as nanosized tools used for diagnosis, prevention, and treatment of diseases with an increased understanding of the complex pathophysiology associated with it [1]. Nanocarrier system reigns over a vast area in nanomedicine. Hence, we mostly focus on nanocarriers in the current discussion. Due to the nanoscale size, enhanced surface area to volume ratio, and new surface properties such as super magnetism, surface charge, etc., the nanosize drug delivery systems are considered to achieve prolonged circulation time, greater stability, improved accumulation at the target site, and controlled release of drug in a sustained manner that enhances their therapeutic efficacy with reduced cytotoxicity to normal tissues. Nanocarriers have been reported for the treatment of cardiovascular diseases, different types of cancer, brain tumor, HIV/AIDS, gastrointestinal diseases, skin disorders, respiratory diseases, pulmonary fungal infections, neurological diseases, stroke, hypertension, leishmaniasis, visceral diseases, epilepsy, ocular diseases, and many more. For the last few decades, emerging research on nanomedicine evolves various biocompatible and biodegradable nanoscale materials which include nanoparticles, nanoliposomes, dendrimers, micelles, polymer-protein conjugates, albumin-drug conjugates, DNA-drug conjugates, antibody-drug conjugates, ligand-conjugated polymeric nanoparticles or nanoliposomes for targeting specific organs of interest, and so on. Nanoliposomal formulations have a great advantage for their ability to include both hydrophilic and hydrophobic drug types in the delivery system and to cross various physiological barriers. Designing of the nanomedicinal targeted anticancer drug delivery systems are of great scientific interest in the field of biomedical research. Many of these nanoparticulate medicines have been translated into clinical trials and some already been introduced commercially, reflecting the successful outcome of laboratory research on nanomedicinal formulations. Despite their well-versed medical applications and fast-developing future prospect, some unavoidable toxic outcomes often limit their extensive use. Evidence in animal studies suggested that certain nanomaterials can interact in cell constituents in vivo in a different manner than small molecules [2]. They can produce a wide range of alterations such as oxidative stress, induction of inflammatory responses, protein aggregation, mitochondrial perturbation, blood coagulation and cell death, induction of autophagy and apoptosis, complement activation, etc., as observed in experimental models. The ability of nanomaterials to cross several biomembranes (e.g., blood–brain barrier) may produce off-target effects and unpredictable dose-response profile. Assessment of environmental exposure to nanomaterials in humans, animals, and our ecosystem and their potential hazards, if any, their persistent use causing nanomaterial-related effect, their immediate effects, and long-term risk should be monitored minutely. Development of suitable devices, testing methods, and guidelines for assessment are in need and currently under the supervision of many

regulatory authorities throughout the world to ascertain the safety concerns. Different strategies are required to develop to avoid nanomedicine-mediated in vivo toxicity in humans and animals.

In this chapter, we have mainly provided recent reports on several nanomedicinal drug carriers used for treatment, diagnostic, and theranostic purposes. The toxicity profiles of many such nanomedicinal materials observed in various experimental models were also discussed to understand the problems associated with current research in the field and requirements to cross the hurdles of clinical trials. Nanomaterials under clinical investigations and their outcomes and safety concerns are also discussed.

18.2 Polymeric Drug Nanocarriers

Polymeric drug nanocarriers have been successfully used for cancer drug delivery since last few decades. Polymers can be natural, synthetic, or pseudosynthetic. In the design of commercial nanocarriers, size, surface morphology, and characteristics have been used to achieve successful delivery. The design of optimized formulation for effective delivery to target sites of action with minimum off-target effects remains a vital research objective. Hence, it is in an urgent requirement to develop new chemotherapy using polymer-based nanocarriers to enhance therapeutic efficacy. Polymer nanoparticles can be fabricated in a wide range of varieties and sizes from 10 to 999 nm. They can range in size from a single polymer chain used directly as a therapeutic or as a modifying agent for a drug or diagnostic agent to large aggregates within the nanoscale [1, 3, 4]. Polymer nanodrugs can be categorized as: (1) degradable polymer forms for controlled release applications and (2) polymer–drug conjugates that increase circulation time and drug half-life or improve biocompatibility/solubility [1].

Two of the top ten best-selling drugs in the U.S. in 2013 were polymeric drugs, Copaxone (glatiramer acetate injection), approved in 1996 for the treatment of relapsing-remitting multiple sclerosis, and Neulasta (pegfilgrastim), approved in 2002 for chemotherapy-induced neutropenia [1]. Polymer (NPs) can facilitate drug release for weeks without accumulating in the body. Therefore, polymeric NPs are considered promising carriers for numerous medications, including treatments for cancer, cardiovascular disease, diabetes, bone-healing therapies, and vaccinations [1, 3]. One of the most well-established polymers is polyethylene glycol (PEG). Plegridy, a pegylated interferon beta-1a formulation, has been approved in 2014 for the treatment of relapsing forms of multiple sclerosis, showed improved drug half-life and exposure. Another such pegylated form is Adynovate (antihemophilic factor [recombinant]), which was approved in 2015 for bleeding prophylaxis and hemophilia A [1, 5]. Rebinyn (coagulation factor IX [recombinant], glycopegylated), was approved in 2017 for treatment and control of bleeding episodes and perioperative bleeding management in patients with hemophilia B [6, 7]. Apart from pegylated polymers, biodegradable polymers are also of prime interest because they can be fully metabolized and removed from the body. Poly-(lactide-co-glycolic acid)

(PLGA), Polyhydroxyalkanoates (PHAs), and cyclodextrins (CDs) are the most commonly used polymer for core fabrication [8]. Polyvinyl alcohol (PVA), PEG, and monomethoxy poly-(ethylene glycol) (mPEG) have been applied in surface modification of polymer-based nanocarriers which gives nontoxic hydrophilic outer shells and outstanding blood biocompatibility. The United States Food and Drug Administration (USFDA) has approved biodegradable polymer such as PLGA and PLA for human use [9]. Poly lactic-co-glycolic acid (PLGA) is an especially intriguing example of a biodegradable polymer because relative proportions of polylactic acid (PLA) and polyglycolic acid can be used to tune finely the biodegradability of PLGA [3]. Zilretta is extended-release injectable suspension microspheres consisting of crystals of triamcinolone acetonide embedded in a PLGA copolymer matrix used for the treatment of osteoarthritis knee pain [6, 7]. In addition to increasing half-life, polymer conjugation can improve passive tumor targeting by increasing the size of a drug [10]. Abraxane® and Transdrug® are the clinically approved passively tumor-targeted nanoparticles in cancer therapy. Abraxane®, a solvent-free, albumin-bound nanoparticle of paclitaxel which is also known as nab-paclitaxel presently used in breast cancer and Transdrug® contains cytotoxic drug doxorubicin currently used to treat hepatocarcinoma clinically [11].

In a recent study, Nosrati et al (2019) developed a mono methoxy poly (ethylene glycol)-poly (ε-caprolactone) (mPEG-PCL) co-polymer based on novel methotrexate sodium (MTX) drug delivery carrier with the objective of enhancing the loading efficiency of the drug in nanocarrier as well as achievement of an effective control release rate of the drug. They showed that these polymersomes provided an ideal carrier for the delivery of MTX to breast cancer cells (MCF-7) [12]. In another research article, Zheng et al (2019) reported that PEGylated poly (lactic-co-glycolic acid) nanoparticles conjugated with LFC131 (a peptide inhibitor of CXCR4) co-delivery of sorafenib and metapristone via the CXCR4-targeted nanoparticles showed a synergistic therapy against hepatocellular carcinoma. Here, they showed enhanced cytotoxicity, colony inhibition, apoptosis, and caspase signaling pathways. Their results also suggested combinational treatment of chemotherapeutics enhanced circulation and target accumulation at tumor sites and consequently inhibited tumor growth in an animal model [13]. Many polymer-containing nanodrugs are being investigated in clinical trials and are discussed in the relevant section of this text. CRLX101 (camptothecin conjugated cyclodextrin-PEG formulation) is currently under clinical trial for lung cancer and solid tumors [3].

18.3 Liposomal Drug Nanocarriers

Liposomes were initially described in 1965 and its drug delivery properties were first proposed in the 1970s. It is a spherical vesicle composed of a self-assembling lipid bilayer membrane arranged around an empty core which carries and delivers both hydrophilic and hydrophobic molecules within the cores. This special characteristic, along with biocompatibility and biodegradability, makes liposomes more unique as a drug delivery carrier. In a structural point of view, liposomes can be divided into

unilamellar vesicles (UVs) and multilamellar vesicles (MLVs) on the basis of lipid bilayers. Each of UVs is composed of an aqueous core enclosed by a lipid bilayer, separating the inner aqueous core from the outside, and MLVs consist of various layers of lipid bilayers along with the aqueous core. Fabrications of liposomes are the first drug delivery system in the field of nanoscale to make the transformation from concept to clinical practice [14]. USFDA approved lipids used for the preparation of liposomal vesicles are 1, 2-distearoyl-sn-glycero-3-phospho-ethanolamine (DSPE), hydrogenated soybean phosphatidylcholine (HSPC), phosphatidylglycerol (eggPG), and 1, 2-distearoyl-sn-glycero-3-phosphocholine (DSPC) [15]. Most of the conventional chemotherapeutic agents circulate nonspecifically in the body and have poor pharmacokinetic profiles and systemic toxicity associated with major side effects. Hence, the establishment of nanodrug delivery systems able to target the tumor site is becoming a genuine challenge [16]. Liposome-based nanomedicines showed the ability to circulate in the bloodstream for an extended time thus providing a longer treatment to affect and accumulate more drugs at the site of a tumor or infection [1, 3, 10]. Attachment of polyethylene glycol chains at liposomal surface proved a 4- to 16-fold enhancement in drug delivery during malignancies, in contrast to prior non-liposomal trials [17]. Liposomes can reach tumor site passively through the leaky vasculature surrounding the tumors by the increased permeability and retention effect although ligands modified at the surface of liposomes allow specific targeting by binding to the receptors overexpressed by cancer cells or angiogenic endothelial cells [16]. By using lipids of different fatty-acid-chain lengths, liposomes can be made temperature or pH sensitive, thereby controlling the release of their contents under specific environmental conditions [3, 10]. Drugs with low bioavailability or high toxicity have been successfully delivered by liposomes [1, 4]. Co-encapsulation of drugs in nanoformulations can also provide a novel means of drug delivery. More specifically, these formulations can deliver drugs sequentially and at specific molar ratios within the tumor microenvironment, allowing for maximal synergy that is not possible with conventional drug delivery methods [10].

Starting with the approval of Doxil in 1995, many nanodrugs incorporating liposomes have been approved, including antifungal agent, anticancer drug, and analgesic [1, 10, 18]. Myocet® is a remarkable example presently used to treat breast cancer clinically in combination with another chemotherapeutic agent (cyclophosphamide). Doxil is used to treat metastatic breast cancer and AIDS-related Kaposi's sarcoma. Other liposomal non-PEGylated systems have been approved such as DaunoXome® and Onco-TCS® for drugs daunorubicin and vincristine [15, 19]. Recently, in 2017, Vyxeos, a liposomal formulation of cytarabine and daunorubicin in a 5:1 fixed molar ratio, also got FDA approval for the treatment of acute myeloid leukemia (AML) [20, 21].

Guan et al (2019) reported doxorubicin-loaded 8-mer and 16-mer D-peptide ligand-modified liposome preparation and studied systemically enhanced immunocompatibility. The biodistribution and biosafety of two different peptide-modified liposomes were assessed in healthy BALB/C mice and antiglioblastoma effect was determined in nude mice bearing intracranial glioblastoma [22]. In

another research work, Awad et al (2019) showed that human serum albumin (HSA) modification of pegylated liposome remarkably increased their binding to the surface of the breast cancer cell line MCF-7 and MDA-MB-231, resulting in the increased uptake of the drug by cancer cells. Therefore, the HAS-coated liposomes coupled with ultrasound-mediated enhanced drug release indicate desirable prospective in breast cancer chemotherapy [23].

18.4 Nanocrystal Drug Nanocarriers

Nanocrystal is formed by an optically active core which emits tunable, narrow, symmetric, photochemically stable spectrum and is surrounded by a shell which makes nanocrystal less sensitive to photo-oxidation and medium changes [24]. These nanocrystal-based medicines are composed entirely of drug compound (s); therefore, the surface area of these drugs is increased and their dissolution speed and saturation solubility are also enhanced. Due to increased saturation solubility, they get absorbed through the gastrointestinal tract easily [1, 4]. Nanocrystal formulations improve the Pharmacokinetic/Pharmacodynamic (PK/PD) properties of poorly water soluble organic or inorganic drug by increasing their bioavailability and solubility [4, 25]. However, the mechanism behind their oral absorption and behavior after subcutaneous injection are not fully understood.

First FDA-approved (2000) organic nanocrystal medicine was Rapamune that contains bacteria-derived immunosuppressant sirolimus and acts to prevent organ rejection (particularly kidney) after transplantation. This formulation makes poorly soluble sirolimus into an extended release drug. This technique is also used in other types of formulation such as tablets, oral suspension, and intramuscular injections [1]. After the approval of Rapamune, several other nanocrystal medicines were marketed using the techniques like Tricor, Emend, etc., and provided the potential solution for solubility issue of many compounds [18]. In comparison to organic nanocrystal formulation, FDA approval of inorganic nanocrystal formulations are only limited to hydroxyapatite and calcium phosphate nanocrystal as a bone-graft substitutes [1].

18.5 Micelle Nanocarriers

Micelle nanoparticles contain a hydrophobic internal core for easy encapsulation of poorly aqueous soluble drugs but adequate polarity on its outer surface helps them to dissolve in aqueous solution [1]. These are self-assembling polymeric amphiphilic structures and may be customized for slow and controlled delivery of hydrophobic drugs as well as their structures can be finely tuned to get desired particle size, drug loading, and release characteristics [10]. A huge number of new chemical entities coming out of research laboratories suffers from low water solubility, making them slightly challenging to the manufacturer for administration and often causing delay in drug formulation and development. Additionally, so many poorly soluble drugs

have not achieved their potential on the market due to intolerable levels of toxicity from the drug or the excipients in the formulation. The most common hydrophilic block used to make the hydrophilic shell is the FDA-approved excipient poly (ethylene glycol) (PEG) or poly (ethylene oxide) (PEO) [26]. Research on nanocarriers composed of block copolymer micelles is a rapidly developing and exciting area of drug delivery. These systems are being studied for stabilization, solubilization, and delivery of the most challenging therapeutic agents. The distinctive architecture, small size, stability, and ability of block copolymer micelles to be modified for good compatibility with the drugs are best preferable properties for a drug delivery system [27]. To target abnormal cancer cells actively, special types of ligands are used to modify the micelle surface, namely aptamers, folic acid, carbohydrates, peptides, and antibodies. The core of the micelle can be functionalized to release the drug at the right concentration to the target site. The stimuli used in smart drug delivery systems based on micelles are changes in temperature, pH gradients, ultrasound, enzymes, and oxidation [28].

Optimized doxorubicin polymeric micelles (NK911, Nippon Kayaku, Co.) were the first clinically evaluated in 2001. NK911 micelles have been tested for metastatic pancreatic cancer in Phase II clinical trials, but the results have not been reported [29]. Paclical (orphan drug status in 2009 by the FDA) and Genexol-PM (approved in South Korea) are two examples of micellar formulation of paclitaxel for ovarian cancer and metastatic breast cancer and advanced lung cancer treatment, respectively, with significantly less toxicity [4, 10]. Genexol-PM consists of low-molecular-weight amphiphilic diblock copolymer, monomethoxy poly (ethylene glycol)-block-poly (D, L-lactide) (mPEG-PDLLA) and drug paclitaxel [30]. Micellar formulation of estradiol (Estrasorb) got FDA approval in 2003 and used for the treatment of menopause-related moderate-to-severe vasomotor symptoms. As it is administered transdermally, gastrointestinal side effects may be avoided [31]. Toxicity of Kolliphor-based paclitaxel drug can be reduced by micellar formulation. Nephrotoxicity of cisplatin drug also reduced due to micellar formulation [32]. Therefore, due to this broad applicability of micellar-based nanoformulations, we can expect many new products in the near future.

Recently, Seo et al (2015) revealed a co-delivery scheme based on the temperature-responsive micelle that can carry genes along with anticancer drugs [33]. Doerflinger et al (2019) developed a targeting aptamer ligand to functionalize polydiacetylene micelles [34].

18.6 Protein-Based Nanocarriers

In protein-based nanocarriers, proteins are used as a carrier, active therapeutic agents as well as for targeted delivery and reduction of toxicity [1]. In the last decade, albumin is mainly studied as a drug carrier and many albumin-based nanomedicines are at present in clinical trials. The advantages of these albumin-based nanomedicines are higher passive accumulation in the tumor site and also facilitating the cellular uptake of the drug by the albumin receptor [35]. Abraxane is an early

albumin-based nanoparticle conjugated with paclitaxel drug and got FDA approval in 2005. This formulation eliminates the use of toxic Kolliphor solvent required to make paclitaxel soluble as it causes immune reaction [1]. Therefore, an improvement in infusion time, drug efficacy, drug PK, and reduction in toxicity was observed during Abraxane use [36]. After the successful entry of Abraxane into the market, several other albumin-bound nanoparticles are in clinical trials for improving the efficacy of the drugs like docetaxel, rapamycin, heat shock protein inhibitor, etc. [31]. Nowadays, apart from unmodified proteins, engineered particle complexes are also being designed to enable active targeting and Ontak is an example of this engineered fusion targeting proteins with cytotoxic molecules. It is an interleukin (IL)-2 receptor antagonist and used to treat non-Hodgkin's peripheral T-cell lymphomas and helps to suppress overexpression of IL-2 receptor on T cells [1]. Ontak also showed a significant reduction in organ toxicity and thus may be an effective treatment for many hematological malignancies which are related to overexpression of IL-2 receptor [37].

18.7 Dendrimers

Dendrimers are well-defined, multivalent molecules with a nanometer size branching structure. There are three distinct components of dendrimer: core, branching dendrons, and surface-active groups. Conventional dendrimers face immune system clearance and lower uptake by cancer cells. Modification of the conventional dendrimer is the solution to these limitations. Chemical modification, linear polymer copolymerization, and hybridization with other nanocarriers are some of the choices for overcoming these limitations as reported so far [28]. To target the cancer cells, peptides, proteins, carbohydrates, aptamers, antibodies, etc., can change the surface of the dendritic structure. The surface of the dendritic structure can also be modified for different stimuli-responsive systems, such as light, heat, pH change, protein, and enzyme transformation [38, 39].

Most of the successful nanocarriers were synthesized using classical linear, random coil polymers, such as polyethylene glycol (PEG), poly (glutamic acid) (PGA), N-(2-hydroxypropyl) methacrylamide (HPMA) copolymers, poly (ethyleneimine) (PEI), and dextrin (a-1, 4 polyglucose). These polymers have produced conjugates and polyplexes that have now been used to develop formulations and that are in the clinical trial [40].

The cationic nature of PAMAM (polyamidoamine) makes it extremely beneficial for the delivery of genetic materials among other dendrimers. The effectiveness of delivery relies on PAMAM generation. In 1993, Haensler and Szoka were the first to report the delivery of PAMAM nucleic acid [41]. The tumor imaging dendritic contrast agent is also very promising [42].

18.8 Other Nanocarriers

Recently, the use of other different nanomaterials has been tried to build effective nanocarriers for drug delivery applications [43]. A large number of inorganic materials, such as metal, metal oxide, silica, carbon nanotubes, etc., can be used to create nanoformulations for therapeutic and imaging applications and metal and metal oxide are being explored intensely.

18.8.1 Carbon Nanotubes

Carbon nanotubes (CNTs) are cylindrical large molecules composed of a hexagonal structure of hybridized carbon atoms that may be composed of one sheet of graphene (single-walled CNTs) or by rolling up various sheets of graphene in a concentric manner (multi-walled CNTs) [44]. CNTs have some unique physicochemical and biological features and high surface modification capabilities that make them a successful drug delivery carrier. The distinctive shape of the nano-needle is particularly interesting, as it enables endocytosis to cross the cell membrane, while CNTs with size ranging from 50 to 100 nm are easy to be engulfed [45]. Any drugs can either be encapsulated in the internal space or be attached (covalent or noncovalent functionalization) to the surface of the CNTs.

The main issues with CNTs are their low water solubility, nonbiodegradable, and cytotoxicity. Although, CNT nanocarriers have the potential to be surface-functionalized (chemically or physically) which render them water-soluble, biocompatible, and nontoxic or less toxic. While PEGylation is used to improve solubility, prevent reticuloendothelial system (RES), and reduce toxicity, their surface functionalization with poly (N-isopropylacrylamide) polymer could be used to modify the CNTs for stimulus-responsive (temperature) nanocarriers. Castillo et al (2013) reported the formulation of a graphene electrode modified with peptide-conjugated nanotubes and folic acid for improving target specificity of human cervical cancer cells overexpressing folate receptors [46]. Lu et al (2019) developed anti-IGF1R antibody (IGF-1R Ab) coupled carbon nanotubes for photothermal therapy of orthotopic pancreatic cancer guided by optical imaging [47].

18.8.2 Metal and Metal Oxide Nanoformulations

Gold nanoparticle (AuNP) is the most distinctive inorganic material in nanotechnology with a wide range of biological and biomedical applications. It has been suggested for drug delivery and gene delivery applications as nontoxic carriers [48]. Passive targeting is well known to be accomplished by using AuNP as a carrier due to its better tumor cell accumulation (Enhanced Permeability and Retention) effect [49]. In fact, AuNP particular characteristics, such as its elevated surface to volume ratio, unique optical properties, simple synthesis, and flexible surface functionality, are committed to cancer therapy in the clinical sector [50]. In addition,

AuNP has optical properties that can be readily adjusted according to their form and structure to desirable wavelengths, enabling photothermal and imaging applications. Battogtokh et al (2019) reported glycol chitosan-coated near-infrared photosensitizer-encapsulated gold nanocages for glioblastoma in vitro and in vivo [51]. In another article, Liu et al (2019) reported that zwitterionic gadolinium (III) complex dendrimer entrapped AuNP showed target specificity to αvβ3 integrin expressing cells and enhanced CT/MRI imaging of lung cancer metastasis model in vivo [52]. Albertini et al (2019) developed RGD-link pentapeptide decorated AuNP for diagnosis and treatment of cancer. The peptide conjugation was selected for its ability to recognize the αvβ3 integrin receptor [53]. Most of the gold nanoparticles are at the in vivo stage (preclinical), and few have reached clinical trials.

In several studies, iron oxide nanoformulations have been considered for examining their use as contrast enhancement reagents for magnetic resonance imaging (MRI) [1, 4]. The iron nanoformulations for treating chronic kidney disease (CKD)-associated anemia are Venofer (iron sucrose injection), Ferrlecit (sodium ferric gluconate complex in sucrose injection), Infed (iron dextran injection), and Dexferrum (iron dextran injection). These formulations avoid toxicity and thus are administered rapidly in large doses, without increasing free iron levels in the blood [1]. Superparamagnetic iron oxide nanoparticles (SPIONs) have low toxicity, more half-life, and are biodegradable that respond strongly when exposed to a magnetic field; used both as targeted and nontargeted contrasting MRI agents to target specific tumors [3, 10]. Three FDA-approved SPION drug formulations are—Feraheme, Feridex, and GastroMARK. Feraheme is used to treat CKD and is also being deliberated as an imaging agent in clinical trials [32]. SPIONs also release energy in a magnetic field, permitting them to be used as promising hyperthermia agents against tumors in preclinical and early clinical studies. Nanotherm is one of such SPIONS to treat glioblastoma tumors; the subsequent injection in the tumor causes programmed and non-programmed cell death due to local thermal heating [1, 10].

Several metals, including silver, are known to be potent antimicrobials as they can easily penetrate bacterial cells and induce toxic effects. Cornell dots are inorganic silica nanoparticles that are being developed at Cornell University as a diagnostic and therapeutic tool in cancer treatment [1, 4, 10]. Although designed for lymph-node mapping in cancer patients, these nanoparticles have also been found to induce cancer cell death in vitro and reduce the size of tumors after multiple high-dose injections were administered to mice. They are composed of an internal silica core labeled with a near-infrared fluorescent dye, a targeting moiety, and an antifouling polymer layer. This design has created a nanoparticulate system that is more stable and 20 to 30 times brighter than a conventional solution of the constituent dye. Various FDA approved nanomedicines used in cancers and non-cancerous diseases have been given in Tables 18.1 and 18.2.

Table 18.1 FDA-approved nanomedicines available for cancer treatment

Drug	Nanomedicine	Manufacturer	Delivery system	Indications	Benefits over free drug	Route of administration	FDA approved Year
Liposomal							
Doxorubicin	Doxil	Janssen	PEGylated liposomes	HIV-associated Kaposi's sarcoma, ovarian cancer, metastatic breast cancer, multiple myeloma	Increased delivery to disease site, decreased systemic toxicity of free drug	i.v.	November 1995
Daunorubicin	DaunoXome	Gilead Sciences	Liposomes	First-line treatment for patients with advanced HIV-associated Kaposi's sarcoma	10 times higher accumulation in tumors than free drug.	i.v.	April 1996
Daunorubicin and cytarabine	Vyxeos	Jazz pharmaceuticals	Liposomes	Acute myeloid leukemia (AML), AML with myelodysplasia-related changes	Increased efficacy through synergistic delivery of co-encapsulated agents	i.v.	August 2017
Vincristine	Marqibo (OncoTCS)	Spectrum Pharmaceuticals	Liposomes	Adult patients with lymphoblastic leukemia	Increased delivery to tumor site, decreased systemic toxicity	i.v.	August 2012
Irinotecam	Onivyde	Ipsen biopharmaceuticals	Liposomes	Metastatic pancreatic cancer	Increased delivery to tumor site, decreased systemic toxicity	i.v.	October 2015

(continued)

Table 18.1 (continued)

Drug	Nanomedicine	Manufacturer	Delivery system	Indications	Benefits over free drug	Route of administration	FDA approved Year
Verteporfin	Visudyne	Bausch and Lomb	Liposomes	Age-related macular degeneration, myopia, ocular histoplasmosis	Increased delivery to site of diseased vessels, photosensitive release	i.v.	April 2000
Cytarabine	Depocyt	Sigma-tau	Liposomes	Lymphomatous meningitis	Increased delivery to tumor site, decreased systemic toxicity	i.v., i.t.	August 1999
Polymer							
Pegademase bovine	Adagen	Leadiant biosciences	PEGylated adenosine deaminase enzyme	Severe combined immunodeficiency disease associated with adenosine deaminase deficiency	Longer circulation time, decreased immunogenicity	i.m	March 1990
Pregasprase	Oncaspar	Baxalta U.S.	PEGylated L-asparaginase	Acute lymphoblastic leukemia	Greater protein stability	i.v., i.m.	July 2006
Pegfilgrastim	Neulasta	Amgen	PEGylated granulocyte colony stimulating factor	Neutropenia associated with cancer chemotherapy	Greater protein stability	s.c.	January 2002

Pegvisomant	Somavert	Pfizer	PEGylated human growth hormone receptor antagonist	Acromegaly	Greater protein stability	s.c.	March 2003
Leuprolide acetate	Eligard	Tolmar	Polymeric nanoparticles	Prostate cancer	Longer circulation time, controlled payload delivery	s.c.	January 2002
Protein							
Denileukindiftitox	Ontak	Eisaii Inc	Protein-drug conjugate	Cutaneous T-cell lymphoma	Targeted T-cell specificity, lysosomal escape	i.v.	February 1999
Paclitaxel	Abraxane	Abraxis bio science, AstraZeneca	Protein-drug conjugate	Breast cancer, NSCLC, pancreatic cancer	Greater solubility, increased delivery to tumor	i.v.	January 2005
Monoclonal human EGF receptor-2antibody and DM1	Kadcyla	Genentech	Protein-drug conjugate	Metastatic breast cancer	Selectively deliver to EGF receptor-2-expressing cells	i.v.	February 2013
Nanocrystal							
Sirolimus	Rapamune	Wyeth pharmaceuticals	Nanocrystals in tablets	Immunosuppressant	Greater bioavailability	Oral	August 2000

Table 18.2 FDA-approved nanoparticles available for treatment of diseases other than cancer

Drug	Name of nanoformulation	Manufacturer	Delivery System	Indications	Benefits over free drug	Route of administration	FDA approved Year
Liposomal							
Poractant alfa	Curosurf	Chiesi USA	Liposomes	Respiratory distress syndrome	Increased delivery with smaller volume, decreased toxicity	Intratracheal	October 1998
Morphine	DepoDur	Pacira pharmaceuticals	Liposomes	Postoperative analgesia	Extended release	Epidural injection	May 2004
Amphotericin B	Fungizone	Apothecon	Surfactant-based nanoformulation	Systemic fungal infections	Increase solubility	i.v.	March 1966
	Abelcet	Sigma tau	Lipid-based (non-liposomal)	Invasive fungal infections	Decreased toxicity	i.v.	November 1995
	Amphotec	Alko Pharma USA	Lipid-based (non-liposomal)	Fungal infections	Decrease infusion time	i.v.	November 1996
	Ambisome	Gilead Sciences	Liposomes	Fungal and protozoal infection	Significantly lower nephrotoxicity, and infusion-related chills/ rigors	i.v.	August 1997
Bupivacaine	Exparel	Pacira pharmaceuticals	Liposomes	Post-surgical analgesia	Sustained release, increase safety	Local/ Depofoam	November 2011

Polymer							
Glatimer acetate	Copaxone	TEVA pharmaceuticals	Polymeric drugs	Multiple sclerosis	Controlled clearance	s.c.	January 1997
Pegaptanib	Macugen	Bausch and Lomb	PEGylated anti-VEGF aptamer	Age-related macular degeneration	Greater aptamer stability	Intravitreal	December 2004
Antihemophilic factor (recombinant)	Adynovate	Shire	PEGylated polymer protein conjugate	Hemophilia	Greater protein stability, longer half-life	i.v.	November 2015
Certolizumab pegol	Cimzia	UCB	PEGylated humanized antiTNF-alpha antibody fragment	Rheumatoid arthritis, Crohn's disease, psoriatic arthritis, ankylosing spondylitis	Longer circulation time, greater stability in vivo	s.c	May 2009
Pegloticase	Krystexxa	Horizon	PEGylated polymer protein conjugate	Chronic gout	Greater protein stability	i.v.	September 2010
Epoetin beta	Mircera	Hoffman-LaRoche	PEGylated polymer protein conjugate	Anemia associated with CKD	Greater aptamer stability	i.v., s.c.	November 2007
IFN alpha-2a	Pegasys	Genentech	PEGylated polymer protein conjugate	Hepatitis B, hepatitis C	Greater protein stability	s.c.	October 2002
IFN alpha-2b	PegIntron	Merck	PEGylated polymer protein conjugate	Hepatitis C	Greater protein stability	s.c.	January 2001

(continued)

Table 18.2 (continued)

Drug	Name of nanoformulation	Manufacturer	Delivery System	Indications	Benefits over free drug	Route of administration	FDA approved Year
IFN beta-1a	Plegridy	Biogen	PEGylated polymer protein conjugate	Multiple sclerosis	Greater protein stability	s.c.	August 2014
Coagulation factor IX (recombinant)	Rebinyn	Novo Nordisk	GlycoPEGylated	Hemophilia B	Longer half-life, greater drug levels between infusions	i.v.	June 2017
Poly(allylamine hydrochloride)	Renagel and Renvela	Genzyme	Polymeric drug of sevelamer hydrochloride and Sevelamer carbonate	Chronic kidney disease (CKD) on dialysis	Longer circulation time and therapeutic delivery	Oral	October 1998
Triamcinolone acetonide ER	Zilretta	Flexion therapeutics	Polymeric drug	Osteoarthritis knee pain	Extended release	Intra-articular	October 2017
Nanocrystal							
Aprepitant	Emend	Elan, Merck	Nanocrystal	Antiemetic	Greater absorption and bioavailability	Oral	March 2003
Megestrol acetate	MegaceES	Par pharmaceuticals	Nanocrystal	Anorexia, cachexia	Lower dosing	Oral	July 2005
Fenofibrate	TriCor	Elan, Abbott	Nanocrystal	Anti-hyperlipidemic	Greater drug loading and bioavailability	Oral	May 2004

Morphine sulfate	Avinza	Pfizer	Nanocomplex	Psychostimulant	Greater drug loading and bioavailability, extended release	Oral	March 2002
Methylphenidate HCl	Ritalin LA	Novartis	Nanocomplex	Psychostimulant	Greater drug loading and bioavailability	Oral	June 2002
Dexamethylphenidate HCl	Focalin	Novartis	Nanocomplex	Psychostimulant	Greater drug loading and bioavailability	Oral	May 2005
Hydroxyapatite	EquivaBone	Zimmer Biomet	Nanocrystal	Bone substitute	Mimics bone structure	Bone graft	September 2009
	NanOss	RTI surgical	Nanocrystal	Bone substitute	Mimics bone structure	Bone graft	2005
	Ostim	Heraeus Kulzer	Nanocrystal	Bone substitute	Mimics bone structure	Bone graft	2004
	OsSatura	IsoTis Orthobiologics	Nanocrystal	Bone substitute	Mimics bone structure	Bone graft	2003
Calcium phosphate	Vitoss	Stryker	Nanocrystal	Bone substitute	Mimics bone structure	Bone graft	2003
Paliperidone Palmitate	Invega Sustenna	Janssen	Nanocomplex	Schizophrenia, schizoaffective disorder	Slow release	Oral, i.m	December 2006
Dantrolene sodium	Ryanodex	Eagle pharmaceuticals	Nanocomplex	Malignant hypothermia	More rapid rate of administration at higher doses	i.v.	July 2014

(continued)

Table 18.2 (continued)

Drug	Name of nanoformulation	Manufacturer	Delivery System	Indications	Benefits over free drug	Route of administration	FDA approved Year
Tizanidine HCl	Zanaflex	Acorda	Nanocomplex	Muscle relaxant	Greater drug loading and bioavailability	Oral	August 2002
Inorganic							
Iron dextran	Infed	Actavis Pharma	Nanocomplex	Iron deficiency in CKD	Increased dose	i.v.	August 1995
	Dexferrum	American regent		Iron deficiency in CKD	Increased dose		February 1996
Iron oxide	Feraheme	AMAG pharmaceuticals	Metal oxide nanoparticles	Iron deficiency in CKD	Prolonged, steady release with less frequent dosing	i.v.	June 2009
Sodium ferric gluconate	Ferrlecit	Sanofi-Aventis	Nanocomplex	Iron deficiency in CKD	Increased dose	i.v.	February 1999
Iron sucrose	Venofer	American regent	Nanocomplex	Iron deficiency in CKD	Increased dose	i.v.	November 2000
Emulsion							
Estradiol	Estrasorb	Novavax	Nanoemulsion	Vasomotor symptoms associated with menopause	Controlled delivery	Topical and transdermal	October 2003
Difluprednate	Durezol	Sirion therapeutics	Nanoemulsion	Eye inflammation, uveitis	Sustained release, increase absorption	Ocular	June 2008

Cyclosporine A	Restasis	Allergan	Nanoemulsion	Dry eye syndrome	Sustained release, increase absorption	Ocular	October 2003
Imaging agents							
Iron oxide	Feridex	AMAG pharmaceuticals	Metal oxide nanoparticles	Liver/spleen lesion magnetic resonance imaging	Vertical irritant effect	i.v.	February 1996
Silicone-coated iron oxide nanoparticles	Lumirem	AMAG pharmaceuticals	Silicone-coated, superparamagnetic iron oxide	Imaging agent	Vertical irritant effect	Oral	May 1996

Source References: [7, 31, 54–59]

18.9 Toxicity Aspect of Nanomaterials

In the following section, we have described in brief the observed toxicity of nanomaterials used for medicinal purposes in an organ-specific/physiological system-dependent manner.

18.9.1 Neurotoxicity

Several nanomaterials such as polymeric nanoparticles, liposomes, inorganic metallic nanoparticles, carbon nanotubes, dendrimers, quantum dots, etc., have been applied for diagnosis and treatment of brain diseases. They can enter the brain by penetrating through the blood–brain barrier with the help of a series of transporters or receptors expressed on the endothelial cells of brain capillaries, or through adsorption-mediated transcytosis, or through the intranasal route; bypassing the blood–brain barrier [60]. Nanosized drug carriers, if permeating through the blood–brain barrier can interact with the hippocampal cells of the brain and can cause alteration of brain functions [61]. The common mechanisms of nanoformulation-induced neurotoxicity involves oxidative stress, induced cell apoptosis and autophagy, and immune response and inflammation resulting in activation-specific signaling pathways which can subsequently alter the neuronal structure or activity and also can alter the function of the blood–brain barrier [60]. Charge of nanoparticles has an impact on its permeability through the blood–brain barrier. For example, anionic wax nanoparticles were found to penetrate the blood–brain barrier better than its neutral or cationic counterparts [62]. Considering the role of surfactants in penetration of nanoparticles through the blood–brain barrier and toxicity of polymeric nanoparticles in the neuronal system, one study revealed that Polysorbate 80-modified chitosan nanoparticles after intravenous injection in rats causes a dose-dependent accumulation of the nanoparticles in the frontal cortex and cerebellum, with neuronal apoptosis, mild inflammation, increased oxidative stress, and loss of body weight [63]. Liposomes are considered to be superior over many other formulations in delivering drugs through the blood–brain barrier but empty liposomes had also found to possess toxic effects on the neuronal system. In a comparative study of the cisplatin containing liposomal formulation, free-drug and drug-free liposomes, it was found that cisplatin-loaded liposomes increased in vitro cytotoxicity against glioma cells and high tumor retention in glioma-bearing rats compared to the free cisplatin but the drug-free liposomes also induced minimal to severe neuroinflammation and necrosis in control rats. This study suggested the intrinsic toxicity of the liposomes alone [64]. Dendrimers of different generations with various surface groups were used to assess the neurotoxic effects on human neural progenitor cells. It was found that cationic dendrimers of higher generation altered mitochondrial activity, induced oxidative stress, apoptosis, and subsequent DNA damage; also can interfere with neuronal differentiation, and gene expression. Metal nanoparticles were also studied extensively to elucidate their neuronal toxicity [65]. Gold nanoparticles may accumulate in the brain where through transportation

via the BBB or olfactory nerve and can induce neurotoxic effects such as increased seizure activity, cognition effects, and astrogliosis [66]. The neurotoxicity of silver nanoparticles involves increased ROS generation, caspase activity, and cytokine release resulting in inflammation and cell death. Additionally, the release of silver ions from nanoparticles of silver or its oxides can directly trigger necrosis through the disruption of membrane integrity [67]. Iron oxide nanoparticles were also found to alter synaptic transmission and nerve conduction leading to several inflammatory responses [68]. TiO_2 nanoparticles were also reported to induce similar toxicities resulting in the alteration of synaptic plasticity and disrupted signaling pathways [69]. The intranasal administration of silica nanoparticles also leads to the accumulation of nanoparticles in the brain and subsequently to cognitive dysfunction and impairment, synaptic changes, and pathologies similar to neurodegeneration. Carbon nanotubes enter the brain through olfactory or systemic administration [70]. Studies have shown that the inhaled carbon nanotubes can accumulate in the olfactory bulb, causing activation of microglial cells and subsequent inflammatory responses. Multi-walled carbon nanotubes (MWCNT) were reported to induce higher neurotoxic effects than single-walled carbon nanotubes (SWCNT) [71]. Neurotoxicity associated with carbon nanotubes can also result in neurobehavioral changes such as anxiety and depression [60]. Oberdörster et al (2004) studied the effect of colloidal fullerenes on the neuronal system of largemouth bass and observed increased lipid peroxidation in brain and reduction of glutathione in gills after 48 h of dosing [72]. The neurotoxic effects of quantum dots are similar to the general neurodegenerative toxicity, including increased oxidative stress and cell function damage; and are dependent on their size, surface charge, concentration, surface coating, the nature and the solubility of the constituent materials [60].

18.9.2 Cardiotoxicity

Metal nanoparticles were most extensively studied to elucidate their toxicity to the heart. Using a zebrafish model, it was revealed that titanium nanoparticles can translocate between organs and can accumulate in the heart through crossing the blood-heart barrier. Thinning of cardiac muscles, tissue inflammation followed by cell necrosis and cardiac biochemical imbalance are the consequences of chronic exposure to TiO_2 nanoparticles. Molecular analysis revealed that TiO_2 nanoparticles can bind with lactate dehydrogenase (LDH) thereby increasing its activity along with the activity of some other enzymes such as AST (aspartate aminotransferase), CK (creatine kinase), HBDH (α-hydroxybutyrate dehydrogenase), which leads to myocardial injury [73]. The other reasons found to be the elevation of ROS (reactive oxygen species) level: mitochondrial swelling, increased activity of caspase-3, and augmentation of DNA peroxidation in cardiac muscle [74]. ZnO nanoparticles are mainly bioaccumulated by the interaction of Zn and sulfur-containing proteins. ZnO nanoparticles were found to be toxic in both in vitro and in vivo studies. Apart from heart, acute oral exposure to ZnO nanoparticles also targets liver, spleen, pancreas, and bone [75]. Long-term exposure of rats to ZnO inhalation was reported to cause

both cardiac damage and lung inflammation [76]. In order to find a correlation between cardiac and respiratory toxicity of ZnO nanoparticles, Bessemer et al (2015) conducted a study on freshwater fish model *Catostomus commersonii*. They concluded that myocardial damage was due to increased parasympathetic input in the heart, which is a consequence of gill neuroepithelial cell damage by ZnO [77]. While studied at the molecular level, increased level of troponin T, CPK-MB (creatine phosphokinase-MB), and myoglobin were found to be responsible for ZnO-induced myocardial damage [78]. Like TiO_2 and ZnO, Ag nanoparticles can also induce ROS and upregulation of cytokine activity, thus producing oxidative stress and inflammation. While searching for molecular marker behind Ag-induced myocardial injury, in a study on chicken, it was found that Ag nanoparticles downregulate FGF-2 (fibroblast growth factor-2, a modulator of cardiomyopathy) and upregulate VEGF-A (vascular endothelial growth factor-A, an angiogenesis modulator) [79, 80]. Inhaled carbon nanoparticles and carbon nanotubes can also cause cardiac damage through depletion of serum thiol content and an increase in lipid peroxidation products. Administration of multi-walled carbon nanotubes (MWCNT) through intratracheal instillation can worsen ischemia/reperfusion (I/R) injury [74]. Both intravenous and intratracheal administration of fullerene was found to cause myocardial infarction [81]. Exposure to silica nanoparticles can also create inflammatory responses in the cardiovascular system and is associated with an increased level of eotaxin-1, LDH (lactate dehydrogenase), and CKMB (creatine kinase MB) [82].

18.9.3 Pulmonary Toxicity

The main toxic effects of nanoparticles on the pulmonary system are inflammation, oxidative stress, and functional disturbances. Inorganic metal nanoparticles such as Co, TiO_2, SiO_2, Ni, and ZnO were found to induce lung epithelial damage, leading to inflammation and this effect is prominently higher in case of nanosized particles compared to their macrosized congeners. Carbon black nanoparticles can also generate similar inflammation and its effect is worse than ZnO nanoparticles. Release of interleukin-8 is responsible for such inflammatory conditions [61]. Heavy metal nanoparticles like cadmium, iridium, and gold showed variable toxicity depending on their solubility and reactivity to the tissues. Inhalation of insoluble iridium and gold nanoparticles did not induce pulmonary inflammation, while soluble cadmium nanoparticles at high doses were found to induce pulmonary injury via translocation from the lung to the liver [83]. Exposure to carbon nanoparticles are mainly through inhalation in occupational level, hence lung is the primary target for carbon nanoparticles. But inhaled carbon nanoparticles and carbon nanotubes can distribute in heart, liver, kidney, and brain and cause multiple dysfunctions such as necrosis of liver and kidney tissue, inflammation, depletion of serum antioxidants such as GSH (glutathione) and SOD (superoxide dismutase), and abnormalities of alveolar microvessels [84]. When instilled in the lung, SWCNTs (single-walled carbon nanotubes) can be phagocytosed by lung epithelial cells and

these result in both local and systemic inflammation [74]. A study about carbon black nanoparticle toxicity revealed that these nanoparticles can increase intracellular calcium by controlling cellular ion channels, resulting in impaired phagosome transport and cytoskeletal dysfunction [85]. Presence of airway inflammation, asthma, and obstructive pulmonary diseases can increase the retention of inhaled nanoparticles and this could worsen the situation. This hypothesis was also tested in animal models [86]. Smaller sized nanoparticles were found to be superior in the induction of inflammatory responses compared to larger nanoparticles or microparticles. Tumorigenesis associated with the inhalation of nanoparticles is invariably related to its size and the smaller congeners are often more severe than their larger analogs. This is supposed to be due to poor detection of smaller nanoparticles by macrophages thereby improper clearance, resulting in nanoparticle buildup, chronic inflammation, fibrosis, and eventually tumorigenesis [87].

18.9.4 Hemotoxicity

Hemotoxicity of nanoformulations is directly related with their circulation half-life, interaction with RBC and macrophages, ability to escape from hepatic reticuloendothelial system, and affinity for enzymes present in serum. The size of nanoparticles regulates their persistence in circulation in a variable way. Nanoparticles with 5–10 nm diameters are rapidly cleared from systemic circulation after systemic administration but those with 10–70 nm diameters can penetrate through blood capillary wall and distribute easily throughout the body. Nanoparticle with 70–200 nm diameters needs longer time to penetrate the blood capillary wall and also can persist in systemic circulation for a longer period. Hence, their toxicity also varies depending on their circulation half-life. Senior and Gregoriadis found that neutral liposome with a diameter of less than 100 nm had circulation half-life up to 20 h while the anionic liposomes had a half-life of less than 1 h [88]. The high surface to volume ratio nanoformulations provide a large exposure of surface molecules toward the circulation system, and this is one of the major reasons behind nanoformulation-related RBC damage. Another important factor is the surface charge which interacts with the cell membrane of RBC. Cationic polystyrene nanoparticles were found to cause hemolysis and blood clotting while such effects were absent in case of its anionic counterparts. Carbon nanotubes were found to cause platelet aggregation and in vivo thrombosis in experimental models while carbon fullerenes with almost similar diameter had not produced such effects. This can be explained with the difference of their shape which plays an important role in binding with platelets [61].

18.9.5 Hepatotoxicity and Nephrotoxicity

Polymeric nanoparticles used in drug delivery have variable effects on liver depending on their physicochemical properties such as functional groups present,

size, biodegradability, etc. For example, polyalkylcyanoacrylate nanoparticles were found to produce mild and reversible inflammatory condition to the liver in animal model due to their biodegradable nature and rapid clearance from systemic circulation. Polystyrene nanoparticles indeed, produce severe toxicity in the liver due to their nonbiodegradable nature and prolonged circulation [61]. Polyamidomamine dendrimers can induce lysosomal dysfunction in the liver, resulting in vacuolization of hepatocytes as observed in the experimental mice model [89]. Acrylic nanoparticles like cyanoacrylate and isobutylcyanoacrylate nanoparticles showed high accumulation in kidneys in experimental rats and this can cause renal injury and proteinuria [90]. Metal nanoparticles were studied extensively to elucidate their effects on hepatorenal system. Isoda et al (2017) reported size-dependent toxicity of Pt nanoparticles with a comparative study of 1 nm and 8 nm Pt nanoparticles and found that 1 nm Pt nanoparticle can cause acute hepato-renal injury in mice by increasing serum aminotransferases and blood urea nitrogen [91]. They also concluded that upregulation of interleukin-6 and interleukin-1β is responsible for such hepatic and renal injury, respectively. Similar findings were also reported by Yamagishi et al (2013) who found that administration of Pt nanoparticles less than 1 nm diameter to mice for several weeks produces urinary casts, tubular atrophy, and accumulates inflammatory cells [92]. Negatively charged superparamagnetic iron oxide nanoparticles were found to significantly damage actin cytoskeleton of kidney and brain cells in both in vitro and in vivo studies [74].

18.9.6 Genotoxicity

Nanoparticles when entered inside the cell can interact directly with the nucleus and transport into the nucleus through the formation of nuclear pore complexes (NPC). As the diameter of NPC is around 30 nm, nanoparticles with a diameter of 30 nm or less can cross nuclear envelope through NPC. Nanoparticles larger than 30 nm enter into the nucleus during mitotic division when the nuclear membrane dissembles. Inside the nucleus, nanoparticles can interact with DNA and affect replication and transcription of DNA. In a study, Li et al (2014) showed nanoparticles of 3–46 nm size have a high affinity for DNA and strongly inhibit replication of DNA [93]. Tsoli et al (2005) reported gold nanoparticle of 1.4 nm size interacts with major grooves of DNA in a unique manner which could account for its genotoxicity [94]. Not only size but also the charge of NPs can affect its transportation into the nucleus. In a study using THP-1 cells, Nabiev et al (2007) demonstrated that green quantum dots (2.1 nm) can enter the nucleus through NPC while the red ones (3.4 nm) cannot, and concluded such transportation is mediated by histone binding [95]. Apart from direct interaction with DNA, nanoparticles can interfere with DNA repair through the interaction of DNA repairing molecules in BER and NER pathways (base excision repair and nucleotide excision repair). Carbon nanotubes due to their similarities with cellular microtubules can interact or mimic mitotic spindle, resulting in loss or gain of chromosomes (known as aneugenic effect). Such aneugenic effects were also observed in CuO and gold nanoparticles [96, 97]. Transition metal nanoparticles

(like Fe, Ag, Cu, Mn, Ni NPs) can release free metal ions which can directly induce ROS generation through Fenton reaction and this accounts for a major metal nanoparticle-induced genotoxicity as a consequence of oxidative stress. This phenomenon is also known as inflammation-induced or secondary genotoxicity. International Agency for Research on Cancer (IARC) evaluated that amorphous silica is not carcinogenic to humans but the crystalline is carcinogenic. Several in vivo studies using amorphous SiO_2 have supported this with mild or no genotoxicity induction (DNA damage) [98]. For crystalline silica, the secondary inflammation-driven genotoxicity mechanism is recognized as an important mechanism for its carcinogenicity. In several in vitro studies, it was found that smaller SiO_2 nanoparticles induce more toxicity due to higher penetration and ultimate lysosomal overload. Although most of the in vivo studies reported TiO_2 anatase did not induce micronuclei formation in hepatic reticulocytes and leukocytes but some of the studies reported significant micronuclei formation in bone marrow cells and peripheral blood cells (erythrocytes), which is a hallmark of genotoxicity [98]. However, intratracheal or inhalation administration of TiO_2 nanoparticles to mice and rat has been reported to induce inflammation in the lung but not significant genotoxicity in both lung epithelial lung cells and erythrocytes [99]. Intraperitoneal administration of TiO_2 NPs has been found to accumulate titanium in liver, kidney, and bone marrow and to induce oxidative stress-mediated genotoxicity in those organs [100]. In the case of gold nanoparticles, high genotoxicity was found in the administration of larger nanoparticles compared to smaller ones. Both acute and chronic intraperitoneal administration of differently sized AuNPs (10 nm and 30 nm, citrate coated) in rats induced DNA damage in blood and liver cells as evaluated by comet assay [101]. As gold can cross the blood–brain barrier, while studying genotoxicity of AuNPs in rat it was found to cause DNA damage in the cerebral cortex. Toxicity of gold nanoparticles greatly depends on its surface chemistry and manufacturing methods. For example, AuNPs prepared in aqueous media did not produce cyto- or genotoxicity but those prepared from pure acetone solution produced remarkable genotoxicity as studied by Di Bucchianico et al (2015) [102]. They concluded the presence of amorphous carbon and enolate ions on the surface of acetone-derived gold nanoparticles were responsible for this effect. Genotoxicity of silver nanoparticles was reported to be mainly oxidation-induced and was prominent in case of larger nanoparticles (200 nm). Several in vitro studies revealed genotoxicity and mutagenicity of silver nanoparticles in vitro, and were confirmed by micronuclei formation, DNA double-strand break, and comet assay [98]. Intranasal administration of MWCNT was found not to induce genotoxicity in lung up to 90 days of treatment but produced pulmonary inflammation in animal models (rats) [103]. Even they (MWCNTs) have not induced DNA damage or micronuclei formation in peripheral blood leukocytes or bone marrow erythrocytes. On the other hand, SWCNT and carbon nanofibers even after single-dose administration can persist in the lung for a long period and can induce micronuclei formation, nuclear protrusions, and pulmonary fibrosis [84]. Catalán et al (2016) showed that the structure and dispersion of carbon nanotubes and their administration routes can give variable results. They showed straight-walled MWCNTs were able to induce DNA damage in mouse BAL (broncho-alveolar lavage) cells after inhalation but not after pharyngeal

aspiration, while both straight-walled and tangled MWCNT can induce DNA damage in lung cells (alveolar) irrespective of aforementioned routes of administration [104]. When functionalized by carboxylation, both SWCNT and MWCNT induced chromosomal aberration in bone marrow cells [98]. This suggests the presence of important interaction of COOH groups with chromosomes in vivo. Dendrimers can form complexes with DNA and this complexion can damage DNA through distortion or complete separation of double-stranded DNA. G4 and polyamidoamine dendrimers were reported to induce considerable genotoxicity [61].

18.9.7 Methods of Assessment of Toxicity of Nanomaterials

Nanotoxicity refers to the biological adverse effects caused by nanomaterials. Toxicity assessments of nanomaterials should follow a standardized set of rules to avoid confusion and misconduct in designing nanomaterials for biomedical applications [60]. Overall walkthrough of nanomaterial toxicity assessment involves (a) characterization of nanomaterials, (b) in vitro and in vivo studies, and (c) final clinical trials. Subsequently, we discussed these aforementioned analyses in detail.

(a) Characterization of nanomaterials: There are several essential physicochemical characteristics to be studied. They include but not limited to, particle shape and size, distribution, surface charge and reactivity, surface area, chemical composition, solubility and partition properties, aggregation tendency in relevant media, crystallinity, porosity, and sample purity. Chemical reactivity, surface chemistry, redox potential, and photocatalytic activity are some of the chemical analysis necessary to identify the chemical nature of nanomaterials. Nanomaterial characterization is generally achieved through spectroscopy, electron microscopy, X-ray diffraction, differential light scattering, magnetic resonance, mass spectrometry, chromatography, zeta potential measurement, thermal techniques, and circular dichroism [60]. Sterility of the formulations is generally assessed by endotoxin test through kinetic turbidity LAL assay [105]. Standardized guidelines should be implemented on the physicochemical characterization of nanomaterials to generate reproducible nanomaterials with desired physiochemical properties. The results obtained in this step should be relevant to the objective and end-point target of the study. Interaction of nanomaterials with body components, especially with proteins and receptors can change its surface characteristics. So, there is a possibility of discrepancies between cellular studies and theoretical predictions based on physicochemical properties and care should be taken to assess such evaluations. Furthermore, combination with biological macromolecules can promote intracellular uptake, reducing body clearance and can lead to chronic and degenerative changes.

(b) In vitro studies: In vitro toxicity assessments are crucial for investigating the mechanism of nanomaterial-induced toxicity on biological entities. Conventional in vitro models consist of different cell culturing systems, xenograft models, and studies on tissue sections. Choice of cultures and tissues depends on the organ of interest on which toxicity needs to be evaluated. In vitro toxicity assessments can be categorized into cell proliferation, apoptosis, necrosis, cell cycle, oxidative stress,

and DNA damage assays. The most common methods involved in in vitro experiments are fluorescence, chemiluminescence, analytical, and molecular marker-based detection systems; often accompanied by chromatographic separation techniques [60]. Cell proliferation analysis is the primary study to detect both the efficacy of cytotoxic agents and unwanted toxicity toward cells of 'not interest'. Cytotoxicity is generally assessed by MTT assay and LDH assay [106]. Cell death can occur through two different routes, namely apoptosis and necrosis. Apoptosis is a programmed cell death, characterized by changes in the nuclear morphology owing to chromatin fragmentation and condensation and identified by specific biomarkers (translocation of phosphatidylserine), occurrence of apoptotic bodies, and cell shrinkage. The main mechanisms of apoptosis involve caspase activation, mitochondrial swelling, release of cytochrome c, and DNA fragmentation. On the other hand, necrosis represents accidental cell death due to trauma, hypoxia, or pathogens; characterized by nuclear swelling, chromatin flocculation, loss of organelle function, membrane break, extracellular release of cytoplasmic content, etc.; and can be identified by microscopic studies. Another necessary assay involves the detection of oxidative stress as a response to the exposure of cells to nanomaterials. Major in vitro studies involve estimation of ROS (reactive oxygen species), activity of GSH (glutathione), and SOD (superoxide dismutase). In vitro studies in the evaluation of genotoxicity involves mutation testing in bacteria and mammalian cells, in vitro cytogenic effects and micronuclei analysis, micronuclei testing in erythrocytes, comet assay, and chromosomal aberration in bone marrow cells [98]. The major limitations of in vitro assay using cell culture involve the inability of the cell to mimic the native tissue microenvironment and reactivity of formulation ingredients with the assay components. However, the recent development of organ culture established in vitro studies more reliable in evaluation of actual phenomena happening in vivo on exposure to nanomaterials and also opened a new era of diverse biological experiments [60].

(c) In vivo studies: In vivo experiments are mandatory for investigation of nanomaterial toxicity and are superior over other methods as they allow assessment of physiological action for the whole organ and cannot be modeled in vitro. Several in vivo models and techniques have been developed to assess the organ distribution of nanomaterials. The most widely used invasive techniques are analysis of blood and tissue sampling after intravenous injection, microdialysis, quantitative autoradiography, and autopsy. The most popular noninvasive techniques are fluorescent and radio-imaging [60]. Assessment of organ-specific toxicity involves the measurement of various parameters specific to the organ system. For example, assessment nanomaterial-induced neurotoxicity primarily involves evaluation of behavioral changes regarding movement, learning, memory, motor coordination or reflexes, tremor, or paralysis; analysis of synthesis, release, and uptake of neurotransmitters; and histopathological observation of neuronal system [107]. Regarding cardiotoxicity assessment, the primary studies involve echocardiography, cardiac magnetic resonance imaging, Speckle-Tracking imaging, and analysis of several biomarkers such as troponin I, CKMB (creatine kinase-MB), LDH (lactate

dehydrogenase), myoglobin, myeloperoxidase, FGF-2 (fibroblast growth factor-2), NT-proBNP (N-terminal pro-B type natriuretic peptide), etc. [74].

18.10 Theranostic Applications of Nanomedicines

The term "Theranostic" was first coined by Furkhouser in 2002. It refers to "any material" with a dual ability of therapeutic and diagnostic potential. The application of nanotechnology in the field of therapeutics gives rise to the development of nanotheranostics which provide significant promise to develop much effective precision medicine by tuning the treatment depending on the molecular understanding of the disease and genetic makeup of the patients leading to protection of patients from adverse side effects. Thus, nanotheranostics help to monitor simultaneously the bioavailability of therapeutics and noninvasive evaluation of therapeutic efficacy in real time. Thus, nanotheranostics belong to a platform which provides integration between molecular therapy and molecular imaging. This integration offers myriads of promising characteristics such as early detection of disease, disease staging, therapy selection, planning and scheduling of treatment, identification of adverse effects at early stages of the treatment, and finally, planning of follow-up therapies. Plenty of researchers prefer to see the nanotheranostics as an integrated platform of nanomedicine and nanosensor due to the ability of nanosensor to identify significant numbers of biomarkers from a small sample volume and nanomedicine can extravasate from the blood vessel and deliver the therapeutic payloads predominantly into the target tissue by receptor-mediated active targeting. Plethoras of materials used for the development of nanomedicines are explored for the production of theranostic nanomedicines as described below.

18.10.1 Drug-Polymer Conjugate

Covalent interaction between drug and polymer depending on the functional group present in drug and polymer carrier resulted in the formation of drug–polymer conjugate. N-(2-hydroxypropyl) methacrylamide (HPMA) polymer is predominantly explored for the formation of drug-polymer conjugate because of its stability, nontoxicity, and biocompatibility for in vivo application. I-131 labeled HPMA-doxorubicin conjugate (HPMA-DOX conjugate) had been studied in Phase-1 clinical trial.

18.10.2 Polymers, Liposomes, Micelles, and Dendrimers

Nanocarriers made up any of these platforms have been widely explored to deliver the drug to the central nervous system (CNS) through the blood–brain barrier, neoplastic cells, and to remote organs such as lungs due to their biocompatibility, stability, cellular membrane-mimicking properties, and their ability to release the

drug in a sustainable manner. They can be converted into theranostic nanomedicine by dual loading of imaging modalities and therapeutic entities. Examples of imaging modalities include magnetic resonance imaging (MRI) contrast agents, radioactive agents for radionuclide imaging via positron emission tomography (PET) or single-photon emission computed tomography (SPECT), fluorescent agents for fluorescent imaging, and nano/microbubbles for ultrasound imaging. Each imaging modality has its own advantages and disadvantages and therefore their usage depends on their suitability for the maximum desired outcome.

18.10.3 Noble Metal Nanoparticles

Gold and silver at their nanodimension acquire optical properties known as surface plasmon response which occurs due to excitation and relaxation from the surface of nanoparticles and the surrounding solution. Optical property can be modulated by tailoring their size, shape, and surface properties. The use of surface plasmon response for cancer detection has limited to superficial sites due to their inability to penetrate deep even in the presence of near-infrared region where the absorbance of tissue is minimum. In contrary, noble metal nanoparticles provide promising outcome X-ray computed tomography (CT) imaging as they are highly dense in comparison to human soft tissues due to the presence of certain vital characteristics such as higher X-ray absorption coefficient, long circulation time in blood, and high surface area for easy attachment of targeting and therapeutic agents. They create high-contrast regions by dampening the amplitude of X-ray leading to much better, noninvasive real-time molecular imaging of solid tumors as compared to iodine, the commonly used CT contrast agent.

Heo et al (2012) synthesized gold nanoparticles (AuNPs) functionalized with PEG, biotin, and rhodamine B-linked beta-cyclodextrin with an objective to function as a theranostic system for the treatment of glioma. Among the two types of nanoformulations developed by them, AuNPs-5 showed a more promising result as it exhibited much better interaction with cancer cells as compared with normal cells. Further, the authors revealed that the developed theranostic system can simultaneously monitor pharmacokinetic profiles of loaded-drug and detection of cancer cells upon induction by laser light [108].

18.10.4 Quantum Dots (QDs)

Pioneer work by Brus and his coworkers at the Bell laboratories gave the birth of QDs in the year 1983. QDs are inorganic semiconductor nanocrystals which can serve as a versatile tool for molecular diagnostics and nanotherapeutics. The absorption and emission spectra of QD are predominantly dependent on size and thus optical spectrum can be finely adjusted by tailoring the size of the nanoparticulate core. Among the various types of QDs, cadmium selenide (CdSe)/zinc sulphide (ZnS)-based QDs are most popularly explored for diagnostic purpose and they

contain a core made up of CdSe which is overcoated with layers of ZnS. QDs offer long-term repetitive bright imaging as they are devoid of photobleaching, overcoming the disadvantages associated with the organic chromophore. One serious drawback associated with QDs is the toxicity of cadmium and their inability to penetrate to a deeper part of tissues leading to the detection of cancers at superficial sites such as skin cancer, esophageal cancer, etc.

Yang et al (2017) developed photostable and multifunctional carbon QDs (known as carbon dots) which was tailored with polyamine containing organosilane molecules for simultaneous cell imaging and anticancer drug delivery. The amine groups of polysilane allowed extremely high loading of doxorubicin (DOX), i.e. 62.8%. Further, the surface hydroxyl groups ensured its significantly good dispersibility in water and the fluorescence property enabled to dynamically trace the drug-release characteristics. Results of in vitro investigations revealed carbon dot-doxorubicin complex (CDs –DOX) was effectively internalized by MCF-7 cells and upon internalization, DOX detached from the complex and moved to the nucleus whereas CDs resided in cytoplasm. Findings of the in vivo investigations revealed that CDs-DOX complex showed much improved performance as compared to free DOX. Further, in vivo investigation revealed that CDs–DOX showed negligible systemic toxicity and was able to successfully illuminate fungal, bacterial, and mammalian cells, signifying it to function as a universal cell imaging reagent. Finally, they concluded their investigations might accelerate the development of carbon dots as a novel nanotheranostic for various biomedical applications [109].

18.10.5 Carbon nanotubes

Cylindrical shaped carbon nanotubes (CNTs) are considered as allotropes of carbon with hindered biodegradation and poor biocompatibility. They can be branched into different types such as fullerene, CNTs, graphene, and carbon dots. All of these varieties have characteristic electronic and mechanical properties which make them suitable for theranostic applications. Further, both single-walled and multi-walled carbon nanotubes designated as SWCNTs and MWCNTs, respectively, have a high surface area and internal volume which are quite sufficient to simultaneously load the therapeutics and imaging agents.

18.11 Nanomedicines in Clinical Trials

Meanwhile, several nanodrugs are available commercially and many more are at clinical trials. Subsequent test-approval by FDA generally leads to clinical trials which are normally done to govern safety and efficacy in humans. These trials can be classified into phase I (dosing, toxicity, and excretion in healthy subjects), phase II (safety and efficacy in subjects with the target illness), and phase III (randomized, placebo-controlled, multicenter trials). When these trials are accomplished, a new drug formulation can be filed with the FDA for approval [1]. The bulk of the

nanoformulations which are in clinical development are generally based on different types of drug distribution methods such as polymeric, micelles, liposomes, dendrimers, and inorganic nanoparticles [110]. Liposomal nanoformulations of doxorubicin currently being studied in clinical trials are HER2-targeted MM-302 (Merrimack Pharmaceuticals, Inc.) and thermosensitive Thermodox (Celsion Corp.). HER2 targeting is expected to improve efficacy compared to non-targeted liposomal doxorubicin; a phase I clinical trial is well tolerated and the phase II trial of this formulation is ongoing in patients with HER2-positive breast cancer [10]. Thermodox comprehends liposome-bound doxorubicin formulated with thermally sensitive lipids. The combination of this nanodrug with radiofrequency thermal ablation shows site-specific targeted combat in phase III trials in the treatment of hepatobiliary tumors [4, 10, 111]. CPX-351 is another liposomal formulation containing dual drugs, cytarabine and daunorubicin, recently passed phase II clinical trial with improved efficacy and reduced side effects to sensitive patients. Pegylated liposomal formulations of irinotecan IHL-305 and MM-398 were also well tolerated in phase I clinical trial with reduced side effects (such as neutropenia and diarrhea) compared to commercially approved formulation FOLFIRI. MM-398 also crossed the hurdles of phase II and phase III clinical trials and is awaiting FDA approval. A phase II clinical study was conducted using liposomal irinotecan sucrosofate for metastatic pancreatic cancer-affected patients whose success led to a global phase III trial (NAPOLI-1) [112]. But this study was also not devoid of common adverse effects of anticancer agents such as diarrhea, nausea, anorexia, vomiting, alopecia, neutropenia, and leucopenia. Hepatocyte-directed vesicular (HDV) insulin is a nanoformulation of liposomal insulin that provides prolonged delivery of the drug directly to the liver. An oral formulation of HDV insulin is also undergoing evaluation in phase II and III clinical trials [10]. Among the liposomal formulations that underwent clinical trials, many are terminated due to low treatment benefits (in spite of reduced side effects too); e.g. L-NDDP, SPI-77, lipoplatin, and Li-PlaCis; or due to success of other formulations, e.g. LEP-ETU and EndoTAG-1 which were left over because of the success of albumin-based and polymeric formulations of paclitaxel (e.g. NK015).

A protein-based nanoparticle RSV-F (Novavax) containing a respiratory syncytial virus (RSV) fusion protein has completed phase II trial and is being used in healthy women of childbearing age. The formulations of Pulmaquin (Aradigm Corp.) in combination with liposomal and aqueous-phase ciprofloxacin have completed company-sponsored phase II studies in cystic fibrosis (CF) or non-CF bronchiectasis patients. An important candidate SGT-53 (SynerGene Therapeutics) containing anti-transferrin antibody fragment for its binding to glycoprotein receptor on cancer cells have completed phase I and II trials to use in glioblastoma, solid tumors, and metastatic pancreatic cancer [1, 10]. Dendrimer-based nanodrug DTXSPL8783 has been investigated in phase I clinical trials among patients with progressive cancer.

Polymeric nanoformulation can potentially improve chemo-radiotherapy treatment through tumor-specific delivery of the drugs, which increases efficacy while decreasing toxicity in normal tissues. Nanoformulations such as SN-38 and

Genexol-PM have been completed both the phase I and phase II trials in treating triple-negative breast cancer and advanced lung cancer, respectively. Genexol-PM also showed fairly low toxicity and good overall response (40–60%). SP1049C is a polymeric formulation of doxorubicin, which completed phase II clinical trial and obtained the title of orphan drug for the treatment of advanced gastric cancer and currently undergoing a phase III clinical trial. Opaxio and CRLX101 are two promising examples. Opaxio, a polyglutamic acid-conjugated paclitaxel formulation is currently under clinical investigation of ovarian and fallopian tube cancer. But while used in NSCLC (non-small cell lung cancer), Opaxio did not improve survival compared to Taxol, and when used in combination with carboplatin, it worsened the situation causing grade III/IV hemotoxicity (neutropenia, leucopenia) and significant neurotoxicity. CRLX101, a drug–conjugate formulation of camptothecin and a cyclodextran-PEG polymer, is being studied in numerous phase I and II clinical trials in the treatment of lung cancers (SCLC and NSCLC), gynecological malignancies, and solid tumors. Clinical studies of CRLX101 in renal cell carcinoma and gastrointestinal cancers have been completed and have shown promising early clinical results [10, 32, 113]. Nanoparticle Lipoxal that contains the drug Oxaliplatin, has been used in Phase II trials for colorectal cancer and glioma [114]. Docetaxel having the nano preparation LE-DT has completed Phase I/II in cure of solid tumors and pancreatic cancers. Drugs like K105 and Paclical are in Phase III trials to cure gastric cancer and ovarian cancer, respectively [10, 114].

Antimicrobial agents have also been profusely used in nanodrugs trials. Polymer nanoparticles with antibacterial properties are also being investigated in the treatment of active infections. Quaternary ammonium polyethylenimine-based polymers are promising as they have a potent activity to disrupt a number of gram-positive and gram-negative bacteria membranes. Such activity make this polymeric nanoparticle particularly promising [10, 115]. Polymeric nano-form of doxycycline have demonstrated a more sustained release and improved efficacy in the treatment of chronic periodontitis. Two polymeric nanoformulations of antiretroviral agents are being investigated for HIV treatment. Efavirenz, a non-nucleoside reverse transcriptase inhibitor, and Lopinavir, a protease inhibitor, are commonly used in combination therapy against HIV. NANOefavirenz and NANOlopinavir are nanoformulations of these antiretroviral agents that have been developed with the aim of reducing total dosage while maintaining clinical efficacy, thereby improving patient tolerability and decreasing treatment costs [10, 116]. One antifungal agent amphotericin B which has been used as nanoformulation (MAT2203) in phase II trials among chronic candidiasis patients. Another antiviral/antibiotic compound, VivaGel (Starpharma), is now being used in patients having bacterial vaginosis (BV) in phase III clinical trials after being effective in phase II.

Inorganic nanoparticles are also an excellent choice for nanoimaging technology and nanomedicine. Superparamagnetic iron oxide nanoparticles (SPIONs) are used as promising hyperthermia agents in the treatment of solid tumors. One such formulation, MFL AS1 (aminosilane coated SPION) has passed phase I clinical trial with no systemic toxicity following intratumoral injection but skin irritation was observed in some patients due to high heat generation in local region (44 °C).

Aurimune (CytImmune) has been developed as recombinant human TNF which is attached to gold NPs using a PEG linker. During its Phase I trials, Aurimune was shown to be well tolerated in patients with advanced cancer. CYT-6091, another from CytImmune, was the first product in a clinical trial using gold nanoparticles for solid tumor patients [61]. AuroLase® is silica-AuNPs decorated with PEG and is approved by the FDA for a pilot test to treat solid tumors [117]. In February 2017, it has been applied for the treatment of patient's tumors of head and neck cancers. AuroLase was explored for the treatment of primary or metastatic lung cancer in another clinical trial (Phase I) [118]. More recently, in patients with recurrent multiform glioblastoma or gliosarcoma, Nu-0129 has been started the clinical trial using spherical nucleic acid [119]. But till date, the FDA has not yet approved any gold-based nanodrugs [1, 120]. Hafnium oxide is another promising nanoparticle suitable for intratumoral injection and a good candidate for radiation-based chemotherapy. NBTXR3 is a hafnium oxide nanoparticle which is undergoing several phase I clinical trials in patients with soft tissue sarcomas and head and neck cancers. A recent "first in human" trial demonstrated a favorable and safety profile when used as a tumor imaging agent, allowing investigation in additional trials with humans in the near future [1, 121].

For the last few years, several nucleic acid nanotherapies are under progress to address nucleic acid targets in the study of organ-specific diseases. These therapies are generally siRNA mediated. Several such formulations are DCR-MYC, ALN-RSV01, TKM-130803, and AGN211745 which were dismissed after phase I and II trials. Reasons of termination are not always related to safety issues (as in case of AGN211745) but some gave fatal output, e.g., TKM-130803 (9 out of 12 died in a study within 14 days). CALAA-01 and PRO-040201 are used for curing solid tumors, but terminated in a clinical trial due to only modest activity in vivo [10].

18.12 Regulatory Authorities for Monitoring Nanomedicines and Their Adverse Effects and Safety Concerns

In the U.S., USFDA is the main regulatory authority in the approval of foods, drugs and formulations, cosmetics, medical devices, and veterinary products. National Nanotechnology Initiative was programmed by USFDA in the objective of development and regulation of nanoscale products, development of new and world-class nanotechnology as well as academic and industrial progress of nanotechnology. Office of Science and Health Coordination (OSHC) under FDA regularly coordinates information delivered by major experts of different internal organizations under FDA [122]. Apart from USFDA, the Center for Drug Evaluation and Research, the Center for Biologics Evaluation and Research, and the Center for Devices and Radiological Health are some regulatory bodies who shared responsibilities of risks associated with nanotechnology and involved in assessment and regulation of nanoscale products.

Under the control of EU, REACH (Registration, Evaluation, Authorization, and restriction of Chemicals) is a policy for controlling chemicals and is a hoe for proper

evaluation and regulation of nanomaterials in European territory [123]. REACH provides a set of standard tests, testing procedures, and testing requirements which are practically feasible in assessing the safety of nanoscale materials. EMA (European Medicines Agency) has also taken initiatives for the development of nanotechnology-based medicinal products. In the UK, government organization DEFRA (Department of Environment, Food & Rural Affairs) is working in the issue of nanotechnology-related risks and published its report on the risk of engineered nanomaterials on human health and environment. European Nanosafety Cluster (NSC) is a forum for Framework Projects like FP6 and FP7, which are national projects in EU member states and seeks to maximize the synergies between the existing projects by addressing toxicology, ecotoxicology, and exposure and risk assessment, mechanisms of interaction, and standardization issues. The European Academies Science Advisory Council (EASAC) and the Joint Research Centre of the European Commission (JRC) recently published a report entitled "Impact of Engineered Nanomaterials on Health: Considerations for Benefit–Risk Assessment". The report pointed on the limitation of current knowledge and technologies and provides a guideline for further research; focusing on 'safety-by-design' principle for the successful implementation of the emerging nanotechnologies [124].

Airborne nanoparticles are the main occupational hazards in manufacturing units of engineered nanomaterials. UCLA (University of California, Los Angeles, CA) has developed new testing methods for measurement of airborne nanomaterials in manufacturing units, analysis of the exposure of workers to those materials, and its associated health risks; and also suggested guidelines for the safe manufacturing of engineered nanomaterials. Some nongovernment industries like QuantumSphere are also working on the regulation of occupational hazard in cooperation with government agencies such as NIOSH (National Institute for Occupational Safety and Health) in the USA [122]. The concern of adverse effects of nanomaterials on environment and ecosystem motivated many research organizations to conduct individual research on the toxicity of nanomaterials on the ecosystem. CBEN (Center for Biological and Environmental Nanotechnology), under the regulation of Rice University, is one of the leading organizations currently working on water-based ecosystem. The International Council on Nanotechnology was established in 2004 as an extension of CBEN and involved in the exploration of health and environmental risks of nanotechnology, data management and screening of knowledge gathered from nanotechnology-related publications, and also in increasing public awareness of nanotechnology. Environmental Protection Agency is a government organization in the USA sharing the responsibilities on the assessment of toxic effects on the environment [122]. It possesses the authority to regulate the manufacturing, usage, commercial distribution, and disposal of existing chemical substances as well as new chemical entities. Incorporation of engineered nanomaterials in food, medical, and pharmaceutical industry also comes under the scrutiny of this organization.

18.13 Conclusion

Thus, nanomedicines, their potential uses, and even their scientific and commercial aggression before human healthcare system in the near future cannot be ignored. However, their toxicity and safety concerns should not be jeopardized by the enormous possibilities of favorable sea-change in human healthcare.

Acknowledgments The authors want to acknowledge the Department of Pharmaceutical Technology, Jadavpur University, Kolkata, India, for providing them with academic facilities.

Conflict of Interest Authors declare no conflict of interest. All the figures and tables are original and self-made.

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Nanotoxicity and Risk Assessment of Nanomedicines

19

Vivekanandan Palaninathan and Aswathy Ravindran Girija

Abstract

Nanotechnological advances have significantly boosted the quality of human life. However, the underlying risks associated with the engineered nanomaterials (ENMs) express serious concerns about their side effects on living things and the surrounding environment. Evaluation of the direct or indirect risks posed by ENMs through deliberate or accidental exposure to them along with their toxicity and biokinetics is the core strategy adopted in nanotoxicology. The rapid evolution of nanotechnology challenges the nanotoxicology division to emphatically provide a precise understanding of the desirable and undesirable effects associated with the application of nanomaterials to the living things or the surrounding environment. Intelligently designed nanomaterials with a multidisciplinary effort may ensure a better understanding of their toxicity through specific approaches surrounding their physicochemical and surface properties.

Keywords

Nanomedicine · Nanotoxicity · Risk assessment · Nanomaterials · Biomedicine

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M. K. Das, Y. V Pathak (eds.), *Nano Medicine and Nano Safety*,
https://doi.org/10.1007/978-981-15-6255-6_19

19.1 Introduction

Nanotechnology is a study that deals with the controlled manipulation of materials and/or devices in the 1–100 nm range. This is a key enabling technology that has grown exponentially over the years with the simultaneous discovery and upgradation of instrumentation along with the methods, due to which it has created obvious ripples across the scientific community owed to its potential applications irrespective of the fields [1, 2]. Technological advances in engineering, biomedicine, food, environment, agriculture, etc. have become a reality of late, due to some significant nanoresearch that has allowed the transfer of the technology from lab to market, especially in engineering and biomedicine. Now, healthcare has always been the foremost priority worldwide with increasing awareness among masses about various medical conditions, and to fulfill those demands with better efficacy and results, nanotechnology has been applied to yield nanomedicines. The genesis of nanomedicine sprang from one of the most ambitious and visionary ideas that nanomaterials could be designed, synthesized, and delivered in the human body to perform molecular therapy and/or diagnosis [3]. Nanomedicines encompass the use of diverse materials to synthesize distinct nanoparticles and nanorobots for drug delivery, diagnosis, and sensing, which are already an indispensable part of several therapies or diagnoses. More specifically, nanomedicines are meant to enhance their bioavailability and/or while responding to an external stimulus. The outcomes of such nanotechnological advancements in biomedicine range from early disease diagnosis to better treatment and lower costs associated with it.

Nanomedicines hold immense potential in healthcare and research for both in vitro and in vivo diagnostics and treatment due to their unique physicochemical properties. For instance, some metal nanoparticles exhibit superparamagnetism, luminescence, and plasmonic, which can be used for theragnostics, while other nanoparticles based on carbon, micelles, polymers, liposomes, etc. can be used to deliver drugs, peptides, genes, and nucleotides. Nanoparticles may also be used in formulating nanovaccines, implants, and regenerative medicine. However, there is a certain level of risks associated with them, which may lead to several undesirable effects and/or may pose threat to health through different toxicodynamics or pharmacodynamics, different organ and cellular distributions, or altered toxicokinetics or pharmacokinetics. Moreover, the composition, size, surface coatings, and functionality of a nanoparticle can unveil varying toxic potentials across different cell types and organisms. So, a detailed technical know-how about the toxicity and biokinetics of nanoproducts is a must to avert such inadvertent calamities to both humans and their environment.

19.2 Nanotoxicology

Nanotoxicology is an emerging branch of toxicology that deals with the assessment of potentially noxious properties of nanostructures or nanoparticles (NPs) under 100 nm with an intent to determine the magnitude of environmental or societal risk associated with their use. Aforementioned, the inherent properties of NPs,

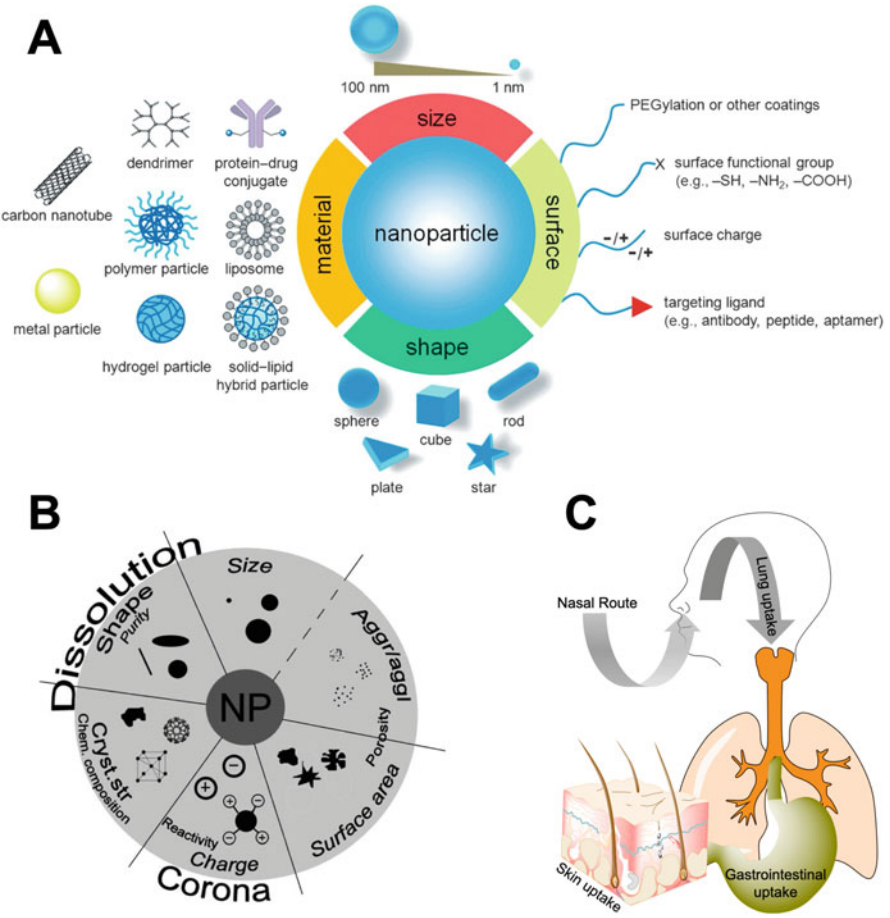

Fig. 19.1 (**a**) An illustration of critical factors responsible for the outcome of diverse characteristics and applications of nanomaterials. Reprinted with permission from Heinz et al. [4] under Creative Commons CC-BY. (**b**) Interplay between distinct properties of nanomaterials that determine the NP-biological system interactions and their impact on nanomaterial toxicity. Reprinted with permission from Madannejad et al. [5]. (**c**) Typical routes of nanomaterial uptake in a human body

physicochemical properties like the size, shape, surface area, surface charge, crystal structure, coating, and solubility/dissolution, and external factors such as the temperature, pH, ionic strength, salinity, and organic matter collectively determine NP toxicity, behavior, and fate inside a biological system, as shown in Fig. 19.1a, b. This very disparity in NP characteristics and properties is the basic hitch in this field of science even though numerous in vivo, in vitro, and in situ models have been studied to estimate the nanotoxicity levels. So, it would be apt to describe nanotoxicology as a branch of science that categorizes the circumstances leading to toxic effects of nanomaterials, which also strategizes to prevent and treat in the event of high-risk exposure.

19.2.1 Nanomaterial Cellular Uptake

The increasing use of NPs in nanomedicine is possibly due to a widely accepted hypothesis that NPs can easily penetrate any tissues or cells escaping through the tight biological barriers, membranes, and other defense mechanisms of the body due to their incredibly small size. The classical routes of nanomaterial intake are shown in Fig. 19.1c, where (1) nasal route allows inhaled NPs to reach lungs, (2) skin allows absorption of NPs through any topical applications of nanomaterial-based creams, and (3) oral ingestion of nanomaterials allows access to gastrointestinal tract. Table 19.1 shows various routes of nanomaterial uptake and its fate. In the following section, we shall discuss the classic nanomaterial intake individually.

19.2.1.1 Nasal Route

Inhalation is one of the recurrent routes of unintentional exposure to nanomaterials, which allows them to enter a biological system. The inhaled nanomaterials may reach deep inside the lungs compromising the barriers like macrophage clearance, bronchial epithelium, and mucociliary system [6]. Thus, direct exposure of NPs through the respiratory tract poses an extreme risk to the human lungs due to a large

Table 19.1 Absorption of nanomaterials through different routes

Material	Size	State	Application	Absorption
Inhalation route[a]				
Porous PLGA microparticles	26–33 μm	–	Inhalation	Lungs
Chitosan NPs	300 nm	–	Inhalation	Lungs
Liposomes	90 nm	–	Inhalation	Respiratory epithelia
PEI	–	–	Inhalation	Lungs
GI tract route[b]				
Fullerene	–	Water soluble	Oral	Intestinal
Gold NPs	4–58 nm	–	Oral	Detected in all tissues
TiO_2	25–80 nm	Suspension	Oral	Liver, spleen, kidney, and lungs
Polystyrene	48 nm	Monodisperse	Oral	Liver, nymph nodes, and spleen
Dermal route[b]				
TiO_2	10–60 nm	Anatase and rutile uncoated	Topical	Stratum corneum
ZnO	80 nm	Emulsion	Topical	No transdermal absorption
Latex particles	50 and 100 nm	Water soluble	Diffusion chamber	Not penetrated skin
Quantum dots	16–19 nm	Water soluble	Intradermal injection	Entered lymphatics

[a]Reference [15]
[b]Reference [16]

surface area [7]. Several studies have been performed both in vitro and in vivo to elucidate the toxicity of different inhaled NPs, of which a report on AgNP exposure in rats resulted in bronchial hyperresponsiveness and eosinophilic and neutrophilic inflammation [8]. Such studies exemplify that inhalation routes can be an effective alternative to the systemic chemotherapy in the case of lung cancer because the inhalation route allows a direct supply of drugs to the lungs, which in turn provides high local concentrations with enhanced anticancer activity and fewer side effects. However, targeted chemotherapy may be well suited for the purpose of preventing localized toxicity [9].

19.2.1.2 Gastrointestinal Uptake

Gastrointestinal (GI) tract is a complex site (mouth, stomach, and intestine) that hosts symbiotic interactions between the host cells and resident microbiome. GI tract presents entry route to nanomaterials that are ingested either intentionally or through unintentional modes like inhalation (secondary ingestion), contaminated water, food, etc. [10]. When a nanomaterial is ingested, it goes through distinct pH and biochemical compositions that may affect its physicochemical properties, bioavailability, and toxicological properties. Particularly, the interaction of nanomaterials with intestinal epithelial cells regulates their bioavailability and the associated systemic effects [11]. Based on these phenomena, oral nanoparticles as therapeutics are proposed for effective absorption from the GI tract. For instance, a study carried out by Kim et al. exhibited specific high-efficiency intestinal uptake of oral NPs [12].

19.2.1.3 Skin Uptake

Skin is the largest organ in the body that is highly dynamic and is constantly evolving. It acts as the first line of defense against the intruding foreign materials and mechanical damage. NP-based topical drug delivery systems like drug patches or sunscreens may provide access to nanomaterials into the skin. For instance, coated-TiO_2 microparticles present in the sunscreen creams as UV filters were found to penetrate the horny layer and the hair follicular orifice [13]. Another study suggests that when the skin was exposed to polystyrene, NPs could penetrate only until stratum disjunctum [14]. In both the cases, intact or partially compromised skin does not allow the penetration of NPs beyond the superficial layers, thus inaccessible to the live epidermis or beyond.

19.2.2 Factors Influencing Nanotoxicity

Nanomaterials have garnered such a huge claim due to their small size that bestows multidimensional properties to several consumer products due to the quantum effect at the nanoscale. While this feature presents tremendous opportunities in the current state and envisages futuristic applications, it may affect human and his surroundings adversely as well. This may be attributed to a large surface-to-volume ratio of NPs compared to their respective bulk form, which makes them highly reactive. Table 19.2 shows the in vitro and in vivo approaches to estimate nanomaterial toxicity.

Table 19.2 Classic in vitro and in vivo approaches to gauge nanomaterial toxicity

Assay type	Classification	Category	Assay principle	Techniques
In vitro	Uptake		Localization of particles	TEM
			Quantitative measurement of uptake and localization within the cell	ICP-AES, ICP-MS, fluorescence imaging
	Viability	Metabolic activity	Assessment of metabolically active cells	MTT, alamar blue,XTT, WST-1, MTS
		Hemolysis	Membrane disruption and necrosis	Spectrophotometric detection of hemoglobin
		Necrosis	Measurement of membrane integrity	Uptake of dyes: neutral red and trypan blue, LDH assay
		Apoptosis	Membrane alterations	Annexin-V, propidium iodide
			DNA fragmentation	Comet assay (SCGE), TUNEL, DNA laddering
	Functional	DNA synthesis	Cell proliferation/ cell cycle arrest	BrdU incorporation, thymidine incorporation
		DNA damage	Single-stranded break	Comet assay
		Proliferation	Double-stranded break	TUNEL
		Gene expression	Increased expression and activation of DNA repair related proteins	Immunohistochemistry, DASL assay, qRT-PCR, Western blot
		Immunogenicity		
		Exocytosis	Number of cell colonies	CFE assay, clonogenic assay
		Oxidative stress	DNA synthesis	Thymidine incorporation
			Cell counting	Flow cytometry, high content image analyzers
			Altered expression of functional genes in cellular processes	DNA microarray, PCR, q-PCR
				ELISA, q-PCR
			Cytokines levels	Carbon-fiber microelectrode amperometry
			Secretion of electro-active small molecules	DCFDA assay, DHE assay, NBT assay,

(continued)

Table 19.2 (continued)

Assay type	Classification	Category	Assay principle	Techniques
			(e.g. serotonin, epinephrine)	dihydrorhodamine 123 assay
			Directly: ROS	C11-BODIPY, TBA assay
			Indirectly: secondary effects of increased cellular ROS	DTNB
				Amplex red
			Lipid peroxidation	NBT,WST-1
			Antioxidant depletion	ELISA
			Presence of lipid hydroperoxides	Immunoblotting
			Superoxide dismutase (SOD) activity	
			8-hydroxy deoxyguanosine levels	
			SOD expression	
In vivo		Lethality	Median lethal dose (LD50): the dose required to kill 50% of the participating animals	ICP-AES, ICP-MS, fluorescence imaging, radiolabeling, TEM, haematoxylin-eosin staining, helium-3/ proton
		Bio-distribution and clearance		MRI, Raman spectroscopy
		Hematology serum chemistry	Following the nanoparticle localization, metabolism, and passage through the animal body	Flow cytometry, hematology analyzers
		Histology/ histopathology		Turbidity or nephelometric measurements, capillary electrophoresis, protein-specific labeling methods
			Examining blood composition, cell population (RBC, WBC, T cell, macrophages)	Light microscopy
			Changes in proteins and enzymes levels: albumin, ALT, AST, alkaline phosphatase, hemoglobin, and total protein	

(continued)

Table 19.2 (continued)

Assay type	Classification	Category	Assay principle	Techniques
			Other analytes; bilirubin, creatinine, glucose, urea, nitrogen	
			Changes in the tissue or cell morphology	

Reprinted with permission from Maddannejad et al. [5]

19.2.2.1 Size

Particle size is of prime importance in nanotoxicology as it plays a vital role in the internalization rate and mechanism. Several researchers have reported the size-dependent toxicity and biodistribution of metallic nanoparticles. For instance, a study by Li et al. showed that larger gold nanoparticles (AuNPs) (40–60 nm) tend to accumulate only in the liver and spleen. However, the smaller AuNPs (6–24 nm) were found not only in the liver and spleen but also in other organs [17]. In another study, the effect of surface capping and size-dependent toxicity of AuNPs on different trophic levels was evaluated, which implied that toxic effects of AuNPs varied under distinct test systems (in vitro and in vivo), emphasizing the importance of size and surface functionalities at different trophic levels [18]. In the case of silver nanoparticles (AgNPs), a decrease in size saw a dip in toxicity, while the reactive oxygen species (ROS) production was dose and size dependent in cochlear cells [19]. So, the particles over 200 nm in size might not reach the venous circulation as they are filtered out by the spleen, and the ones below 5 nm are rapidly filtered out through renal system. It is only the particles that are within the 40–50 nm range, which show maximum internalization in the cell body [4].

19.2.2.2 Morphology

Apart from the distinct size of NPs, the morphology of NPs plays a significant role in the toxicity of nanomaterials. Researchers have synthesized a wide range of NPs with different morphologies (spheres, cubes, rods, star, truncated triangles, flakes, etc.) for specific purposes. Now, the shape of a NP directly affects its transport or kinetics in the body of an organism or in the environment, which is in turn related to its toxicity. For instance, it is known that AgNPs with different shapes and sizes are toxic to aquatic life. In a recent study, the effects of three different Ag-based nanomaterials (like Ag-nanospheres, Ag-nanowires, and Ag-nanoplatelets) with a species of algae, *Chlorococcum infusionum*, were studied to evaluate their growth and photosynthetic activity upon interaction. This study proposed that the toxic potential of the Ag nanomaterials was dependent on their diameter and shape, which resulted in decreased growth and photosynthetic activity of the algae [20]. In another study with alumina nanoflakes and nanorods, the shape-dependent toxicity on rat astrocytes was evaluated. The findings of this study emphasized that the morphology of nanoalumina played a significant role in its toxic potencies and its underlying

mechanisms. Here, both the nanoalumina materials showed dose-dependent cytotoxicity and apoptosis after 72 h of exposure, which was attributed to the elevated ROS generation and inflammation induction. It was noteworthy that the nanorods inflicted much damage to astrocytes than the nanoflakes [21].

19.2.2.3 Surface of Nanomaterial

The surface functionality of a NP is believed to influence its surface charge, where the charge on a NP plays a crucial role in not only the dispersion or aggregation characteristics but also the adsorption of various ions or molecules [22]. Therefore, even though the particle geometry is of prime importance in nanotoxicology, the surface charge, functionality, wettability, and adsorption with polysaccharides or other ligands also hold great significance. Surface functionality confers high flexibility and high hydrophilicity to the NPs. This can be understood with an example of a study that shows that the surface-modified polystyrene nanoparticles (d-α-tocopherol polyethylene glycol (1000) succinate or TGPS) of 100 and 200 nm have higher cellular uptake efficiency across the gastro-intestinal barrier (Caco-2 cells) and the blood-brain barrier (MDCK cells) than 20–100 nm sized particles [23]. This study contradicts the size-based internalization of NPs across the physical barriers of cells as mentioned in the earlier section, which clearly illustrates the need for a case-by-case study and relevance of surface functionality of NPs in toxicology. In another study conducted with the coating of natural organic matter (humic acid) on iron oxide nanoparticles, an enhanced surface charge was observed, which led to the disaggregation of those NPs, which confirmed the influence of surface charge on the aggregation characteristics of NPs [24].

19.2.3 Materials

The usage of different nanomaterials owed to their size and aspect ratios based on distinctive requirements of the wide spectrum of consumer products available today necessitates the need to analyze material-based toxicity evaluations due to growing concerns of their noxious effect in human and other living organisms alike. Carbon allotropes are one of the most sought after supplies in both material-based research and industry. So, it is particularly important to understand the underlying mechanisms of cytotoxicity and some potential factors influencing the detected cytotoxicity involving carbon allotropes. Apart from the carbon nanomaterials, the metallic NPs like gold, silver, platinum, copper, zinc, etc. have also been used extensively in nanomedicine for drug delivery and bioimaging to various biological sites. However, when there is a decrease in size of the certain NPs, the toxicity profile is seemingly enhanced even when they are inactive in their bulk material. The driving force behind the use of multiple variants of nanomaterials is the quest for suitable nanocarrier to achieve desired responses by targeting cells and/or receptors for distinct clinical conditions with minimal side effects. Figure 19.2a gives a brief insight into material interactions with various experimental models and their associated cytotoxicity. While the use of nanomaterial-based consumer products

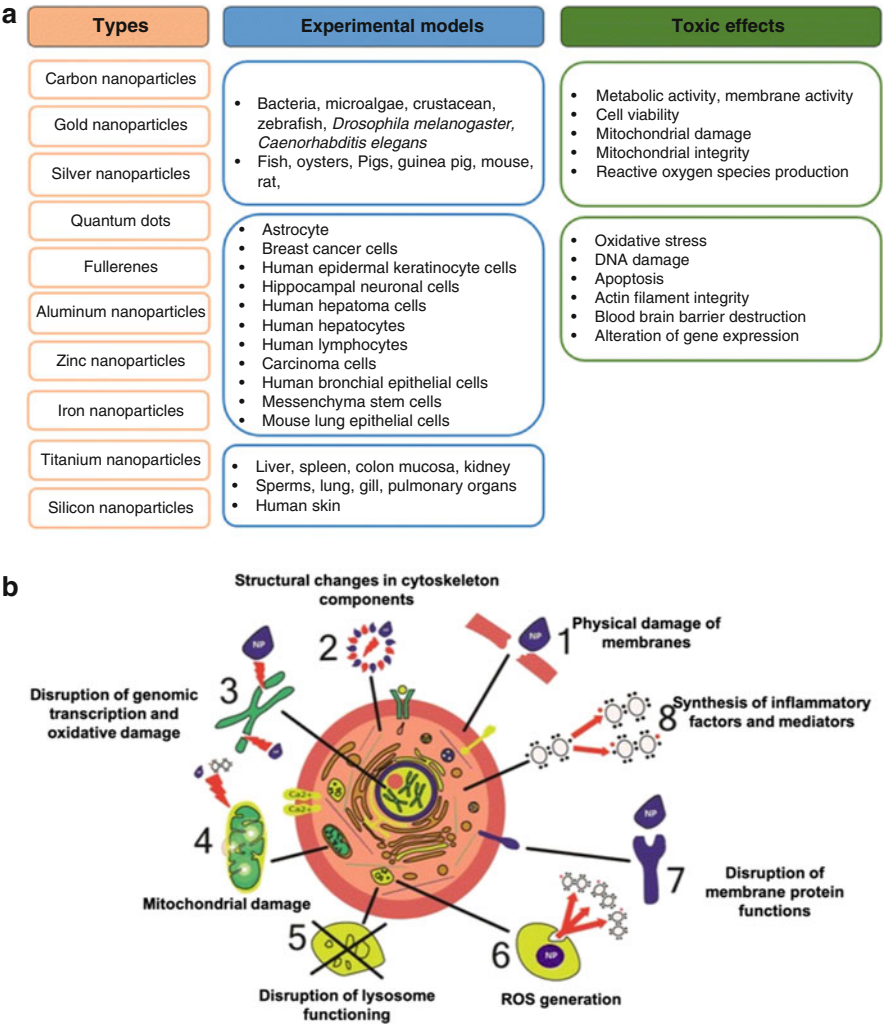

Fig. 19.2 (**a**) Various nanomaterials and their associated noxious effect upon interaction with different experimental models. Reprinted with permission from Kumar et al. [25] under Creative Commons CC BY. (**b**) Possible routes of cell damage inflicted by NPs. Reprinted with permission from Sukhanova et al. [26] under Creative Commons CC BY

has grown significantly over the last decade, it is now also evident from the available reports that exposure to various nanomaterials poses serious risks to biological systems. The following section will discuss the mechanisms behind the possible toxicity of nanomaterials as a result of the interaction with cells.

19.2.4 Underlying Mechanisms Behind NP-Mediated Toxicity

NP toxicity is not limited to the site of administration alone but can be traced to other regions of the body to reach specific target organs due to translocation. Different NPs might favor specific target organs and/or cellular compartments based on the above-mentioned factors that are extremely critical in deciding the fate of NPs in the body of an organism. So, the experimental models both in vitro and in vivo are extremely important to determine the toxicity of NPs because in vitro models provide valuable insight into the toxic effects inflicted by a NP on an individual cell and/or a tissue and/or its components, while an in vivo model allows the estimation of NP toxicity in individual organs or the whole body itself. Additionally, the concentration of NPs including their duration of interaction with a living system, their stability in biological fluids, and the capacity to accumulate in tissues and/or organs may possibly decide their toxic effect. Hence, it is pivotal to have a complete understanding of all factors and mechanisms underlying NP toxicity to develop safe and biocompatible NPs. Figure 19.2 depicts the possible damage caused by NPs when they come into contact with cells. There are possibly eight different ways through which the NPs can inflict damage to cells; however, the most prevalent causes have been discussed below.

19.2.4.1 Oxidative Stress

An interlude in the balance of free radical (reactive oxygen species) generation and the antioxidant defenses that result in tissue damage may be attributed to oxidative stress [27]. Typically, these free radicals are unstable and highly reactive molecules due to the presence of unpaired electrons. As a result, they tend to interact with other nonradical biological molecules, lipids, proteins, nucleic acids, carbohydrates, etc. [28]. Now, under normal circumstances, homeostasis is maintained by the host body; however, when exposed to engineered nanomaterials (ENMs) depending on their physicochemical properties and exposure (high/low), they may either generate free radicals or modulate nontoxic redox signaling [29]. There are numerous ways of nanomaterial uptake like active and passive diffusion, phagocytosis, and receptor-mediated endocytosis. Once the nanomaterials enter a cell, they may interact with various biomolecules inside the cell to cause oxidative stress that may enhance inflammation by upregulating the redox-sensitive transcription factors like NF-κB, activator protein-1, kinases, etc. [30]. ENM localization and interaction with cell organelles especially mitochondria and nucleus contribute to apoptosis and ROS generation, where they may end up in DNA damage, mutagenesis, or cell cycle arrest [31].

19.2.4.2 Autophagy and Disruption of Lysosomal Function

Intracellularly, nanomaterials are predominantly captured and destroyed in the lysosomal compartment. Thus, autophagy and lysosomal dysfunction hold tremendous significance in understanding the mechanisms of nanomaterial toxicity. Both phagocytic and nonphagocytic internalization of nanomaterials into lysosome often exposes them to an extremely hostile environment with varying pH, enzymes that are meant for degradation [32]. Reports also suggest that nanomaterials induce

autophagy (self-eating) [33], which is basically a highly conserved homeostatic process meant to degrade intruding pathogens, impaired organelles, etc. [32, 34]. Here, the researchers had exposed cells to carbon and metal-based nanomaterials, which showed an increase in autophagic activity with elevated autophagic vacuoles toward nanomaterial clearance, which contributed to cell death. Other reports suggest that cellular autophagy may get affected upon exposure to nanomaterials like CNTs, quantum dots, and gold nanoparticles [35].

19.2.4.3 Necrosis and Apoptosis (Cell Death)

The pathophysiological or the likely cytotoxicity that may be incurred as a result of using nanomaterials has often questioned their prospects, which is directly related to their physicochemical properties and dose that may either generate ROS or autophagy that eventually decides the cell fate via necrosis or apoptosis [36]. Non-specific cell injury that often occurs due to trauma, infections, toxins, or neurodegenerative diseases contributes to cell necrosis that was long considered to be not driven by any distinct signaling and has been clarified by studies that it may also be regulated (necroptosis) and may play a role in physiological and pathological processes [37]. Predominantly, when cell membrane integrity is breached due to nanomaterial uptake, it results in leakage of cytoplasmic contents, which in turn leads to necrosis [38]. In a study on size-dependent toxicity of gold nanoparticles, 1.4 nm gold NPs lead to rapid cell necrosis within 12 h as a result of the oxidative stress and mitochondrial injury after their intracellular uptake [39].

In contrast, apoptosis is a programed cell death that is genetically controlled. In the case of apoptosis, cell and nuclear shrinkage can be observed. Unlike necrosis, the plasma membrane integrity of cell is preserved until late apoptosis. Here, the cytoskeletal proteins are cleaved by death receptor signaling and activation of caspases, which leads to break down of the subcellular components. It also leads to chromatin condensation, nuclear fragmentation, and the formation of plasma membrane blebs [40]. In a study, the copper NPs induce intrinsic apoptosis in mice kidney tissue through ROS and reactive nitrogen species generation, regulation of Bcl-2 family protein expression, release of cytochrome c from mitochondria to cytosol, and activation of caspase-3; however, they also observed the activation of FAS, caspase-8, and tBID, which suggests the involvement of extrinsic pathways, which shows that nanomaterials trigger extrinsic and intrinsic apoptotic pathways [41, 42]. Further investigations on the complexities of the nanomaterial-induced cell death may give a better insight into the consequences of human exposure.

19.3 Risk Assessment

Nanomaterial risk assessment is a global issue that must be addressed owed to the diverse forms of the same material, which means different toxicity. The unavailability of a standard protocol to gauge the toxicity further makes the job difficult. Thus, there arises a significant uncertainty over the ENM toxicity. That is why nanotoxicology becomes an indispensable part of any new nanotechnologies. Table 19.3 shows the newer strategies for risk assessment for nanomaterials.

Table 19.3 Risk assessment frameworks for nanomaterials

Type of framework and aim	Link to current regulation	Advantages and key details	Disadvantages
Risk assessment strategies mainly directed towards regulatory submission			
NanoRiskCat			
Screening level assessment framework	The tool can be used for a first risk assessment of existing consumer products containing nanomaterials, i.e. not as such applicable to substance-based legal frameworks like REACH	Descriptors for the potentials have been listed, and where possible cut-off values are proposed (mostly in analogy to non-nanomaterials)	Qualitative output on exposure and hazard
Aim: a systematic tool that can support companies and regulators in their first tier assessment and communication on what they know about the hazard and exposure potential of consumer products containing nanomaterials. Input is given on how the potential for exposure, human health hazard and environmental hazard can be assessed	Also useful for prioritisation of existing products	The human hazard potential is based on HARN-information (i.e. whether it is a nanomaterial with a high-aspect ratio). Classification and labelling information of the bulk, and information on genotoxicity/mutagenicity, respiratory toxicity, cardiovascular toxicity, neurotoxicity, reproductive toxicity, carcinogenicity and organ accumulation	No integration of exposure and hazard information
	It gives a qualitative exposure potential For professional end-users, consumers and the environment, as well as information on the hazard potential for humans and the environment	For the environment indicators are: Is the nanomaterial reported to be hazardous to environmental species? Is the nanomaterial persistent or bioaccumulative? Could use of the nanomaterial lead to potential irreversible harm? Is the nanomaterial readily dispersed? Is the nanomaterial novel?	

(continued)

Table 19.3 (continued)

Type of framework and aim	Link to current regulation	Advantages and key details	Disadvantages
DF4nanoGrouping framework			
Specific framework focussed on human health hazards, via inhalation	No direct link to current regulations	Clear framework with description of tools, decision criteria and specific nanomaterial cases	Regulatory acceptability unclear
Aim: efficient strategy to sort out nanomaterials that could undergo hazard assessment without further testing	Proposal by a group of industries organized within the European Centre for Ecotoxicology and Toxicology Of Chemicals (ECETOC)		Independent evaluation of triggers and protocols used has not been conducted
			Focus on local inhalation toxicity (not all endpoints)
MARINA risk assessment strategy			
General strategy	Generally applicable on high level	Generally applicable	General strategy, it provides a blueprint of a risk assessment strategy. It is not specific and not very elaborated with regard to requirements on basic information set, decision criteria, etc.
Aim: to develop a flexible and efficient approach for data collection and risk assessment. The generated information should be sufficient to assess the risks of nanomaterials	Information on physicochemical properties, exposure, toxicokinetics/ fate and hazard are to be integrated	Different potential applications of grouping and read-across within the MARINA Risk Assessment Strategy have been discussed in detail	
	No direct link to regulations		
Nanomaterial categorization for assessing risk potential			
Screening level assessment framework	Prioritisation methodology to target materials of high concern that need additional scrutiny, while material categories that pose the least risk can receive expedited review	Nanomaterials for which no exposure or potential hazard is expected require no further testing	General strategy without clear information on how to proceed from one tier to another
Aim: prioritise nanomaterials for further testing. To avoid 90-day inhalation studies when possible		Subsequently, hazard is investigated with alternative testing strategy assays, and the adverse outcome pathway is investigated (tier 1). If further testing is required, a short-term bolus administration in vivo study is performed (tier 2). If needed in tier 3 an aerosol inhalation (90 days) study is performed	Focus on inhalation

		Presents a case that focuses on CNTs and health risks under the Toxic Substances Control Act of USA	Chance of false negatives (no further testing required whereas it in reality poses a health risk) cannot be assessed
		Provides general framework or evaluation	The suitability of alternative testing strategies and of the in vivo short-term bolus approaches to inform on chronic toxicity is not clearly addressed
Test strategy for assessing the risks of nanomaterials in the environment			
Towards a specific framework for nanomaterials in the environment	Aligned to the risk quotient (PEC/PNEC) as applied in environmental regulations	Generally applicable for environmental risk assessment of nanomaterials	Still a conceptual framework (although more concrete)
Aim: to develop a lest and risk assessment strategy for nanomaterials which specifically addresses environmental fate and effects	The strategy is not yet sufficiently developed to fulfil the information requirements of specific legislation (e.g. plain protection act, biocide regulation, REACH)	Bioaccumulation is taken into consideration as an alternative endpoint delivering additional information on ecotoxicity	Screening tests are required to identify a potentially significant effect, but no suitable screening tests have been identified
		Different stages along the life cycle of the nanomaterial(s) are considered by assessing whether there is a potential for the nanomaterials to be released into the environment	Trigger values have not yet been set
		Test systems and strategies of data collection, and evaluation are provided. A screening on durability (i.e. the extent to which nanomaterials remain intact) in the initial compartment needs to be performed as a first step. In case of medium or high durability, in tier 1	

(continued)

Table 19.3 (continued)

Type of framework and aim	Link to current regulation	Advantages and key details	Disadvantages
		the risk quotient for environment is assessed in an initial compartment for the pristine material. In tier 2, pristine and aged nanomaterials are considered and secondary compartments are included	
A strategy towards grouping and read-across			
General strategy	Comprises testing strategies for nanomaterials that are in compliance with REACH	Generally applicable	General strategy
Aim: to develop testing strategies for nanomaterials in order to characterise the potential risks to human health and the environment		Two hypothetical case studies	Life cycle changes are not considered
Risk assessment and grouping strategy based on clouds of predefined test strategies			
General strategy	Sketch of issues on nanomaterials in current regulations and general outline of potential ways to deal with those issues	The clouds mainly relate to modes of action (MOA) and adverse outcome pathways (AOP)	Details on how clouds of predefined test strategies can be formed are lacking
		The need for pragmatic solutions is indicated	Not clear how and when read-across between members of the same 'cloud' can be substantiated and performed
			Limited considerations on fate and toxicokinetics
NANoREG nanospecific approach for risk assessment			
Screening level assessment framework	The proposed approach provides alternative ways to address the risk assessment of nanomaterials, by prioritising those applications with the highest potential health risk	A generally applicable approach to prioritise nanomaterials for potential risk	Can currently only be used for prioritisation and directions on type of data needed for scientific justification to perform risk assessment across different nanoforms (e.g. using (Q)SARs, grouping or read-across)

Aim: to prioritise those nanomaterial applications that may lead to high risks for human health. To identify those aspects of exposure, kinetics and hazard assessment that are most likely to be influenced by the nanospecific properties of the material under assessment	Approaches for (Q)SARs, grouping and read-across are integrated	Provides a more concrete framework by listing and discussing which aspects of exposure, kinetics and hazard of nanomaterials are most relevant and how they can be taken into consideration using several flow charts	Illustration by case studies not yet performed
		Provides a sorting of nanomaterials into 3 classes for further human health assessments: soluble, high aspect ratio nanomaterials, and all others for which case-by-case analysis criteria are generally described	Cut-off values still need to be defined
Risk banding framework			
Screening level assessment framework for inhalation route	Output is qualitative risk banding, which can be used to advice on risk management measures in occupational setting	Integrating scientific knowledge to give an estimate on risk	Qualitative output
Aim: development of a scientifically based risk banding tool by combining information on deposition of particles in the respiratory tract, lung burden and clearance, diffusion through lung mucus layer, translocation and cellular uptake and local and systemic toxicity		Transparent description on the assumptions behind the framework	Only for inhalation route
		Available information on relationships between physicochemical properties and the processes mentioned above is used	
NANoREG framework for the safety assessment of nanomaterials			
Review of current framework in view of nanomaterials, and outlook to screening level assessment framework	To analyse the applicability of the current EU regulatory framework for nanomaterials, with focus on REACH	Comprehensive overview of safety assessment of nanomaterials under REACH, including nanospecific considerations	No clear recommendations for nanospecific adaptations of the current regulations are provided

(continued)

Table 19.3 (continued)

Type of framework and aim	Link to current regulation	Advantages and key details	Disadvantages
Aim: to analyse the applicability of the current EU regulatory framework to nanomaterials and to giving concrete, practical direction to industry and regulatory authorities on how to address nanomaterials in a legislative context, with focus on REACH		Outlook scenario, are addressed, which comprise (1) the NANoREG nanospecific approach for risk assessment and safe by design, as also described in other NANoREG deliverables	
Sustainable nanotechnology decision support system (SUNDS)			
Conceptual decision framework in which various tools and models have been integrated	Tools (e.g. PROAST on dose-response) and exposure models (such as Stoffenmanager Nano and ConsExpo) are part of the overarching framework. Links to REACH have been established	It considers links to risk governance and risk management approaches	The present assessment is on main features as the final SUNDS framework has not yet been published
		It provides the user a broader overview	
Risk assessment strategies mainly directed towards the innovation chain			
LICARA nanoSCAN			
Generally applicable risk comparison framework	The tool is preferably to be used at an early stage in the innovation chain with the aim to facilitate the development of sustainable and competitive nano-enabled products	Risk and benefits are addressed in a transparent manner	Not providing information that can be used directly in regulatory frameworks
Aim: A screening tool fur SMEs that provides a qualitative evaluation of the potential benefits and risks of a new or existing nano-enabled product. A comparison against a reference product or "doing nothing" is made		Easy to use	
		Relevant tool to support safe innovation	
Alternatives assessments for nanomaterials			
Alternative assessment framework	Alternatives assessment is not obligatory	Principles for the design of safer nanotechnology (adapted from Morose 2010) have been discussed	Link to innovation chain not clearly made

Aim: To assess the overall applicability of alternatives assessment methods for nanomaterials and to outline recommendations	The decision context might allow more use of high-throughput data (comparison vs. assessment). It is concluded that although science may not (yet) be in the position to predict or explain nanotoxicology, science may be (more) ready for making better and safe(r) choices	Examples of alternatives assessments have been discussed (mitigating oxidative stress, encapsulation, doping approaches, surface properties)	How the actual comparison with and decision making on alternatives is performed remains unclear
Alternatives assessment for nanomaterials is complicated by the sheer number of nanomaterials possible			
NANoREG D6.04			
Generally applicable risk screening framework	If the indicator for a specific 'risk potential' suggests low risk, risk assessment can be performed in the conventional, i.e. non-nanospecific, way. If the potential is high, nanospecific data/testing is required	Generally applicable for nanomaterials and products containing nanomaterials al early stages of innovation	Some information on the relationship between the parameters and the risk potential is provided, but clear direction on how to assess and integrate the experimental information of the parameters is lacking
Aim: Strategy to efficiently screen for indicators of potential risks during early stages of an innovation process. Based on six key risk potentials: solubility/dissolution rate, stability of coating, accumulation, genotoxicity, inflammation and ecotoxicity		Detailed in the sense that the parameters relevant for the key risk potentials are described, and methods to investigate these parameters are listed	
		Parameters relevant for the key risk potentials are listed and discussed. Analytical methods suitable to investigate these parameters are also listed and referenced	

The aim and scope of the presented frameworks for risk assessment are very broad and lack decision criteria. However, the listed risk assessment strategy could be used to prioritize nanomaterial hazards in human health.

19.4 Conclusion

Nanotechnology, nanomedicine, and nanotoxicology aim to improve human life, where nanomedicine drives the development of novel and superior diagnostic, therapeutic, and preventive measures, while nanotoxicity details the necessary safety assessment for nano-enabled products. It is now understood that the size, shape, surface chemistry, and aggregation of nanomaterials are the key aspects that influence their toxicity. With the rising use of nanomaterials in different sectors, nanotoxicology plays a vital role in guiding through the challenges to get it right and recognize and avoid potential risks associated with ENMs. It is noteworthy that in vivo studies can provide adequate information regarding ADME of ENMs; however, long-term studies require systemic knowledge.

Another important limitation in understanding the toxicity of nanomaterials is posed by the discrepancies in assessments under in vitro and in vivo conditions. This is largely due to the findings that reveal toxicity under in vitro conditions; however, the same may fail to reciprocate under in vivo conditions. Therefore, clearly, more understanding is needed to compare in vitro and in vivo testing systems. Intelligently designed nanomaterials and appropriate methodology combined with proper experiments may ensure better understanding of toxicity of nanomaterials, which may further find their way into futuristic applications for the welfare of mankind.

Acknowledgments The author (ARG) thanks the University of South Australia for providing support. VP would like to thank ARG for her immense support extended during the period of this study. Also, VP would like to thank BNERC, Toyo University for their continuous support throughout this work.

Conflict of Interest The authors declare no conflict of interest.

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Clinical Toxicity of Nanomedicines

20

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Abstract

Nanomedicine (NM), the use of nanoparticles in diagnosis, prevention and treatment of human disease has drammatically improved the practices of medicine in the past two decades. Approximately 50 NM products have received US-FDA approval for a variety of human diseases and even greater number are currently under clinical evaluation. One of the main advantages of NMs is explored in drug delivery owing to thier unprecedented ability to facilitate high payload drug delivery thereby reducing the systemic toxicity associated with free drug administration. Despite their widespread popularity, the NMs receive several toxicological and regulatory apprehensions. Clinical toxicity of NMs is one such inevitable challenge limiting the success of a large number of NMs in clinical translation. Regulatory agencies ensure that any NM must demonstrate rigorous safety profile during preclinical and the clinical studies prior to considering its commercialization. Outcomes from toxicological research revealed that the clinical toxicity of NMs is governed by multiple factors and not merely a drug-dependent response. In this chapter, the role of key factors such as physicochemical properties of NMs (namely size, surface area, charge, shape, and composition) and route of administration contributing to the clinical toxicity of NMs are elaborated. This chapter also provides mechanistic insights into the potential mechanism of toxicity of NMs in different systems. Towards the end, the chapter summarizes different pros and cons of well-established models for testing NM toxicity. Lastly, the globally acceptable safety guidelines and roles of various regulatory bodies in promoting research on nanomedicine are outlined. The understanding from this chapter will help in designing a safe and effective NM for specific application.

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M. K. Das, Y. V Pathak (eds.), *Nano Medicine and Nano Safety*,
https://doi.org/10.1007/978-981-15-6255-6_20

Keywords

Nanomedicine · Nanoparticle · Nanocarriers · Toxicity · Clinical toxicity · Toxicity mechanism

Abbreviations

ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
DOPC	1,2-Dioleoyl-*sn*-glycero-3-phosphocholine
DOPS	1,2-Dioleoyl-sn-glycero-3-phosphoserine
DPPC	1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine
GGT	Gamma-glutamyl transferase
GM-CSF	Granulocyte-Macrophage Colony Stimulating Factor
IL	Interleukin
TNF-α	Tumor Necrotic Factor- Alfa
US-FDA	Food and Drug Administration

20.1 Nanomaterials in Medicine: Nanomedicine

According to the most widely adapted definition, a material having at least one of its dimensions between 1 nm and 1000 nm scale is classified as a *nanomaterial* [1]. Humans are always surrounded by nanomaterials, for example, viruses (diameter 20–400 nm), bacteria (diameter ~400 nm), or tiny particles generated from anthropogenic sources, e.g., volcanic ash, dust storm, or industrial waste [2–4]. These particles have a great impact on human health. Also, engineered nanomaterials or nanoparticles (NPs) are synthesized for diverse applications in multiple areas of science and technology.

In a biological perspective, a material when brought to nanoscale dimention acquires two unique features which cause it to behave dramatically different from its bulk state. The first one is a tremendously high surface area-to-volume ratio which creates a vast platform for incorporation or functionalization with a large amount of molecule of interest and secondly, owing to its inherently small size, it can comfortably cross biological barriers such as moving out from blood vessels, crossing the blood–brain barrier, and entering into cells (diameter 10–100 μm) via different mechanisms of endocytosis. Taking advantage of these properties, researchers have explored an array of inorganic and organic nanomaterials for diagnosis, prevention, and treatment for a variety of human diseases, and such nanomaterials are known as nanomedicine (NM). The term NM was first used during the late 1990s, although scientists have been studying these from multiple decades [5, 6].

20.1.1 Biodegradable or Nonbiodegradable NPs

A group of NPs when placed under physiological conditions when undergo subsequent degradation due to hydrolytic or enzymatic action are known as biodegradable, and the rest are nonbiodegradable. Most popular biodegradable materials used in developing NMs include polymer-based [natural (chitosan, alginate, dextran) and synthetic (poly (lactic-*co*-glycolic acid; PLGA or polycaprolactone; PCL], lipid-based [natural (phospholipids, cholesterol) and synthetic (DPPC, DOPC)], protein-based (albumin, gelatin) materials, and few others such as silica, synthetic dendrimers, and cyclodextrins. Popular examples of nonbiodegradable materials include gold (Au), silver (Ag), iron (Fe), aluminum (Al), and copper (Cu) [7–9]. These materials have been processed in various nanoforms such as porous particles, liposomes, micelles, cochleates, nanofibers and some nonspherical shapes (rod-shaped, diamond, cuboidal, etc.) specially made using metallic material.

20.1.2 Bioactive or Carrier Function of Nanoparticles in NMs

A large number of nanomaterials, mostly nondegradable, show specific bioactivity which has been leveraged by biomedical scientists for developing effective diagnostics or therapeutic NMs. For instance, AuNPs have been exploited for their evident antimicrobial, anti-inflammatory, or anti-arthritic properties [10, 11]. Similarly, AgNPs (anti-microbial), CuNPs (bone resorption) [12], and CNTs (anti-cancer) [13] also achieved substantial interest in therapeutics. Additionally, these NPs when combined with other therapeutic agents result in overall improved efficacy [14]. Also, metallic NPs behave differently when exposed to electromagnetic radiation, which makes them preferred NMs for theranostics purpose. For example, when illuminated with radiation of specific wavelength, metallic particles produce light or heating effect in the body. The ability to produce light [e.g., fluorescence or near-infrared fluorescence] has been strategically utilized in developing a variety of diagnostic devices or nanosensors for tracking/monitoring distribution and selective accumulation in the body [15]. Also, many of these NPs have attracted considerable interest as contrast-enhancing agents in ultrasound imaging [16–18]. On the other side, the heating effect has been exploited for targeted killing of cancer cells in patients, a phenomenon well known as *hyperthermia-based killing* [19].

Nanomaterials particularly those which are biodegradable also serve as drug delivery carriers where they enhance the biodistribution and absorption of their encapsulated cargo which is therapeutic. This property or carrier function helped in significantly reducing toxicity associated with free drug administration and also improving therapeutic efficacy [20–22].

Nearly 50 NMs have already been approved by US FDA including both nonbiodegradable as well as biodegradable formulations. Among the approved products, nonbiodegradable particles have been primarily used for diagnostics purpose (e.g., superparamagnetic iron oxide nanoparticles; SPIONs) and some therapeutic

applications such as cancer treatment using hyperthermia in combination with radiotherapy (e.g., variety of metallic NPs). Whereas, biodegradable particles have been mostly used for their carrier function (Table 20.3) [23, 24].

20.2 Clinical Toxicity of NMs

Ideally, any NMs intended to be administered in the body essentially need to be *biocompatible in nature*, i.e., they should not elicit any undesirable immune responses or toxicity when injected or implanted in the body [25]. Despite the utmost care being taken in designing the material and dose, NMs may elicit clinical toxicity when administered into the body. For example, Shi et al. tested safety and chemotherapeutic potential of monomethoxy polyethylene glycol–poly (D, L-lactic acid) (mPEG-PDLLA) polymeric micelles-paclitaxel particles in the treatment of cancer. They injected formulation in 18 patients with confirmed advanced malignancies in low (175 mg/m^2) to highest (435 mg/m^2) dose. The dose was intravenously injected for 3 h without premedication on day 1 (on a schedule of 21 days). Among the six patients receiving high dose (300 mg/m^2), 4 showed grade-4 neutropenia and 1 patient showed grade-3 numbness. Also, 6 out of 18 patients (33.3%) having prior exposure to chemotherapy showed partial hypersensitivity reactions [26].

Therefore, increasing applications of NM research has given rise to critical safety and regulatory concerns. Certainly, it is unethical to conduct toxicity studies on humans; most of the knowledge about toxicology of NMs is apparantly generated using cell culture and preclinical animal models. The OECD guidelines particularly in Sect. 20.4 (health effect) emphasized rigorous evaluation of toxicity in preclinical models. Specifically, for toxicological profiling, the subject animal should be monitored for any altered respiration, salivation, weight loss, muscle spasm, diarrhea, convolution, lacrimation, numbness, tremors, and loss of a reflex. If any of these symptoms appear during the first 24 h or within 14 days of single/multiple exposures, then it is considered as *acute toxicity*. These symptoms can also extend to 28 days (*subacute*), ~3 months (*sub-chronic*), or 6 months and above (*chronic toxicity*). Additionally, organ-specific toxicity such as hepatotoxicity, cardiotoxicity, neurotoxicity, and hemotoxicity are needed to be evaluated. The first-in-human toxicity and safety studies of developed NMs are conducted in phase 1 clinical trials, while phase II and III clinical trials focus on evaluating efficacy (Table 20.1).

20.3 Factors Responsible for Clinical Toxicity of NMs

The clinical toxicity of NMs results from multiple factors such as individual factor (e.g., genetic variability, disease condition, and immunity), physicochemical properties of NPs, and its route of administration [2]. Additionally, some other factors such as inadequate handling/storage of formulation, and inappropriate dosing might also result in acute to sub-chronic toxicity in patients receiving such

Table 20.1 Stages in clinical development of nanomedicine

Stage	Objective	Design	Number of volunteers	Outcome (if successful)
Preclinical studies in small animals	Development of investigational new drug (IND)	NA		Application to FDA on IND
Phase I	Safety profile and pharmacology. To study dose-limiting toxicity (DLT)	"3 + 3" cohort expansion design[a]	20–80	Dose and schedule decided for further evaluation or recommended phase 2 dose (RP2D)
Phase II	Efficacy against placebo Also, validation on toxicity and tolerability	Single arm OR randomized trials	Several hundreds	Progress to phase 3 Or conditional FDA approval[b]
Phase III	Efficacy over 'gold standard'	Randomized	Hundreds to thousands	FDA approval
Post-marketing surveillance	Safety studies during sales	NA		Data on small risk, benefit, and optimal use. Recall in case of adverse effects

[a]Dose escalation by increasing 3 + 3 volunteers in each step
[b]In case of robust Phase II and interim Phase III data and unmet medical need

formulations. Thus, a sound understanding of all factors contributing to clinical toxicity is essential for designing safe and effective NMs for human applications. In this section, the role of physicochemical properties and routes of administration of NMs on its clinical toxicity are discussed.

20.3.1 Physicochemical Properties

The physicochemical properties including size, shape, surface charge, coating, stability in biological fluids, and agglomeration rate have been considered the key factors leading to clinical toxicity of NMs[3]. Physicochemical properties of NMs can affect the particle's ability to cross biological barriers (e.g., skin or mucosal barriers), distribute, accumulate, and eventually get cleared from the body which has substantial impact on its clinical toxicity (Fig. 20.1).

20.3.1.1 Effect of Size, Surface Area, and Shape

The size and surface area of NMs play an important role in deciding how the body responds to, distributes, and eliminates the particles. Particle surface area to volume ratio increases exponentially with decreasing particle size which makes the particle

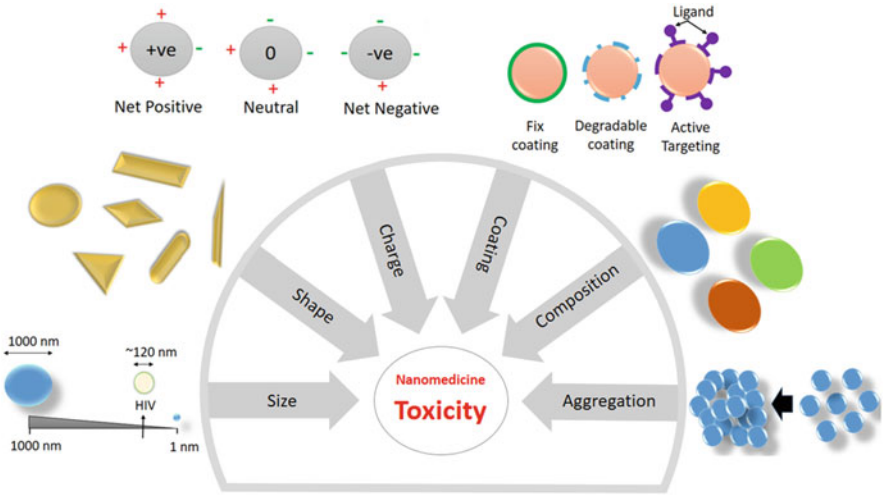

Fig. 20.1 Illustration of various physicochemical properties of nanomedicine

surface chemically more reactive. As a consequence, particles undergo more agglomeration and interact differently with biological fluids. Particle agglomeration results in serious toxic effects in body [27]. Small size particles have the ability to easily cross biological barriers hence, they get distributed to multiple organs importantly in brain, liver, kidney, intestine, pancreas, and spleen, hampering their function thus leading to acute and chronic toxic effects in body. Caster et al. studied the role of particle size on the efficiency and toxicity in chemoradiotherapy (CRT) of xenograft tumor model. In a comparison between 50, 100, and 150 nm particles, the authors demonstrated that in contrast to larger particles (100 and 150 nm), the 50 nm particles avoided liver and spleen accumulation and penetrated homogenously in the xenograft tumor model. The small size particles caused more small bowel toxicity in animals [28].

The particle shape and aspect ratio have important effects on the clinical toxicity of NMs [29, 30]. Almost all the NMs that reached the clinical stage have been fabricated in spherical shape. Spherical shape has distinct advantages such as ease and reproducibility of fabrication with most existing techniques and favorable interactions with cells. In literature, few shapes such as flakes/needle shaped (e.g., single or multi-walled carbon nanotubes, SWCNTs/MWCNTs), irregularly spherical (dendrimers and cyclodextrins), and other shapes including rod-shaped, elliptical, cube, diamond, clusters (mostly AuNPs) have been explored for developing NMs. The shape of particles affects absorption and phagocytic clearance from the body. The endocytosis of spherical particles is relatively easier and more efficient when compared to the rod-shaped particles [31, 32]. For example, the toxicity of SWCNTs and MWCNTs (both being rod-shaped) is attributable to their adverse effect on the process of endocytosis, particularly, influencing the membrane wrapping and formation of endocytic pocket [33]. Due to this, in contrast to

spherical fullerene, the CNT due to tubular shapes caused more platelet aggregation and vascular thrombosis in rat carotid arteries [34].

20.3.1.2 Effect of Surface Charge and Coating

Surface charge and particle coating play a significant role in the clinical toxicity of NMs. Inability to maintain optimum surface charge (positive/negative) results in precipitation/agglomeration of NPs during storage or inside the body. Agglomerated NPs do not distribute homogeneously in the body and potentially lead to toxicity in a specific organ. In a study, Saxena et al. showed that carboxylated-SWCNT (more anionic) showed profound in vivo toxicity in a mouse model when compared to pristine SWCNT, which might attribute to their homogenous dispersion and eventually higher availability in different organs [35]. Additionally, surface charge (with size) also controls the formation of 'protein corona' (a shell of plasma protein formed on particle surface in biological fluid) on the particle surface [36]. Protein corona determines biological identity of particles in the body, regulates their distribution, and organ-specific toxicity [36]. For initiating phagocytic clearance of NMs from body, protein corona facilitates opsonization, thus helping in reducing toxicity [37, 38]. Moreover, surface charge also controls the affinity of the particles toward oppositely charged biomolecules, e.g., protein, nucleic acid, and lipid inside or on the cell surface. As a general consensus, cationic NPs are considered more toxic over anionic particles. The toxic effect of cationic particles is due to their higher affinity toward anionic head of phospholipid and negatively charged proteins embedded on the cell surface. Excessive binding or adsorption of cationic particles on cell surfaces inhibit associated cellular function eventually causing severe toxic effects. Earlier studies have demonstrated that cationic particles are more likely to cause hemotoxicity by inducing platelet aggregation and hemolysis, in contrast to negatively charged or neutral surface which is considered mostly biocompatible [39]. In a study by Heiden et al., it was observed that cationic polyamidoamine (G4) dendrimers showed time-dependent toxicity in zebrafish embryo model, whereas anionic dendrimers were not toxic [40].

In order to modulate toxicity due to particle surface properties, NP surface is strategically coated by grafting differently charged molecule onto them. Thus, a thin coating or shell on the nanoparticle surface helps in stabilizing particles and making their surface more biocompatible. In some cases, the coating improves solubility, tissue-specific targeting (known as active targeting), or preventing opsonization-triggered phagocytic clearance of NPs from the body. For example, SPIONs show systemic toxicity due to the poor solubility and high rate of agglomeration under physiological conditions which potentially impede blood flow. For NM applications, SPIONs have been coated with various biocompatible materials (e.g., silica or polymers) for improving their solubility hence, retention in the body, and simultaneously reducing hemotoxic effect [41–45]. Similarly, for active targeting, particles' surface is functionalized or coated with a ligand which specifically recognizes the cells of the target tissue, thus minimizing toxicity at nontarget locations. For example, coating the particle surface with folic acid improves their uptake by cells overexpressing folate receptor-β (FR- β), such as cancer cells [46] and

pro-inflammatory macrophages [47]. In most NM products, phagocytic clearance due to opsonization often results in premature clearance of the particles from the body. To prevent this, particle surfaces are grafted with polyethylene glycol (PEG) coating (known as PEGylation). PEGylation turns NP surface biologically inert preventing their phagocytosis, as a result the stealth NPs retain longer in circulation without showing toxic effects [48]. PEGylation is a biocompatible coating approved by the FDA for reducing the toxicity of various NMs. PEGylation has been successfully used for reducing the toxicity of multiple NM products approved by US-FDA, e.g., PEGylated liposomal doxorubicin (anti-cancer), PEGylated GM-CSF (Neutropenia), and PEGylated TNF-α inhibitor (Crohn's disease) [24].

20.3.1.3 Effect of Composition and Degradability

The chemical composition of NPs is another important factor leading to toxicity. It has been observed that, in contrast to degradable NPs in which toxicity is mostly attributable to encapsulate drugs, nondegradable NPs show inherent toxicity by accumulation at vital organs. Moreover, NPs prepared using different materials (similar size) can have different toxicity effects. In a study, aimed at understanding the toxicity due to composition of NPs, Herper et al. used 11 different types of NPs fabricated using different materials (although comparable sizes) and evaluated their toxicity on the embryo of the zebrafish model. The range of NPs included titanium oxide, aluminum oxide, gadolinium oxide, zirconium oxide, samarium oxide, dysprosium oxide, holmium oxide, and erbium oxide (all positively charged) and silicon dioxide, yttrium oxide, and alumina doped, and cerium oxide (all negatively charged). These NPs were individually exposed to zebra fish embryo by supplementing in water (waterborne exposure) at 10–250 ppm concentration and subsequent toxic effects were evaluated. Through this study, the authors interestingly observed that NPs different in composition (even with comparable surface charge and size) had different toxic effect on the morphology as well as mortality of zebrafish embryo [49].

Degradability of NMs is another factor that is associated with clinical toxicity. Toxicity of nondegradable NMs such as metallic or latex particles, injected into the body for a therapeutic or diagnostic purpose, is attributable to their long-term accumulation in vital organs and inefficient clearance from the body. Also, nondegradable metallic NPs due to small size are known to accumulate inside the cell, disrupting normal cellular function ultimately leading to uncontrolled production of toxic free radicals (e.g., ROS) [50]. On the other hand, toxicity of biodegradable nanoparticles depends on the toxicity of released degradation product (monomers). For example, NMs developed using PLGA are most biocompatible as the degradation products such as lactic acid and glycolic acid are safely absorbed by the body. It was demonstrated by Semete et al., where they injected PLGA NPs (200–300 nm) in blood, and 7 days postinjection, they observed that nearly 40% particles were accumulated in the liver, while rest were localized in brain and kidney without showing any toxic effect [51]. Additionally, the rate of degradation (or rate of releasing encapsulated drug) can also lead to toxicity. For instance, burst-releasing formulations can show acute toxic effects in the body which is mostly attributable to

the loaded therapeutic agent. Thus, depending on the requirement, either burst releasing (e.g., micelles), fast releasing [e.g., albumin-based or polylactic acid (PLA) or polyglycolic acid (PGA) particles], or slow releasing (e.g., PLGA, PCL) nanocarriers are selected. Besides, material type, the rate of degradation also depends on NP size and percent porosity. Small size particles with more porosity degrade faster due to more accessible surface area for hydrolytic and enzymatic actions during degradation [52].

20.3.1.4 Other Factors

There are certain other properties of NPs such as surface roughness, surface elasticity of particle assembly, surface acidity or basicity, and surface density which have been understood up to some extent on in vitro systems. However, their role in toxicity is not clearly understood thus far [53–56].

20.3.2 Route of Administration

The choice of route of administration plays a substantial role in controlling toxicity of NMs. Each route results in a unique biodistribution profile in different bodily sites and associate to different toxicities depending on the physicochemical properties of NMs. Thus, the inherent toxicity of NMs can be strategically minimized by the selection of an appropriate route of administration with the intent to achieve a high benefit to risk ratio. In this section, the toxicological impact of various routes of administration of NMs is discussed (Table 20.2).

20.3.2.1 Systemic Routes of Administration

Intravenous

Intravenous drug administration is most successful in delivering NMs at accessible sites in body, also incurred as the most dangerous route due to associated systemic toxicity. Organs rich in morphonuclear-phagocytic cells (e.g., liver, lungs, spleen, and bone marrow) are the most common biodistribution site for the passive accumulation of NPs [58–60]. Close proximity with blood and lymphatic nodes potentially causes hematological, immunological, cardiac, and renal toxicities [60]. Following intravenous administration, the silica NPs (SiO_2NP) showed toxicity in liver, spleen, and lungs with substantial mononuclear infiltration and hepatic necrosis [61]. In another study, systemic administration of AuNPs elicited acute inflammatory responses in rat liver and kidneys involving enhanced production of IL-6, IL-1β, and TNF-α cytokines[62] and significant alteration in the levels of liver enzymes (AST, GGT, ALP, and ALT) [63]. Moreover, there is evidence of NP extravasation through blood–brain barrier (BBB) following intravenous injection inferring their neurotoxic potential. Intravenous delivery of polysorbate 80-coated polybutylcyanoacrylate NPs (containing dalargin peptide) in Swiss mice showed breaching of BBB with potent neurotoxicity leading to decreased locomotor activity and increased mortality [64].

Table 20.2 Routes of administration with their advantages, disadvantages, and potential toxicities

		Route of administration	Advantages	Disadvantages	Potential toxicity
Local routes	Local response	Skin (topical)	• Patient compliant • Quick local response • No systemic toxicity	• High drug loss	Skin irritation or dermatitis
		Intra-articular, intra-tumoral, intravesical, intradermal, intracranial, intratracheal, intravaginal, intrarectal, intrauterine, intraocular	• No systemic toxicity • High bioavailability at the desired site	• Patient noncompliant	Local tissue toxicities
Systemic routes	Quick systemic response	Intravenous	• Quick therapeutic outcome with fast accumulation at the target site • Potential to deliver drugs through BBB	• Patient noncompliant • Systemic bioaccumulation • BBB breaching can give rise to CNS toxicity	Systemic toxicity • Blood and lymphatic system-associated toxicities • Hepatotoxicity • Pulmonary toxicity • Splenic toxicity • Cardiotoxicity • Brain toxicity
		Oral	• Patient compliant • Prolonged effect and maintenance of high plasma concentration • Quick therapeutic outcome	• First-pass metabolism • Poor bioavailability • Delayed response	• Gastrointestinal toxicity • Liver associated • Systemic toxicity if nanosystem reaches the circulation in high concentration
		Respiratory	• Patient compliant • High local dose accumulation • Quick therapeutic outcome and fast systemic absorption • Bypass first-pass metabolism of liver • Potential to surpass BBB	• Systemic bioaccumulation • BBB breaching can give rise to CNS toxicity	• Pulmonary toxicity • Systemic toxicity if nanosystem reaches the circulation in high concentration

	Prolonged systemic response	Skin (Transdermal, Subcutaneous depots)	• Patient compliant • High and steady plasma concentration • Long-term therapeutic effect • Surpass BBB	• High drug dose requirement because of poor penetration • Loss of NMs • Delayed systemic response	• Skin irritation and allergic reactions [57] • Systemic toxicity if nanosystem reaches the circulation in high concentration

20.3.2.2 Slow Absorbing Systemic Routes of Administration

Routes other than systemic administration facilitate gradual entry of NMs/released drug in circulation (Table 20.2).

Oral

Owing to convenience factor and high patient compliances, oral NM delivery remains one of the most preferred and widely accepted noninvasive routes. However, due to the combined effect of gastric and mucosal barriers along with first-pass metabolism in the liver, the bioavailability of drugs at various organs is considerably poor. Bednarski et al. observed that oral administration of AuNPs resulted in excretion of ~55.8% and 3.7% AuNPs in feces and urine, respectively, in contrast to only 1.02% and 1.9% excretion after intravenous administration. Moreover, of the total orally administered particles, nearly 40% were adsorbed on bowel walls and other organs leading to acute toxic symptoms in mice [65].

Respiratory Tract

Intranasal mucosal surface presents another vast surface area for noninvasive delivery of aerosolized NMs. Unlike oral route, it avoids first-past metabolism. Exposure to large or small particles leads to toxicity in different ways. Large-sized particles (3–5 μm) get retained in the trachea while the smaller NPs (0.1–1 μm) infiltrate to the deepest part of the lungs, i.e., alveoli and enter into the systemic circulation [66]. However, even with a smaller size of NPs, when compared to degradable counterparts, the toxic effect is more profound with nondegradable NPs. Nondegradable NPs get conveniently accumulated deep into the lung tissues and lead to various toxic effects. Miller et al. reported that an increase in the accumulation of different sized AuNPs (nondegradable) in vascular inflammation sites after inhalation enhances incidence and risk of cardiovascular diseases [67]. Sub-chronic inhalation of 4–5 nm AuNPs in rats showed a dose-dependent increased bioaccumulation involving active infiltration of inflammatory cells (macrophage, neutrophil, lymphocytes) in lungs and kidneys when compared to blood, liver, and olfactory bulb [68]. Moreover, due to instant access, high surface area with abundant blood supply, fenestrated endothelium and permeability to BBR, intranasal delivery of NMs can also associate with toxicity in brain [69]. For example, intranasal instillation of different sized nondegradable TiO_2 NPs in mice showed brain accumulation through olfactory bulb with increased oxidative damage and inflammation contributing to toxicity [70].

Skin

Skin is another attractive noninvasive site for NM delivery. The uppermost keratinized layer of the skin, i.e., stratum corneum, poses the biggest challenge for NMs for deep extravasation to the epidermis and dermis, which have accessibility to fine blood vessels. NM delivery via skin (topical, subcutaneous, and transdermal administration) is more often associated to local toxicity involving acute allergic symptoms, irritation, redness, and swelling, but sometimes systemic toxicity by a gradual entry in circulation [71]. Subcutaneous depots [72] and transdermal delivery

creams, lotions, patch containing NPs in them [73], and micro-needles [74] have been used for prolonged systemic delivery of nanotherapeutics. The accumulation of NPs in hair follicle (root), though help their intrafollicular migration to deep skin layers, also may be associated with toxic effects depending on the nature of the nanoparticle. Ilver et al. locally applied zinc oxide NPs to treat local skin inflammation in atopic dermatitis mice model. Although ZnO particles diminished skin inflammation, they evoked allergy by inducing systemic IgE antibody production [75].

20.3.2.3 Route for Local Delivery at Diseased Site

Locations in the body where systemic bioavailability with either enteral or parenteral route is considerably poor are often administered with nanoparticles locally. Most common locations are brain tissue, eye, ear, articular joints, skin, terminal GI tract, vagina, deep muscles tissue, lungs, and interior of solid tumors. For visualization and deep access in body/tissues, local injections are sometimes used as imaging or endoscopic probes [76]. However, as local injections suddenly provide a high dose at a confined location, it often results in acute inflammatory responses. Warheit et al. showed that intratracheal administration of 300 nm titanium dioxide NPs (TiO_2) and nanorods (200 × 35 nm) resulted in local pulmonary toxic effects including inflammation and cell injury 24 h postinstillation [77]. Also, intra-articular administration of solid-lipid-NPs (SLNs) and hyaluronic acid functionalized-PLGA NPs in the rat knee was considered safe without showing notable sign of joint inflammation [78]. Clinical study on intraocular retinoblastoma showed reduction in systemic toxicity and periocular toxicity associated with administration of free carboplatin through periocular application of carboplatin-loaded polymethylmethacrylate NPs [79].

20.4 Clinical Insights into the Mechanism of NM Toxicity

Understanding the mechanism of clinical toxicity is essential for improving the strategy for NM development. As already discussed, NMs due to small size and unique surface properties can easily penetrate biological barriers and cause changes at the cellular, subcellular, and molecular levels that eventually determine the toxic outcome of NMs.

20.4.1 Cellular Mechanism

NPs elicit various toxicological responses in cells which dysregulate cellular homeostasis by multiple mechanisms. Among these, induction of free radical generation (reactive oxygen species; ROS) is central to most toxicological outcomes (Fig. 20.1). This has been observed with several NPs like Au, Ag, Fe, and SiO_2 that high ROS (peroxide, superoxide, singlet oxygen, and hydroxyl ion) production in the cell results in net oxidative environment disrupting cellular functions. As a consequence, cell undergoes shrinkage leading to aberrations in cytoskeleton network affecting the function of actin and tubulin fibers ultimately leading to cell death by necrosis or

apoptosis [80]. Additionally, loss in cell membrane integrity manifested by leakage of lactate dehydrogenase (LDH) enzyme in extracellular space is another predominant mechanism of cellular toxicity of NMs [81]. Multiple NPs, AuNPs for instance, exert their cytotoxic effect by inhibiting the production of focal adhesion complexes (FAC) which serve as a link between cytoskeletal actin filaments and transmembrane integrin receptors [82]. Warren and Payne studied CHO and HeLa cells exposed to NP treatment and found an alteration of the membrane potential. They observed that positively charged polystyrene NPs blocked potassium channels; as a result, a positive charge was accumulated inside the cells leading to membrane depolarization [83]. Also, NPs may cause toxicity by affecting the process of endocytosis which is critical for phagocytic clearance of foreign particles from the body. For example, rod-shaped particles are difficult to be engulfed by macrophages, as the longitudinal shape of these particles inhibits the formation of endocytic pocket, thus ultimately slowing down overall endocytosis [84].

20.4.2 Subcellular Mechanisms

Depending on their physicochemical properties, the NMs interact differently with the plasma membrane (PM) and organelles such as mitochondria, lysosomes, endoplasmic reticulum (ER), Golgi bodies (GB), and nucleus disrupting associated function and thus overall cellular physiology (Fig. 20.1). A group of hydrophobic NPs accumulate inside lipid bilayers due to affinity for hydrophobic tails of phospholipids. Accumulation or interaction of NPs with PM may affect their efficiency of endocytosis. Following endocytosis, NPs are mostly stored inside lysosomes. Storage of NPs in a lysosome may disrupt the efficiency of lysosomal degradation by alkalinization of lysosomal lumen and accumulation of autophagosomes [82, 85]. Other studies have shown that NP treatment, using AgNP for instance, activates oxidative stress in ER by altering redox state of cells. The ER stress stimulates upregulation of genes involved in cellular protein unfolding responses [86]. On the other hand, platinum NPs and AgNPs showed dose-dependent reduction in mitochondrial membrane potential and also altered the redox state of mitochondria [87]. Damaged mitochondria undergo leakage and release cytochrome c, a key component of the intrinsic pathway leading to cell death by apoptosis [88] (Fig. 20.2).

20.4.3 Molecular Mechanisms

Detailed mechanism of NM toxicity is understood by analyzing the interaction of NPs with biomolecules, e.g., proteins, glycans, lipids, and nucleic acid inside the cell. Binding of NPs to biomolecule inhibits their cellular function to maintain morphology, cellular homeostasis, and redox potential [89]. Prolonged exposure with carbon nanotubes is known to damage calcium (Ca^{2+}) homeostasis in cells [90]. Small-sized NPs can potentially translocate through nucleopores directly

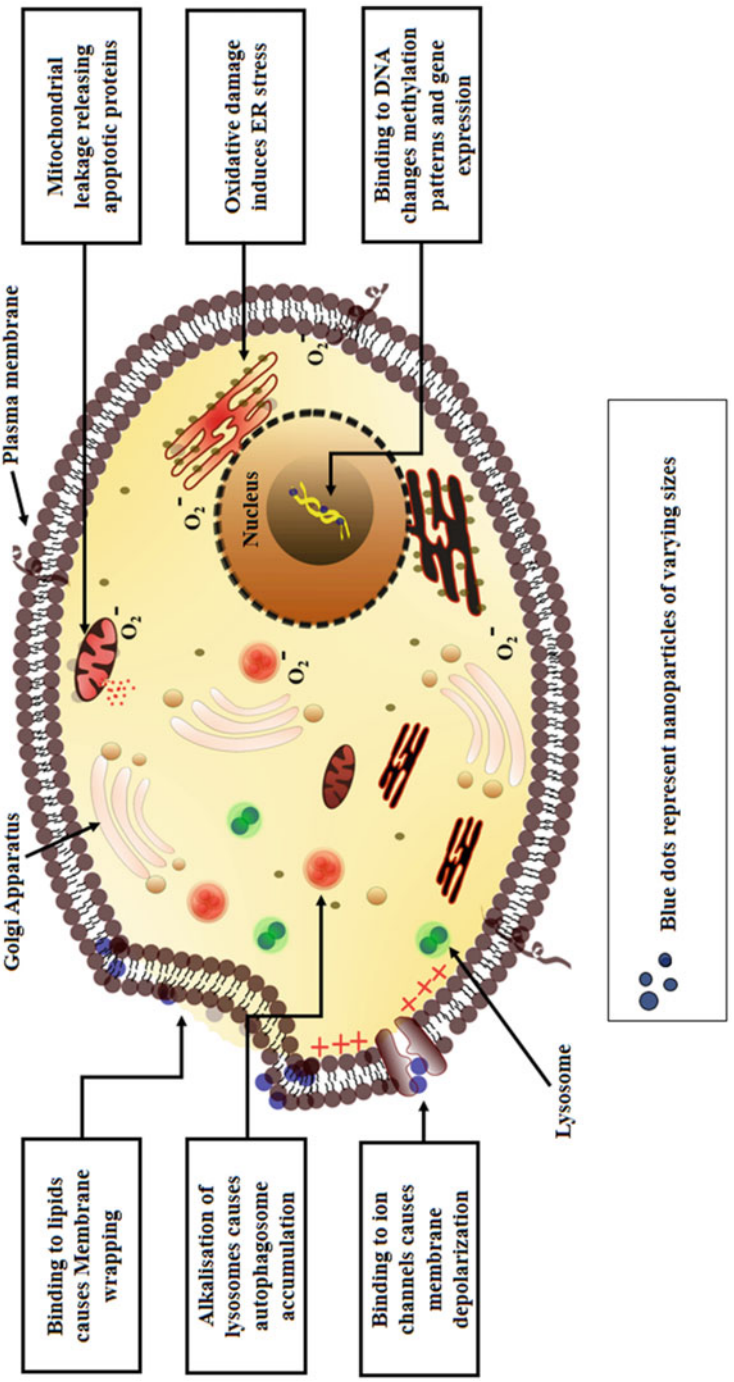

Fig. 20.2 Illustration of different mechanisms of nanoparticle-mediated toxicity in cell. (ER; endoplasmic reticulum)

interacting with nucleic acids. Interaction of NPs with DNA is a key mechanism that regulates the toxicity of a variety of NMs. NPs binding to DNA may cause physical damage, affecting methylation pattern, leading to the formation of micronuclei (MN) and nuclear buds ultimately affecting gene expressions (NP-associated genotoxicity) [91]. Comet assay and cytokinesis-block micronucleus test revealed that exposure to TiO_2-NPs increased both DNA damage and formation of MN in endothelial cells [71].

20.5 Models for Assessing Toxicity

A range of in vitro and in vivo (preclinical) models have helped in understanding the toxicity of NMs to a great extent. For simplification, the models are broadly classified into two groups, namely cell-based and animal models.

20.5.1 Cell-Based Models

The in vitro cell culture system is an excellent and well-established model for evaluating the toxic potential of NMs under investigation. Under laboratory conditions, cells can be conveniently handled, grown, and manipulated. Moreover, most of the assays required for studying even a slight change in their morphology, behavior, proliferation, and differentiation have already been established. Overall, cell-based evaluation of clinical toxicity is an economically viable, rapid, and effective approach for reproducibly understanding the toxicity of NMs. Under in vitro conditions, cells can be grown either on 2-dimensional (2D) surface or in 3-dimensional (3D) microenvironment.

20.5.1.1 2D Cell Culture Models: A High-Throughput Screening Approach

Advancements in cell culture techniques and instrumentation have provided the researchers with an unprecedented ability to simultaneously monitor multiple parameters in a toxicity study. For evaluating the toxicity of CNTs on 2D cells, Movia et al. performed high content screening and analysis (HCSA) of the treated ThP-1 cells. The authors could study multiple cellular properties such as cell viability, cell membrane permeability, lysosomal mass/pH, and nuclear staining at the same time. The comprehensive set of data obtained on CNT toxicity was reproducible, consumed less time, and helped predicting expected toxicity in live organisms [92]. Additionally, high-throughput analysis also facilitates analysis of free radicals (e.g., ROS) production, cytokines, and change in cell membrane potential as a result of NM exposure.

Further, in order to predict clinical toxicity better than cell lines, researchers have used human-derived primary cells as screening models. Although growing primary cells is challenging as the cells lose viability beyond a few generations, the results are more relatable to the clinical scenario. In this context, pluripotent and multipotent

stem cells (SCs) have achieved substantial interest. As SCs tend to differentiate into specific lineage (for instance, bone, cartilage, muscles, neuronal) when exposed to specific physical, chemical, or biological cues, they have attracted huge interest for studying NP influence on differentiation. Moreover, the majority of cellular signaling pathways triggering their differentiation have also been investigated in detail. Thus, SCs have been considered an excellent model for understanding the specific toxicity of NMs on cellular proliferation and differentiation behavior. Coccini et al. have used mesenchymal stem cells (MSCs) derived from human umbilical cord for toxicity screening of iron-based magnetite NPs and observed enhanced senescent phase with decreased mitochondrial activity [93].

20.5.1.2 3D Monoculture and Coculture Models: Physiological Relevance

3D cell culture models are widely adapted for their ability to recapitulate tissue microenvironment by facilitating near-native cell–cell and cell–matrix interactions, thus more closely mimicking original tissue morphology and function [94]. Models et al. developed small aggregates of human monocytes (Thp-1) to monitor cytotoxic potential of SWCNTs. SWCNT exposure on 3D cellular aggregates did not result in a severe inflammatory response, which otherwise was seen on 2D culture of the same cells. This emphasizes a poor correlation between 2D and 3D cell culture models [92].

Besides conventional monoculture (using one cell type), researchers have also developed 3D coculture models involving more than one cell type. These models have been designed to mimic unique anatomical, morphological, and/or functional features of any organ such as GI tract, liver, placenta, kidney, and skin [94–96]. Interestingly, these models help better understand the toxicological effects of NMs on the specific functions of the particular organ. For example, Muoth et al. studied the effect of cadmium telluride (CdTe) and copper oxide (CuO) NPs on 3D coculture microtissue placenta model containing fibroblasts core surrounded by a trophoblast cell layer resembling the in vivo placenta. Cell viability and rate of hCG release were considered as the parameter of acute toxicity [97]. In line with this, human artificial skin models such as EpiSkin™ and SkinEthic™ (EpiSkin Research Institute, France) and EpiDerm™ (MatTech Co., USA) have been commercialized. Kim et al. used EpiDerm™ for evaluating dermal toxicity of metallic NPs. Measurement of cell viability and cytokine release suggested that FeNPs, AlNPs, TNPs, and AgNPs are noncorrosive and nonirritant to human skin [98].

20.5.2 Animal Models for Toxicity Testing

Despite enormous advancement in cell culture-based models, the results of clinical toxicity barely correlate to results obtained on live animals. The data from cell culture studies are useful for primary screening, however, should be essentially validated on animal models. Animal models are suitable for evaluating acute,

subacute, sub-chronic, and chronic toxicity potential of developed NMs with more reliable and relatable observations to clinical scenario.

20.5.2.1 Small Animal Models

Rats and mice are the most popular animal models for toxicological studies but sometimes rabbit, guinea pig, and dogs are also used. There are guidelines for safe and ethical handling of animals for toxicological studies. These animals have been suitably administered NMs via systemic, oral, nasal, skin, or any local route for studying symptoms of acute to chronic toxicity. Small animals have been used as gold standard for studying biodistribution, accumulation, and organ-specific toxicities [99]. Small animals were also used for evaluating the genotoxic effect of NMs studied by monitoring chromosome aberrations, mitotic index, and micronuclei test.

Owing to numerous ethical and regulatory concerns for using small animals for toxicology study, there was a pressing need for having alternative models for bridging the gap between in vitro and in vivo models. In this context, *zebrafish* (*Danio rerio*) emerged as a prominent alternative animal model. Zebrafish offers several advantages including: (*a*) it is a live animal unlike cell culture, (*b*) simple and cost-effective culture maintenance, (*c*) easy to grow in laboratory, (*d*) easy to handle and manipulate due to small size (4–5 cm), (*e*) rapid testing due to high fecundity and rapid development of embryos, (*f*) well-characterized development stages and optical transparency, (*g*) no limitation of upper limit of replicates per test unlike animal, (*h*) high-throughput screening is possible, and interestingly (*g*) being a vertebrate animal, it shares up to 70% genetic homology, anatomical, and pathological features with humans [100]. Importantly, there are no strict ethical concerns for using zebrafish for toxicity testing [101]. Overall, zebrafish has been considered as the 'gold standard' for biosafety assessments of chemicals and is gaining recent interest even for NM toxicity testing. Lee et al. observed size-dependent toxicity of AgNP on zebrafish embryos. Similarly, there have been multiple studies using zebrafish model for understanding the role of physicochemical properties of NMs on their toxicity [102].

Besides, zebra fish, *Drosophila melanogaster* also achieved considerable interest for evaluation of toxicity of NMs [103].

20.5.2.2 Nonhuman Primate Models

Proportionally, the use of nonhuman primates (e.g., capuchin, cynomolgus monkeys, rhesus monkey) for the toxicological study is remarkably low when compared to small animals. As these animals most closely resemble human, hence enable best prediction of clinical toxicity of newly developed NMs. However, due to limitation in numbers, tedious handling, and high cost of maintenance, their use is recommended only under special circumstances (Fig. 20.3).

In a study, Ye ling et al. compared the gestational toxicity of nanocrystals in rodents and primates [104]. In a study, Sedic et al. observed that intravenous administration of cationic lipid-based NPs in cynomolgus monkeys showed mild

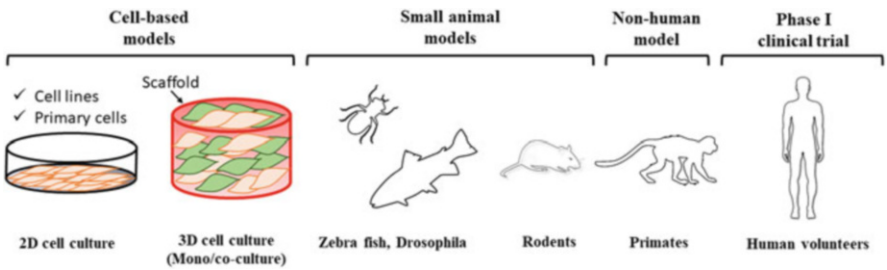

Fig. 20.3 Schematic presentation of different models for testing the toxicity of Nanomedicines

splenic necrosis and lymphocyte depletion, accompanied with reversible complement activation [105].

20.6 Regulatory Guidelines for Clinical Toxicity of NMs

Assessment of clinical toxicity or safety of NMs in human is on utmost priority and a number of standards and guidelines have been developed for ensuring it. In most of the cases, the toxicity of NMs is evaluated in accordance with the regulation policies and guidelines developed for chemical drugs. Regulatory guidelines impose rigorous evaluation of NMs for their potential to cause acute to chronic toxicity. Whereas, on the other hand, guidelines from animal welfare restrict the use of animals to the most desired and permitted studies. In addition to toxicity profile, regulatory guidelines also ensure efficacy and quality by periodic assessment of critical quality attributes (CQAs) of NMs and postulation of regulatory accepted standardized test methods and strategies [106].

20.6.1 Some of the Challenges Associated with Nanomaterial Regulation Are [107, 108]

1. Diverse regional legislative framework and pharmaceutical classification.
2. Lack of consistent terminology and classification of NMs.
3. Lack of single and harmonized regulatory framework for NMs.
4. Limited number of approved products and their heterogeneity.
5. Lack of robust datasets necessary for designing common guidelines and standardized procedures for their quality and safety testing.
6. Lack of regulatory framework for ‘Nano similars’ (products similar to the ones already approved), ‘Borderline products’ (products that impart therapeutic action physically rather than chemically like metal oxides), and ‘Combination products’ (complex products possessing both diagnostic and therapeutic properties).

20.6.2 Current Global Regulatory Status

Regulatory authorities across the world realized the need for harmonization of information on nano-specific properties and requirement of an independent NM characterization facility. This would essentially support regulators in assessing the performance of new and existing test methods for the evaluation of nano-systems [109]. In USA, the Nanotechnology Characterization Laboratory (NCL) established by the National Cancer Institute (NCI) has been providing assistance to regulatory agencies such as US-FDA and product developers. This interaction is tremendously improving the quality and safety of NMs being approved. Similarly, six European laboratories with NCI-NCL have founded European Nanomedicine Characterization Laboratory (EU-NCL) to offer services and expertise to European regulatory agency including European Medical Agencies (EMA) and the developers at the same time [109].

Further, in order to quantify the importance of information acquired from the methods developed/validated under EU-NCL for regulatory, decision-making agencies periodically conduct survey. The survey encompasses questionnaires from regulatory scientists involved in NMs Working Group (NWG), established by International Pharmaceutical Regulators Forum (IPRF). The NWG acts as a platform for the dissemination of information on the work related to NMs/nanomaterials and supports regulatory harmonization and consensus finding on standards. Around 9 agencies including EMA, US-FDA, Health Canada (Canada), Pharmaceuticals and Medical Devices Agency (PMDA, Japan), Swissmedic (Switzerland), National Institute of Public Health and the Environment (RIVM in Dutch, Netherlands), Centre for Drug Evaluations (CDE, Taiwan), Medicines and Biological Products Office (MBPO, Brazil), and Ministry of Food and Drug Administration (MFDA, Korea) have responded to the surveys conducted by IPRF chaired by EMA[109] (Table 20.3). The results of the survey showed the consensus of participating agencies in attributes like stability, particle size and distribution, surface properties, solubility, and drug release kinetics as relevant for the evaluation of NMs. Particle dispersity, endotoxin testing, agglomeration behavior, and empty carrier-associated inherent toxicity testing were considered highly relevant before clinical trials being conducted [109].

Present regulatory guidelines require robust data on physicochemical properties and nano-specific toxicity on target tissues to identify the hazardous potential during preclinical testing itself [110, 111]. Such information will be beneficial to assess the need for additional toxicity testing before clinical trials. Specific in vitro tests need to be developed for toxicity and efficacy assessments to minimize the expenses involved and cruelty toward animals during preclinical testing.

Table 20.3 Present global status of NM regulatory guidelines

Region	Initial guidance document
USA	FDA's draft guidance for industry on • Liposome drug products (2002) • Drug products, including biological products, that contain nanomaterials (2017) • Liposome drug products (revised) (2018)
European Union	Reflection papers • Data requirements for intravenous iron-based nano-colloidal products developed with reference to an innovator medicinal product (EMA/CHMP/SWP/620008/2012) • Surface coatings: General issues for consideration regarding parenteral administration of coated NM products (EMA/325027/2013) • Data requirements for intravenous liposomal products developed with reference to an innovator liposomal product (CHMP/806058/2009/Rev.02) • Development of block-copolymer-micelle medicinal products (EMA/CHMP/13099/2013) • Nonclinical studies for generic nanoparticle iron medicinal product applications (EMA/CHMP/SWP/100094/2011)
Japan	Reflection papers • Development of block-copolymer-micelle medicinal products (2013) [Joint with European Medicine Agency] • Nucleic acids (SiRNAs)-loaded nanotechnology-based drug products (2016) Guidelines for development of liposome drug products (2016)
Canada	Nanotechnology-based health products and food-related general guidance
Brazil	Not available
Taiwan	Draft guidance on technical review on chemistry, manufacturing, and control (CMC) of liposomal drug products
Korea	Not available
India	• Guidelines for the evaluation of Nanopharmaceuticals • Guidelines for the evaluation of Nano-Agri input products and Nano-agriproducts
Switzerland	Not available

20.7 Summary and Challenges

The clinical toxicity of NMs has a great impact on their success in clinical translation. One of the main reasons for exploring NM platform is to reduce toxic effects associated with free drug administration. As discussed in this chapter, the toxicity of NMs heavily depends on their physicochemical properties and preferred route of administration. The chemistry of particle such as size, surface area, charge, and composition has been found to play a pivotal role in governing clinical toxicity of NMs. Efficient targeting and preventing early clearance of drug from body are essentially two important ways by which NMs help reducing toxicity while improving efficacy. Analysis at cellular, subcellular, and molecular levels evidently revealed that body responds differently with all NMs having varied physicochemical

Table 20.4 Some examples of FDA-approved NM products

Approved NMs	Year of approval	Nanocarrier systems	Therapeutic molecule	Target disease
Zoladex	1989	PLGA	Goserelin acetate	Prostate and breast cancer
Lupron Depo	1989	PLGA	Luprolide acetate	Prostate cancer and endometriosis
Doxil	1995	PEGylated liposome	Doxorubicin	Multiple cancer types
Feridex	1996	SPION coated with dextran		Imaging agent (also 2008)
Ambisome	1997	Liposome	Amphotericin B	Fungal infection
GastroMARK	2001	SPION coated with silicon		Imaging agent (also 2009)
Estrasorb	2003	Micelle	Estradiol	Vasomotor symptoms of menopause
Abraxane	2005	Albumin	Paclitaxel	Breast cancer
Sometulin	2007	PLGA	Lanreotide	Acromegaly
Cimzia	2008	Polymer-drug conjugate	PEGylated TNF-α	Crohn's disease
Ozurdex	2009	PLGA	Dexamethasone	Macular Edema
Onivyde	2015	Liposome	Irinotecane	Pancreatic cancer

properties, which ultimately define its toxic effects. Majority of NMs elicit toxic response by adversely affecting phagocytosis, free radical-induced oxidative stress, and disrupting cellular physiology by interacting with membrane or cytosolic signaling receptors. Choice of appropriate animal model for assenssing toxicity of NMs formulation remained a debatable topic. However, numerous studies have emphasized the fact that initial assesment of toxicity of developed formulation on cell-based model or zebra fish may help reducing experimental load on animal models. Various regulatory bodies are consistently trying to harmonizing globally acceptable guidelines to ensure this.

Despite substantial advances in nanotechnology and enormous efforts being invested, so far approximatley 50 NMs products succeed in receiving clinical approval in past 20 years (key examples in Table 20.4). Clinical toxicity remained a long-standing challenge that indirectly impede the success in the clinical translation of NMs. In most of the cases, toxicity of NMs is attributable to NP accumulation (mostly metallic NPs) and undesirable drug release (nanocarriers) behaviour at off-target organs. Additionally, availability of suitable model for toxicity testing limits the progress of most NMs. Toxicological results from cell culture and lower animal are rarely reproducible in humans, which ultimately result in discontinuation of clinical trials in early stage. Further, there are very few harmonized regulatory guidelines specific for NMs. There is a pressing need to formulate separate specific set of guidelines for evaluation of NMs under clinical trials.

20.8 Future Prospects

Clinical safety is the first and the most important criteria for clinical translation of NMs. Challenges at biological, technological, economical, ethical, and regulatory grounds cumulatively slow down overall progress in clinical translation. In toxicological perspective, there is great need to accelerate the development and translation of suitable and cost-effective advanced cell-free and animal-free models for toxicity evaluation [112, 113]. Models should be able to provide rapid and high throughput toxicological profiling with anticipated similarity of results in human trials. Such models will help reducing heavy usage and concurrent cruelty associated with animal testing. Alongside, it is necessary to define separate safety and regulatory guidelines about CQA specifically for NMs giving bifurcated division on biodegradable as well as nondegradable products. Results or case studies from toxicity and safety studies on NMs from previously conducted clinical studies could be centralized on a single portal for the public interest in specific domains. It will help expedite the development of NMs generated on a common platform or with a similar ingredient.

Acknowledgments NA would like to thank IIT-Bombay and Ministry of Human Resource Development, Govt. of India for his Institute Postdoctoral Fellowship. PB and EB would like to thank IIT Bombay and MHRD Govt. of India for their research fellowship during PhD.

Conflict of Interest The authors declare no conflict of interest.

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Nanomedicine: Risk, Safety, Regulation, and Public Health

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Abstract

Nanomedicine is a developing area of medicine, which uses the techniques and tools of nanotechnology to deliver the drugs using appropriate drug delivery systems. These are used for diagnosis, prevention, or treatment of acute or chronic diseases. At present, there are three major areas where nanotechnology has been adopted in medicine, diagnostics, theranostics, drug delivery systems, and regenerative medicine. A new branch of toxicology has been proposed called nanotoxicology, and a lot of studies are reported about various risks involved with nanoapplications. This chapter covers various aspects of nanomedicines and risks involved in using nanomedicine, some of the safety concerns are discussed, and it also covers the regulatory aspect of nanoproducts under US FDA as well as approaches adopted by various countries. Finally, it discusses the public health and public opinion about nanoproducts and nanomedicines.

Keywords

Nanomedicine · Nanotoxicity · Nanosafety · Public wellbeing · Regenerative medicine

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M. K. Das, Y. V Pathak (eds.), *Nano Medicine and Nano Safety*,
https://doi.org/10.1007/978-981-15-6255-6_21

21.1 Introduction

21.1.1 NanoMedicine

Nanomedicine is a developing area of medicine, which uses the techniques and tools of nanotechnology to deliver the drugs using appropriate delivery systems. These are recommended for diagnosis, prevention, or treatment of acute or chronic diseases. There are several devices and techniques that are used in surgery, which can also come under nanomedicine if they have the ability to prevent or treat the diseases [1].

The application of nanotechnology in developing new medicines is a part of the research and development worldwide. In USA and European Union (EU), it is recognized as an important enabling technology, capable of providing an innovative medical solution to address medical needs especially in the treatment of chronic diseases [2, 3].

Major applications of nanomedicine:

Three major areas have been identified in the medical field for applications of nanotechnology in medicines:

Diagnostics and Theranostics Nanomaterials have shown significant applications in the development of diagnostic tools for diagnosis, which provide information about the disease. These are also used to analyze or detect diseases or medical conditions, which can be useful in treating thee conditions. Nanodiagnostics *is* used to describe *application* of nanobiotechnology in diagnosis, which *helps in* developing personalized cancer therapy. It is *supported by* pharmacogenetics, pharmacogenomics, and pharmacoproteomic information. It also considers environmental factors that influence the response to patient therapy [4]. Nanoparticles with unique intrinsic physical properties are used as bioimaging agents. Some of these imaging agents included nanoprobes and magnetic metal nanoparticles, which *are* widely used as MRI contrast agents for cancer imaging, helping *to provide* anatomical details and monitoring of the therapeutic response [5].

Theranostics is a new area of therapy, which uses specific targeted therapy-based targeted diagnostic tests. These involve using nanoscience to provide diagnostic and therapeutic applications in a single form allowing diagnosis, drug delivery, and treatment for a particular disease simultaneously. Some of the *examples of* theranostics include Lutetium Octreotate therapy (somatostatin positive tumors), Lutetium PSMA Therapy (prostate cancer), and Yttrium-90 SIRT for liver *cancer*.

Chitosan, a biopolymer, is biocompatible and carries several functional groups, which can be used for delivering drugs to the human body. It is *utilized in* the formulation or coating *of* nanoparticles, thus producing different particles with multiple functions, which can be used *for the* detection and diagnosis of chronic diseases [6, 7].

Lee et al. [8] reported about oleic acid-coated FeO nanoparticles. Oleic acid-conjugated chitosan (oleoyl-chitosan) was reported to be used for examining the accumulation of nanoparticles in tumor cells through the penetrability and retention in the *in vivo* state. Analytical tools, such as near-infrared and *resonance* imaging

(MRI), were used to study the accumulation of these nanoparticles. Both these techniques showed measurable signal strength and enhanced effect in the tumor tissues through a higher EPR consequence after the injection of cyanine-5-attached oleyl-chitosan nanoparticles intravenously in in vivo studies [8].

Yang et al. [9] reported the application of nanoparticles for the diagnosis of colorectal cancer (CC) cells via a light-mediated mechanism. These cells are visible owing to the physical conjugation of alginate with folic acid-modified chitosan. This property led to the formation of nanoparticles with increased 5-aminolevulinic (5-ALA) release in the cell lysosome. The reports confirmed that alginate-engineered nanoparticles were voluntarily endocytosed by the CC cells due to the folate receptor-based endocytosis process [9].

Drug Delivery Systems or Nanomedicine in Therapy Nanomedicine and nanodelivery systems are rapidly growing science where nanoscale materials are employed to serve as diagnostic tools or deliver drugs to specific sites in a sustained delivery [10] Several polymeric and natural materials have been used as carriers in the form of nanoparticles for drug delivery including chitosan, alginate, pectin, PLGA, PLA, Caprolactone, and so on. The nanodrug delivery has been used for buccal, intestinal, nasal, eye, pulmonary, and vaginal therapy. From the 1990s, FDA of the United States has approved several nanotechnology-based products and clinical trials of such products have significantly increased. Even though regulatory mechanisms for nanomedicines, e.g., their safety/toxicity assessments, are in the formative stage, nanomedicine has significantly improved the way we discover and administer drugs [10].

Parnajape and Goymann, 2014 have written a very nice review on Colloidal drug delivery systems and their application in lung diseases. We strongly recommend the readers to refer to this interesting review.

Regenerative Medicine (RM) Regenerative medicine can be identified as the process of "regenerating" human cells, tissues, or organs to establish a normal function of these body parts. RM shows the promise of reviving damaged tissues and organs in the body by substituting or by activating the body's own repair mechanisms to heal in the natural process. RM may also help scientists to grow tissues and organs in the laboratory setting and safely implant them when the body is unable to heal itself due to damaged tissues or cells. Currently, it is estimated that approximately one in three geriatric American population could potentially benefit from RM. It is a biomedical approach that uses nanoscience to build clinical therapy involving the stem cells or other bodily tissues. The examples of such RM include injection of stem cells or progenitor cells in the form of nanoparticles, immunomodulation therapy using nano-embedded biologically active molecules or secretions (infused by cells), or tissue engineering using laboratory-grown cells and tissues for treatment [11].

RM aims *to understand the* functional rehabilitation of body systems injured due to external harm, disease, or aging. Nanotechnology can provide advanced biomaterials with specified morphologies, promoting the adhesion and proliferation

of stem cells and accelerating *somatic cell* differentiation in tissue engineering. Pan et al. wrote a review that summarizes the biological effects of nanomaterials and their RM applications in orthopedic surgery research and *nervous tissue* [12].

Biocompatibility is the fate of biopolymers and their compatibility with body systems. With the application of nanotechnology, new concerns *are associated with* biocompatibility *of these nano*biomaterials. The size and variability of physicochemical properties, nanoparticles' distribution within the body, and interactions with the target cells and tissues can pose challenges to the patient. Zor et al. *have provided a summary* about NPs, the concept of biocompatibility and biocompatibility-related issues in nanomedicine, *and a number of other* different nanoparticles [13]. In *a* review by [14], reported recent applications of nanotechnology to ophthalmology, drug, gene, and trophic factor delivery [14] were discussed.

21.2 Growing Areas for Nanomedicines

Cancer treatment is one of the most important areas of nanomedicine applications, and more than 90% of the products approved by US FDA as commercial products or investigational products are used for diagnosis or treatment of various types of cancers. The next group is infectious diseases that witness many new products for the treatment of various infectious diseases using nanodrug delivery systems. Some other areas include treatment of hepatitis, anesthetics, cardiovascular disorders, inflammatory and immune disorders, endocrinal and exocrine diseases, and neurodegenerative diseases. Some other areas that are employing nanoparticles include in vitro testing, in vivo imaging, in vivo device coatings, bone substitute, dental applications, medical dressing, and preventing bacterial infections of wounds, surgical devices, and nanorobots for minimally invasive surgeries.

21.3 Nanomedicine: Known and Unknown Risks

Nanotoxicity The term nanotoxicology was coined in 2004, which refers to the evaluation of the destructive outcomes of nanostructure interactions with biological and ecological systems [15]. Many research papers are published since then (2004), which address various aspects of nanoparticle toxicology for both biological and environmental systems. The nanosize particles pose different kinds of challenges due to their quantum size and enormous surface area to volume ratio, which may or may not bring toxicity to the living things.

As of today, we do not have a complete understanding of the biointeractions of nanoparticles. The biochemical pathways and biochemical molecule interaction with the quantum size nanoparticles are not well understood. One of the major challenges is the nonavailability of the appropriate analytical techniques to measure the small amount of material in the tune of nanograms to picograms when it is delivered to biological systems. Magnificent growth of healthcare applications of nanoparticles

in the last two decades triggers the concerns of possible potential health and environmental risks due to the use and widespread production of nanoparticles.

The nanoparticle applications creating concern include the deposition and clearing, biocompatibility, systemic translocation and body distribution of nanoparticles, intestinal tract involvement, and direct effects on the central nervous system, which warrants a detailed study. The toxic effects caused due to different physicochemical and structural properties of nanoparticles that result from their nanoscale size may be responsible for a number of material interactions, which need further evaluation.

In the coming decades, we have to develop methods and techniques to understand the toxicology techniques to understand the increasingly sophisticated nanoparticle-based systems that exhibit novel dynamic and multifunctional applications. Today, we are not aware whether these systems may or may not be good for the users or biological systems they will be exposed to in the course of their production to application. In order to better serve the challenges, the scientific community working in nanotoxicology will have to move toward picotoxicology and to treat these sophisticated nanoparticles as a challenging entity.

21.4 Risks for Nanomedicines

1. Nanoparticles due to their size can enter lungs, GIT, Lymph systems, mucosa, and skin and can accumulate there, leading to local toxicity, which needs more data for further evaluation and educated decision about nanomedicine applications and safety.
2. Nanoparticles due to their size can influence Absorption, Distribution, Metabolism, and Elimination, and there is a need to understand how these processes are affected for nanomedicines.
3. The smaller size of Nanoparticles can affect the dose level of the drugs, due to the enormous surface area, and the dose levels can be reduced, leading to reduced toxicity.
4. There is a need to develop techniques and animal models to understand the nanotoxicity and nanosafety, which are not available at present.
5. One has to go carefully as the effects are still unknown and may be quite dangerous to the patients, and so, to protect the safety for patients, we need more data in these areas.

21.5 Nanomedicines in Biological Systems

As the nanoparticles are size-specific, they can range from very small like 10–20 nm to almost 500 nm sizes. For each of these size ranges, they offer significantly different surface area, and individual particles might exhibit different properties. These particles based on their particle size range may exhibit different levels of interactions in the biological systems.

We need to seek answers to these questions:

1. Will nanoparticles gain access to tissues and cells that normally would be avoided by the larger particles?
2. Once nanoparticles enter tissues, how long do they remain there? What is their fate once they enter in the tissues?
3. How are these cleared from tissues and blood? Elimination from the biological system is a major challenge, and very little data are available to find out the elimination route from body systems for the nanomedicines?
4. If nanoparticles enter cells, what effects do these have on cellular functions (transient and/or permanent)? Do the cells have the ability to remove these nanoparticles from the cellular structure?
5. Do the nanoparticles interact differently with different cell types?

21.6 Route-Specific Issues Related to Inhalation

The accumulation of nanoparticles in the alveoli and other lung cellular structures may lead to local toxicity, it can also affect the regular physiological function of Lungs, and these may exhibit some sort of local lung toxicity, which need more data and studies. It is expected that the nanoparticles that carry drugs may release the drug in the lungs and whether the drug can enter into systemic circulation and the extent it will reach systemic circulation is not clearly known yet [16].

21.7 Route-Specific Issues Related to Subcutaneous Sensitization

Nanomaterials can enter the body by different routes, if *they* are nanostructured materials, which become airborne or come into contact with the skin, they get absorbed through the skin surface [17] and can penetrate and enter the subcutaneous levels. Countless nanoparticles float in the environment, which can potentially be absorbed by the human skin surface. There are many consumer products *like* sunscreens, cosmetics, and natural or man-made processes, which contribute to the concentrations of nanoparticles in the environment [18]. These nanoparticles in the air represent a target for potential toxicity for human skin and other body organs. These nanoparticles can also enter the human body through inhalation [19].

21.8 Route-Specific Issues Dermal

21.8.1 Increased Dermal and Systemic Bioavailability

Increased follicle retention and distribution to local lymph nodes.

The dermal absorption refers to the dermis that is the outer covering of the skin classified into two parts, epidermis and dermis. Skin is considered to be the largest

surface area for absorption of material, of the human body, and it also works as a protective layer for the underlying internal organs.

Debilitated skin represents a possible channel for entry of finer and even larger particles (0.5–7 μm) as reported [20]. Accumulation of soil particles in lymph nodes of bear-footed human *related to* elephantiasis was reported. It is observed that quantum dot NPs can penetrate the skin, providing insight into potential danger to individuals involved in the manufacturing of quantum dots [21].

21.9 Other Safety Issues Due to Nanoparticle Exposure

Phototoxicity It is very well exemplified by Titanium Dioxide nanoparticle studies. Titanium dioxide (TiO2) is a major component in sunscreens, soaps, shampoos, toothpastes, cosmetics, paper products, plastics, ink, paint, and building materials [22] in both its bulk form and its nanoform. It is also *utilized in* human food as a colorant and inactive ingredient. From 1916 to 2011, an estimated total of 165,050,000 metric tons of TiO2 pigments were produced worldwide (bulk form and nanoform combined), with a current annual estimated production of more than six million tonnes/year [23, 24].

Titanium dioxide nanoparticles are considered to be photoactive and produce reactive oxygen species under natural sunlight, showing dangerous side effects in human bodies, which warrants further a detailed study.

Hemocompatibility Mayer et al. [25] focused on adverse effects on human blood and its interaction with the blood and blood constituents. Blood-related interactions, which include and need special attention, are clotting, reaction-triggering inflammatory and immune responses, and hemolysis due to interaction with the nanoparticles. They also reported the effect of size and surface charge on the induction of coagulation, thrombocyte activation, complement activation, granulocyte activation, and hemolysis. Using polystyrene particles, they showed the possible interactions between the blood components, the different size and charge of the polystyrene nanoparticles, and variations in the interactions. Positive surface charge induced activation of the complement. Small size caused thrombocyte and granulocyte activation and hemolysis [25].

21.10 Possibilities of Nanoparticle Exposure in Industrial Setting

There are likely possibilities that the resultant environment may increase nanoparticle hazards too:

1. If you work with nanoparticles in solution without adequate protection, you may get exposed to the risk of skin exposure and side effects.

2. In nanoparticle processing where agitation is occurring, it will lead to an increase in p inhalation of the droplets, leading to lung and respiratory tract injuries and side effects.
3. Manufacturing of nanoparticles in the gas phase or an aerosolized system, in nonenclosed systems, will enhance the likelihood of aerosol expose to the workplace, and the nanoparticles may find their way to Lungs and other body organs.
4. People who are responsible for the maintenance of equipment and processes used to produce or fabricate nanosize materials or the cleanup of spills or waste material will be exposed to fine nanoparticles and may show the side effects of such exposure in due course of time [26].
5. Cleaning of dust collection systems may capture NPs, which can pose risk for both skin and inhalation for the cleaning persons.
6. The transfer of nanomaterials in open systems is likely to increase exposure potentials even for relatively hydrophobic NPs [27]. Open systems during NP processing may increase exposure to human beings [28].

With the present amount and extent of knowledge available and the techniques used to understand the nanoparticle toxicity, we still need to be worried about the safety of these nanomedicines in different settings, such as natural nanomaterial exposure or anthropogenic nanomaterial exposure.

21.11 Occupational Hazards After Nanoparticle Exposure

The exposures to nanoparticles might lead to serious inflammation after the deposition of nanoparticles in the respiratory tract because of their large particle numbers, surface areas, complex chemical compositions, sizes, shapes, and electric charges. Several studies such as for different nanomaterials have been reported, and we will recommend to visit these research papers to get in-depth information about occupational hazards due to nanoparticles. Kousake et al. studied the nanosized magnetic nanoparticles and showed the challenges due to the size of the particles to the workers working with these nanoparticles.

Manke et al. [29] reported similar challenges posed by carbon nanotubes, and they used mice model to study the interactions of carbon nanotubes and their effect on the DNA system of mice.

Hamilton et al. [30] examined the effect of size (20 and 110 nm) and surface stabilization (citrate and PVP coatings) on toxicity, particle uptake, and NLRP3 inflammasome activation in a variety of macrophage and epithelial cell lines.

Wang and Fan [31] reported nanoscale titanium dioxide (TiO2), one of the most commonly produced and widely used nanoparticles, as a model to study the nanotoxicity of nanoparticles. The correlation between the lung toxicity and pulmonary cell impairment *associated with* TiO2 NPs and their unusual structural features, including size, shape, crystal phases, and surface coating, is reviewed *thoroughly in this research paper*.

Recent toxicological studies have suggested that they can easily penetrate cells or tissue and may result in many irreversible health effects, such as chronic pulmonary inflammation, epithelial cell hyperplasia, cardiovascular disease, and lung tumors.

21.12 Nanomedicine and the Pharmacokinetic and Pharmacodynamic Considerations

Nanodrug systems *are* developed *for various* routes of administration and materials, which include dendrimers, nanocrystals, emulsions, liposomes, solid lipid nanoparticles, micelles, and polymeric nanoparticles. Nanodrug systems *are* employed *to enhance* the efficacy, safety, physicochemical properties, and pharmacokinetic/pharmacodynamic profile of pharmaceutical substances. This creates a need for understanding of the pharmacokinetic and safety characteristics of nanodrugs and the limitation of each drug delivery mentioned above [32].

Particle size, shape (chemical structure), and surface chemical characteristics affect the pharmacokinetic characteristics *of* nanomedicines [33]. Nanoparticles with sizes less than 10 nm are removed by kidneys, whereas those with particle size more than 10 nm are removed by the liver and/or the mononuclear-phagocyte system (MPS).

By regulating particle size in nanoproducts, their retention in target tissues can be increased and can also be removed rapidly when distributed to nontarget tissues. Choi et al. reported Nanoparticle targeting *supported* chemical properties of nanoparticles and surface coatings comprises active and passive targeting [34]. This is especially applicable to solid cancer tumors *during which* targeting *leads to* increased *vessel* and transporter permeations and retention (enhanced permeability and retention, EPR effect) of nanomedicines and their increased accumulation in tumor tissues [34].

Specific or active targeting is defined as selective transport of nanomedicines containing protein, antibody or small molecule only to specific tissues and/or specific cells. This may occur via homing to overexpressed cell-surface receptors [34].

Some of the questions that can be raised and need to be answered when it comes to the Pharmacokinetic and pharmacodynamics studies of nanomedicines are as follows:

1. Are there differences in the ADME profile, for nanoparticles versus larger particles? Are these drug specific or nonspecific?
2. Are current methods used for measuring drug levels in blood and tissues adequate for assessing levels of drugs delivered through nanoparticles (appropriateness of method and limits of detection)? As the drug concentrations in blood and plasma may be in picogram levels?
3. How accurate are mass balance studies, if doses of drug administered are very low, i.e., can we account for 100% of the drug administered?

4. How is clearance of targeted nanoparticles accurately assessed? If nanoparticles concentrate in a particular tissue, how will clearance be assessed accurately?
5. Can nanoparticles be successfully labeled for ADME studies?

The present data are miniscule, mostly obtained by pharmaceutical companies for their brand products, and little available for the researchers. So, there is a need for developing some database where such information may be made available to the researchers and can be used for further development of the nanomedicines.

21.13 Regulations of Nanoproducts and Nanomedicines

In recent years, the incorporation of nanomaterials into products and components utilized in the pharmaceutical and medical fields has increased significantly. There are a large number of products with nanomaterials already in commercial distribution and many more in the development and conceptual stages. It is an accepted fact that present risk assessment methodologies *are not* sufficient *to deal with* this toxicity because *they typically* consider mass alone and ignore the number of particles and surface area [35–37].

Regulatory bodies are looking at *various possibilities and* factors and trying to develop a rational system, *which will* signal *the general public* that its health and safety interests are being protected while ensuring developers and supporters that their effort *will not* be compromised if certain safeguards are observed [35–37].

21.14 Challenges Ahead for the Regulatory Bodies

Two major challenges are faced by the governmental bodies considering the need to regulate nanotechnology

1. Inadequate reliable data to make any rational decisions related to nanomaterials.
2. Inadequate reliable tools to help to reproducibly characterize and evaluate the nanoparticle-based products.

The question that the regulatory bodies will have to answer is How are the new technology and its characterization, application and established safety to the consumers, and the personnel involved in manufacturing and handling of these products? Regulatory bodies tempted to use the application of existing regulatory provisions for pharmaceuticals and medical products to nanomaterials containing products. This might help to circumvent the delays caused due to nonavailability of specific regulations for nanoproducts. Invariably, the question arises as to whether the present framework is relevant and/or adequate to address issues related to nanoproducts. Nanoproducts in the food sector have the potential to lead to healthier, safer, and better tasting foods, with improved food packaging, but the hesitation of

the food industry and public fears in some countries about tampering with nature *could also be* holding back the introduction of nanofoods [38].

United States FDA generated its own definition in 2011 under guidance issued by FDA for nanoproducts. The FDA highlighted the following aspects: [39].

1. Engineered material or end product
   This term is used to distinguish between products that have been engineered to contain nanoscale materials or involve the application of nanotechnology from those products that contain incidental or background levels of nanomaterials or those that contain materials that naturally occur in the nanoscale range.
2. At least one dimension in the nanoscale range (approximately 1–100 nm).
3. Exhibits properties or phenomena . . . that are attributable to their dimension(s)
   These terms are used because properties and phenomena of materials at the nanoscale enable applications that can affect the safety, effectiveness, performance, quality, and, where applicable, public health impact of FDA-regulated products [39].

21.15 US FDA Guidance Document 2011

FDA intends to incorporate attention to nanomaterials into its product-specific review procedures and apply certain considerations to better understand the properties and behavior of engineered nanomaterials.

For products not subject to premarket review, manufacturers are encouraged to consult with FDA to reduce the risk of unintended harm to human or animal health [39].

We strongly recommend the readers to consult the US FDA guidelines and keep abrest of the new developments and suggestions posted by the FDA on their website.

21.16 US FDA Center for Drug Evaluation and Research (CDER) Guidelines for Nanoproducts

The Center for Drug Evaluation and Research (CDER) has posted a Manual of Policies and Procedures (MAPP), effective June 3, 2010.

The Manual provides CMC section (chemistry, manufacturing, and controls) reviewers in CDER with "the framework by which relevant information about nanomaterial-containing drugs will now be captured in CMC reviews of CDER drug application submissions."

U.S. Food and Drug Administration. 2010. CDER Manual of Policies & Procedures, Chap. 9, "Reporting Format for Nanotechnology -Related Information in CMC Review." http://www.fda.gov/AboutFDA/CentersOffices/OfficeofMedicalProductsandTobacco/CDER/ManualofPoliciesProcedures/default.htm.

We strongly recommend the readers to visit this website for the most updated guidelines for their references.

21.17 The Center for Veterinary Medicine (CVM) Guidelines

The center for veterinary medicine also provides a similar guideline document for veterinary products. CVM writes a procedure that is intended to identify points to consider for technical sections for products containing nanomaterials or otherwise involve the application of nanotechnology, which might require additional data or special steps to address potential safety or quality issues.

The CVM procedure refers to the investigational stage, also the postclinical stage.

We strongly recommend the readers to visit their website for the most updated guidelines for their references.

21.18 The Center for Food Safety and Applied Nutrition (CFSAN) Guidelines

CFSAN has issued two guidelines,

1. For cosmetics and one.
2. For food.

Both these guidelines contain important information about the agency's thinking on nanotechnology-based product regulation.

The first document discusses the proof of safety for cosmetics that contain engineered nanomaterials.

There has been concerted opposition to the utilization of nanomaterials in cosmetics, especially fullerenes because they pose a big theoretical risk to users without a perceived benefit that might be worth any risk.

U.S. Food and Drug Administration. 2012. Draft Guidance for Industry: Safety of Nanomaterials in Cosmetic Products. http://www.fda.gov/Cosmetics/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/ucm300886.htm.

We strongly recommend the readers to visit this website for the most updated guidelines for their references.

21.19 CFSAN on Food and Food Packaging Guidance

- The other guidance document posted by CFSAN is related to food and food packaging.
- CFSAN identifies the purpose of the document "to describe the factors you should consider when determining whether a significant change in the manufacturing process for a food substance already in the market:
- Affects the identity and/or safety of the food substance;
- Affects the regulatory status of the use of the food substance; and.
- Does the alteration in products warrants regulatory submission to FDA.

- U.S. Food and Drug Administration. 2012. Draft Guidance for Industry: http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/IngredientsAdditivesGRASPackaging/ucm300661.htm.

We strongly recommend the readers to visit this website for the most updated guidelines for their references.

21.20 Environmental Protection Agencies (EPA) and Toxic Substances Control Act (TSCA) Recommendations Adopted by FDA

The strategy of EPA (Environmental protection agency), which is somewhat more directed to implementing specific regulations, supported two major statutes it administers.

The law of broader application is the Toxic Substances Control Act (TSCA) that gives EPA authority over "chemical substances," including nanoscale materials.

EPA would like to ensure that nanoscale materials are manufactured and utilized in a manner that protects against unreasonable risks to human health and therefore the environment, and EPA is pursuing a comprehensive four-prong regulatory approach under TSCA.

This approach includes the Pre-Manufacture Notifications (PMN); Significant New Use Rule (SNUR); information gathering authority; and test authority.

We strongly recommend the readers to visit these EPA websites for the most updated guidelines for their references.

21.21 FDA Guidelines on Devices Using Nanotechnology

Nanotechnology may be a new and evolving field for both the medical device industry and therefore the regulating Agency (Such as the US FDA).

At this time, FDA has not adopted nanotechnology-specific criteria to assist manufacturers in determining when a change to a device that contains nanomaterials or otherwise involves the application of nanotechnology rises to the extent of significance that needs submission of a replacement 510(k).

For this reason, FDA recommends that manufacturers consult the agency for any nanotechnology-related changes to devices to work out whether and the way the change may affect the security or effectiveness of the device.

U.S. Food and Drug Administration. Guidance for Industry and FDA Staff—510(k) Device Modifications: Deciding When to Submit a 510(k) for a Change to an Existing Device. http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm265274.htm.

We strongly recommend the readers to visit this website for the most updated guidelines for their references.

21.22 Regulations About Nanoproducts in Other Countries

European Union on Nanomaterials The European Union (EU) has been active in improving regulations to approve nanomaterials, their impact on the environment, and the possible regulation of pharmaceuticals, foods, cosmetics, and other medical products. *Several* reports and descriptions *of these* activities are readily available on *their websites*.

Germany Guidelines Germany is interested to have a dialogue on the effects on the environment from the nanomaterial production and use of such materials for human consumption. They have come up with recommendations regarding regulations and labeling, and there has been a consideration and need felt for *the necessity* for further research.

The German Federal Institute for Risk Assessment has posted its view on the potential for risk in the use of nanomaterials (Figure taken from http://www.european-coatings.com/Raw-materials-technologies/Technologies/Nanotechnology/Survey-on-micro-and-nanotechnology-in-Germany). We strongly recommend the readers to visit this website for the most updated guidelines for their references.

Australia Guidelines In Australia, the federal organization responsible for drugs is known as Therapeutic Goods Administration (TGA), which manages the regulation of medical products, pharmaceuticals, and cosmetics.

TGA has taken *keen* interest in the regulation of nanotechnology, due to controversy of using sunscreens, leading to the high level of skin cancers in Australians. TGA describes its plans on its website:

To date, *the prevailing* regulatory framework of the TGA has proved to be *quite capable of* identifying, assessing, and managing the risks *related to* therapeutic products that incorporate nanotechnologies. (Australian Government Department of Health Guidelines).

21.23 Nanotechnology, Public Health, and Public Opinions

Nanoproducts have been a misleading concept in the general public as they have been hijacked by many different products that have no relationships such as I POD nano, nanocars, or computer nanoproducts, nano/quantum TV, nanopaints, and so on. Worldwide, there are still no clear guidelines on how the regulatory bodies are responsible for consumer product safety (mostly controlled by the local Government) will regulate nanomaterials, and this has led to the reluctance of some nanoproduct manufacturers to use the term nano in their marketing.

It is also creating a fear in these companies that *they could* be targeted by consumer advocacy groups, which may affect their product market However, this reluctance is an overall reflection of uncertainty surrounding nanoproducts. The unclear guidelines lead to a lingering possibility that consumers might reject

nanotechnology products. Based on this concern about the impact of nanomaterials on human health and the environment, therefore, this is likely to be one of the reasons why the commercialization of nanotechnology is not keeping pace with basic research in nanoscience [40].

Even though thousands of research papers are published, which are mostly dedicated to some or other aspects of nanotechnology, there are very few articles that are geared toward the education of public and public opinion about nanotechnology [41].

In a recent publication by [42], it is mentioned that even with the widespread use of nanomaterials in everyday life, consumer knowledge about the functionality, benefits, and possible danger of nanotechnology is still modest. As with any developing technology, its public perception has direct implications on future policies and *has got to* be taken *under consideration* by academia and industry alike. As an interdisciplinary research project, they conducted an online survey using a Citizen Science-guided approach. The main goal was to evaluate the current levels of knowledge and the attitude toward nanotechnology among the general Austrian public and to determine how differing sociodemographic factors may affect these. Over the course of 17 months, they collected a total of 1067 responses and quantitatively analyzed. They found that while Austrians display a generally optimistic view and a positive attitude toward nanotechnology, there are still remaining concerns about its safety and possible risks [42].

Understanding stakeholder views is *an important part of* addressing this uncertainty. This provides insight into the possible social reactions and tolerance of unpredictable risks.

In the field of nanotechnology, due to the existence of uncertainties regarding *the important* and perceived risks, this technology may *wear* society. We need to offer better evidence, which is needed to address various issues. Capon et al. conducted a survey of public, academics, Government officials, and business stakeholders about the perception of the risk with nanotechnology. Capon et al. recommended that policy makers should consider the disparities in risk and trust perceptions. This is happening between *the general public* and influential stakeholders. It places a greater emphasis on risk communication and the uncertainties of risk assessment in these areas. Scientists being *the very best* trusted group are well placed *to speak* the risks of nanotechnologies to *the general public*.

Public acceptance is of utmost importance and a necessary pre-requisite for the sustainable development of nanotechnologies, or for that matter, any new technologies are introduced in the society. A large number of research initiatives and consumer polls have attempted to gauge public awareness and perception of nanomaterials and nano-enabled products [43–45].

Overall, it appears that the public is still largely unaware of nanotechnology and its applications. Of those who are aware, nanotechnology is generally seen in a positive light.

In general, concerns about the perceptions of risks of nanotechnology are low and there is some evidence to suggest that these may be decreasing further; as people are using these products with no apparent risks, the acceptance is growing. However, the

potential for *a big* change *public* opinion, *thanks to* the way *in which* nanotechnologies are portrayed by the media and NGOs or *because the results of* a single negative event, remains high, and media can play an important role in changing these perceptions about nanotechnologies.

Many initiatives on Government and NGO levels are underway to promote interaction between institutions and the public, but what is stressed most is that risk communication strategies should involve "early" or "upstream" public engagement. This will create an opportunity for the public to inform and shape the direction of research and development and helps to generate and maintain trust and confidence in new technologies.

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New Deliveries and Nanomedicines: Commercial Aspects and Business Perspectives

22

Sunita Dahiya and Rajiv Dahiya

Abstract

At present, the global pharmaceutical industry is going through various challenges such as limited and slower translation of medical science advancements in the therapeutic benefits, changing regulatory requirements, escalating costs, and lower estimated returns spent on R&D. These challenges collectively pose the pharmaceutical business sector a less fascinating choice for the investors. The developmental cost for transferring a novel drug candidate to the commercial platform is quite expensive, risky as well as time-consuming; however, the total costs of imitations are considerably low, simple, and less tedious. Under this scenario, counting on the new therapeutic opportunities for existing drugs using "repurposing" or "reformulation" can be considered as the mainstream commercial strategy for the pharmaceutical industry. In addition to clinical success, these strategies offer financial benefits in terms of relatively low cost and shorter development span, patent extension provisions, as well as higher and fast returns as compared to developing an entirely new drug, and therefore, manifest to be powerful strategies for the financial health of the companies. Also, over the last three decades, nano-drug delivery and nanomedicines have drawn considerable attention of the researchers and pharmaceutical companies due to their undeniable advantages over conventional dosage forms which has led to several successful commercial nanoformulations. In fact, first generation nanoformulations were expanded by altering original formulation, i.e. by reformulating existing drugs to overcome associated physicochemical and

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M. K. Das, Y. V Pathak (eds.), *Nano Medicine and Nano Safety*,
https://doi.org/10.1007/978-981-15-6255-6_22

toxicity issues. This approach further revolutionized in the second generation with an emergence of new classes of therapeutic biomolecules including proteins and nucleic acids, or other biotherapeutics, as the therapeutic effectiveness of such molecules relied on effectual nano-based deliveries. Among different repurposing approaches, reformulation can be viewed as the key segment for the growth in product's life cycle management, as it acts as a pivotal resource of outrageous revenue generation and value addition in company's economic health and status. This chapter portrays various economic facets and business perspectives of the new deliveries including nanomedicines, and discusses road maps and challenges to their successful clinical translation and commercialization.

Keywords

New deliveries · Nanomedicines · Commercial · Business · Market · R&D · Investment · Repurposing · Reformulation

22.1 Overview

Advancement of drug delivery systems for enhanced health benefits and patient compliance has been a subject of ongoing interest in the pharmaceutical industry with a specific focus on creating better and smarter dosage forms. This includes not only meeting the therapeutic needs by maintaining the desired plasma concentrations for extended periods, but also the ability of avoiding drug toxicity, thereby making patient compliance less oppressive. Pharmaceutical companies display perspicuous interests to try smart polymers and technologies to achieve performance-driven and competitive differentiation in the market by producing high quality and cost-effective delivery systems. In this milieu, pharmaceutical companies have traditionally converged on developing novel compounds with documented safety and efficacy in treating a disease. In doing so, these companies have relied upon the consortium position held by their patent status. Later, with the continued development of controlled drug delivery systems, a parallel industry emerged that grown their focuses on the improvement of drug delivery technologies by either aiding or enabling the administration of therapeutic compounds. Such delivery systems included various formulation technologies as well as specialized devices such as inhalers, transdermal patches, nano-drug delivery systems, etc. These endeavors led to partnering or merging of pharmaceutical companies with drug delivery companies to co-establish next generation proprietary products. This can be well-exemplified by Alza that developed several sustained-release cardiovascular drug delivery products with the pharmaceutical firms like Bayer AG and Pfizer. With such a co-developed and/or co-launched drug delivery system, the relationship terms between the drug delivery companies and pharmaceutical companies are determined by the virtue of value that the co-developed product or device creates in the market. DiMasi and co-workers proposed methodological approach that estimated the resource values

spent by industry to invent and develop novel therapeutics and biologics, including the degree to which private sectors' expenditure shifted gradually [1, 2]. It was indicated that the new drug development expenditures played an unambiguous role in the research investment incentives necessary for medical innovation.

22.2 Basic Economics and Resources

22.2.1 Profits and Patent Protection

Since a new drug can enjoy its patent protection only for 18 years, the off-patent time period allows any other company to manufacture that product. This leads to a drastic reduction in the profit provoked by that particular new drug molecule. On the other side, the percentage of the cost that is used for the development of a novel drug delivery system is as low as 10% of the cost of the new drug development process. This concept enables a higher investment return and shorter time span for developing new medicinal products which offer fast-track societal benefits in addition to its eligibility to apply for its own patent. Also, when a newly developed entity requires an efficiently designed delivery system, the development of a novel drug delivery system could be carried out not only for that specific new drug, but can be unquestionably applied to other existing drugs for omitting crucial patent regulations [3].

From the pharmaceutical manufacturers' viewpoint, steps to decrease prices in turn lead to lower innovation in the future. This conception is based on the timing of market policies including shortening the patent period, making generic approval process rapid or regulatory tool such as price control. This response from the manufacturer appears valid as lower revenues give rise to reduced research and development (R&D) investment. This can be explained based on the economic law of diminishing returns wherein only a few breakthroughs are expected based on supplemental sources taking part in innovation. In this context, extra sources aiding R&D may not defend the cost that the consumers pay in the form of higher prices to support this unit. Massive societal impacts of pharmaceutical innovation are evidenced from comparative studies within heterogeneous inter-country data for specific disease expenses on prescribed medicines in and outside the United States (US), pointing out the higher community recovery with respect to persistence and overall quality of life [4]. The welfare from health inventions originates largely from patent-related strategies and the market exclusivity for innovative products. From economists' perspective, the drug development industry is a marginal cost industry due to its high-fixed and low-cost nature. Transferring a novel entity from laboratory to commercialization is an expensive, uncertain, and tedious venture. On the other hand, the cost of developing a different system of an existing commercial product is usually relatively low. In spite of conflict on the accurate cost of transferring a new chemical entity to the market, the approximate expenditure for each new product to reach the commercial market is millions of dollars. Contrarily, the costs of imitation are low for most drugs, and the process is untroublesome and economical for

non-innovator pharmaceutical companies. This leads to an indisputable fact that if free competition becomes permissible, companies spending lots of money on new drug development process to make it to market would be insecure about recovering their business due to those market rivalries which would be operating at low process-to-production costs.

22.2.2 Patent Expiration: Brands Versus Generics

Patent expiry is one of the major factors affecting the company's revenue. Apparently, with the entry of the generic competitor of a blockbuster drug in the market, the sales of that blockbuster drug demonstrate a significant decrease. To exemplify, the patent expiry of Pfizer's Lipitor resulted in 90% reduction in the annual sales revenue of the drug. Astra Zeneca lost patents for two major drugs: Crestor and Seroquel XR, which were accounted for a combined revenue of $7.34 billion annually in 2016. In 2016, Merck lost the patents for four drugs: Invanz, Vytorin, Cancidas, and Cubicin, that were estimated to value around $3.4 billion which was 10% of company's revenue of the year 2015. Generic medicines have undertaken a dominating part to play in the pharmaceutical business. With beginning of the modern generics industry in 1984, the Drug Price Competition and Patent Term Restoration Act progressed with a substantial increase in the pace and level of generic substitution [5]. In 1984, an 8% share of the generic medicine manufacturers' prescription drug market increased to 39% within five-year period, and the number of generic medicines' applications multiplied twice from 470 in 1984 to 1985 to 1069 in 1985 to 1990 [6]. This state of affairs empowered the generic companies to acquire reliability and rewarding the health practice through managed care. Also, as the patents guarantee market exclusivity and artificially high premiums, the patent expiration renders rapidly declining sales for brand-name pharmaceuticals. Usually, when the generic versions of drugs are introduced at 20–25% of the branded drugs' prices, the branded drug's market collapses rapidly and loses 60–80% of the total days of therapy within 6 months under the influence of managed care and mandatory substitution laws [7]. Table 22.1 provides information of drugs that turned out off-patent in the year 2018 [8].

22.2.3 Price Controls and After-Tax Returns

A moderate 5–10% price changes are approximated to show a proportionately small influence on the product development motives with an estimated 5% negative effect. However, 40–50% price reduction in the US ranges in 30–60% reduction in R&D projects to be undertaken during the early drug development stages [1]. In recent years, the central concern of business and policymakers displays the growing costs of health care. This began in 2003 after the enactment of Medicare Modernization Act. In the current global scenario, prices of only the US pharmaceutical market remain largely unregulated, unlike in many other countries where governments

Table 22.1 Off-patent drugs of the year 2018

Brand name	Generic name (s)	Indication	Pharmaceutical company	Net business in a year (billion U.S.$)
Lyrica	Pregabalin	Nerve and muscle pain	Pfizer	3.46
Rituxan	Rituximab	Blood cancer and rheumatoid arthritis	Roche	7.9
Cialis	Tadalafil	Erectile dysfunction	Eli Lilly	2.3
Xolair	Omalizumab	Allergic asthma and chronic idiopathic urticarial (CIU)	Genentech, Inc. and Novartis Pharmaceuticals Corporation	51.9
Restasis	Cyclosporine	Dry eye treatment	Allergan	1.41
Advair	Fluticasone propionate and salmeterol	Asthma and chronic bronchitis	GlaxoSmithKline	1.55
Neulasta	Pegfilgrastim	Non-myeloid malignancies	Amgen	3.93
Zytiga	Abiraterone acetate	Prostate cancer	Johnson & Johnson	20.4
Sensipar	Cinacalcet	Hyperparathyroidism	Amgen	1.58
Ampyra	Dalfampridine	Multiple sclerosis	Acorda Therapeutics	5.43

regulate drug prices in a direct or indirect manner [9]. In the opinion of some critics, the pharmaceutical companies are encouraged to charge high product costs to the consumers due to two main reasons: firstly, the freedom to price medicines in the US, as well as declaration of the product's present status as "patent protected". Secondly, the ability to utilize flaws in the established insurance policies. Different studies indicated that the price controls achieved by decreasing the financial recovery through drugs' sales would lessen the number of new drugs to be developed for the market by that company. This sort of economic study indicated that a short-term benefit of consumers could negatively impact huge public welfare in the future. However, it may take decades to assess such a negative impact [10].

One of the major dilemmas for the drug industry revealed that out of every ten products, only two to three are capable of generating after-tax returns considering total averaged and R&D costs. This involves major technical risk since only one out of thousands of investigated compounds may succeed to get the FDA approval [11]. Most of the R&D projects end as flops due to stringent regulatory requirement for efficacy, safety, or commercial feasibility. Very few compounds gain FDA approval for market launch. As per one study reported in 2007, the complete procedure from the discovery stage to product launch takes about 12–15 years with a huge estimated cost of around $802 million on pre-tax basis. An average cost of developing is about $480 million if the company is able to sell tax benefits. Therefore, the company expects profit at the time of a product launch. However, the

company must review the economic background for making a decision about undertaking an R&D project for full clinical translation. This uncertainty factor analyzes the behavior of the industry and may explain the logic behind greater probability of success with minor innovations rather than carrying out more inventive research efforts that pose higher failure risks with enhanced health outcomes [9].

22.3 Drug Repurposing in Pharmaceutical Industry: New Therapeutic Opportunities for Existing Drugs

22.3.1 Rationale and Approaches of Repurposing

Despite tremendous advancements in the field of technology and medical science, the enhanced knowledge of human disease is still not fully exploited to be translated into therapeutic benefits, and the progress to achieve this goal has been far slower than expected [12, 13]. On the other side, the global pharmaceutical industry is facing challenges in terms of excess time and unsteady regulatory requirements to make a new drug to reach the market. These two factors together account for escalating prices and lower estimated returns spent on R&D [14], making the pharmaceutical industry a relatively less fascinating option for investment. In this context, drug repurposing has been a major approach in drug development, although it deals with multiple challenges; one of which is the changing regulatory framework for the repurposed drug. Drug repurposing includes drug repositioning, drug reformulation, and new combination (Fig. 22.1). In its simplest meaning, drug repositioning is a strategy in which an old drug is used for a new disease or condition for a different curative area. Drug reformulation is an alteration in the original formulation and/or a different pharmacological target. Two or more drug components can be combined in a new drug combination [12, 13].

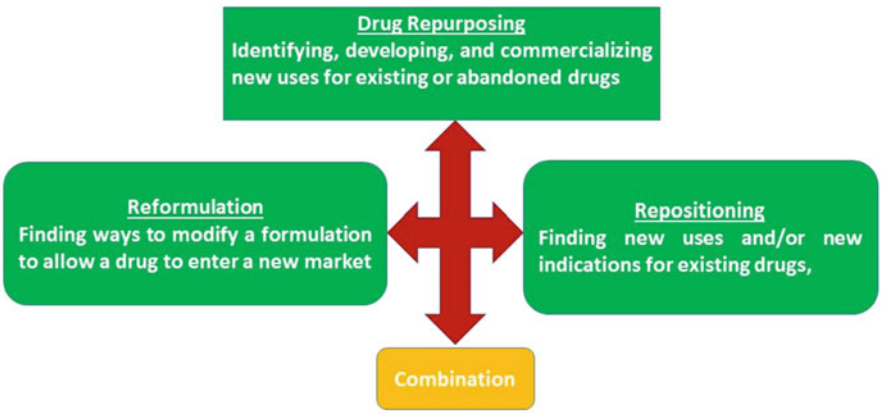

Fig. 22.1 Drug repurposing strategies

The repurposing strategies demonstrate advantages in terms of cost of investment as well as associated risk spent for new drug development for a specific disease or condition. The repurposed drugs are safe to be used in animal models and humans and have less chances of failure in safety and efficacy trials, demonstrating less overall risk of failure. Additionally, the repurposed drug development requires considerably less time, as most of the animal testing, safety assessment, and formulation development of the drug are supposed to be completed in most cases. From the economic viewpoint, repurposed drug development requires less financial inputs, although it may vary based on the development process of the particular candidate to be repurposed [15]. Together, these positive aspects have the promise to achieve a less risky and more rapid return on their development costs. Undeniably, in present time, a repurposed drug-cost estimates to be US$300 million which is much lower as compared to that of ~$2–3 billion for a new molecule to bring it to the market [16]. Repurposing offers valuable market exclusivity for the new product by protecting its new formulation. This is true even when the original product has lost its patent protection for active ingredient or formulation and/or its indication [17]. Repositioning approach is beneficial in the case of both the approved drugs as well as several rejected compounds which have been tested in humans to have adequate information on their pharmacology, toxicity, dose, and formulation [18]. In academic settings, drug repositioning is conducted for redeveloping a compound to be used in a different disease or condition [19].

22.3.2 Reformulation: Key Segment in Pharmaceutical Product Life Cycle Management

The life cycle management of a pharmaceutical product includes the systematic process of developing and managing the sequential events such as research, design, manufacture, service, and disposal of a specific product during its entire development phase. A well-designed life cycle management tool can maximize product sales throughout its life cycle by providing a competitive edge for higher profitability and retaining market share, particularly when the product approaches patent expiry. Since the pharmaceutical companies invest high capital and resources for protecting the product's patent life and its subsequent profits, the companies face a rapid and significant reduction in its returns due to generic competition after patent expiration. This further necessitates refined policies for maximizing a product's lifetime value, once the patent expires. Likewise, the financial health of big pharma firms relies massively on drug portfolios earning more than a billion dollars annually. Such blockbuster drugs require a stupendous expenditure of resources to be spent on their research and development. As per the statement of the Pharmaceutical Research and Manufacturers of America [20], 1 out of 10,000 compounds investigated by America's research-based pharmaceutical companies reaches the development pipeline and approved by the United States Food and Drug Administration (USFDA) for patients' use [14]. In such a scenario, life cycle management planning should be a major focus of the company's attempts to solve diminishing R&D productivity and

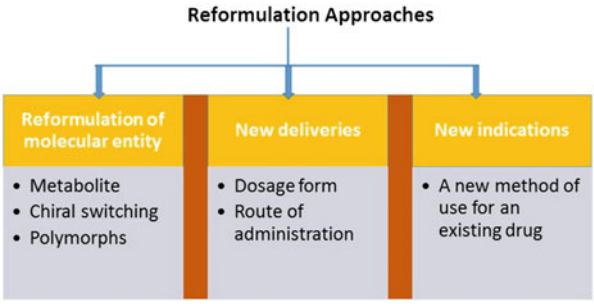

Fig. 22.2 Different reformulation approaches

growing generic-brand rivalry in order to fulfill company's urge for financial compensation against huge prior investment. Because of the high cost and long timespan required for creating a completely new chemical entity [16], it is not a new idea to develop the existing drug as a new drug delivery system. For instance, 60% of the new drug applications (NDAs) submitted to the FDA in the year 1990 were for the existing drug substances [14]. In this context, reformulation is the key segment in the product's life cycle management as it acts as a pivotal resource of great revenue generation and value addition in the company's financial health. Reformulation approaches can be divided into three categories: (1) reformulation of the drug entity; (2) reformulation for new deliveries; and (3) reformulation for new indications. (Fig. 22.2). Within the scope of drug delivery technologies, reformulation using new deliveries finds enormous scope for developing both controlled release and/or nano-based delivery systems which is described in further discussion.

22.4 Prioritization Process for Reformulation Drug Candidates

Once the pharmaceutical company decides to proceed for reformulating an original product, the most critical component is the selection of drug candidate to reformulate. This process mainly includes five explicit steps (Fig.22.3): developing drug candidates' list, examining the delivery technology, analyzing the therapeutic or administrative unfilled needs, conducting a competitive screen, and mapping the market.

22.4.1 Developing Drug Candidates' List

Primarily, the companies often focus on their blockbuster drugs which are either approaching patent expiration or are off-patent. Another source of selecting drug candidates is among those drugs that have clinical potential but could not pass in human trials because of side effects or administrative problems with specific routes [17]. This may be of more value in reviving those drugs which have consumed costly drug discovery dollars but would not gain regulatory approval and/or market acceptance without reformulation.

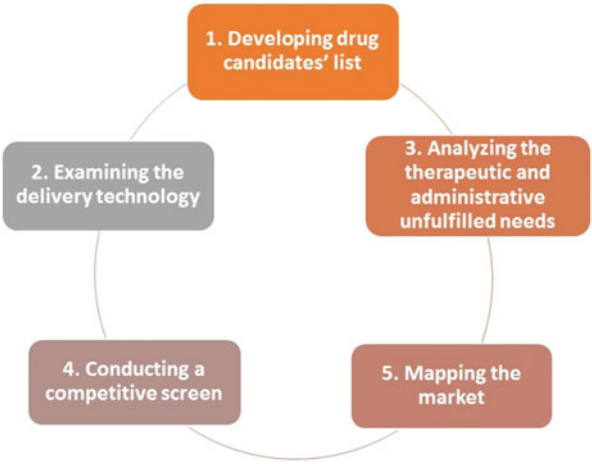

Fig. 22.3 Steps in reformulation process

22.4.2 Scrutinizing the Delivery Technology

Companies must evaluate technical constraints for drugs under consideration for each delivery technology. This can be in terms of relation of physicochemical properties and/or stability aspects of drugs to be suitable with the respective delivery technology [21]. These parameters are molecular weight, water insolubility, thermal or enzymatic stability, absorption window, etc. For instance, insulin has a narrow therapeutic index and the absorbable dose must be accurate. Further, the drugs that are reformulated from injectable drugs to oral drugs demonstrate different pharmacodynamic and pharmacokinetic profiles. Also, some biologics cannot withstand harsh manufacturing techniques such as encapsulation. Compounds unstable in the acidic environment of gastrointestinal tract must be protected to be released in the stomach. Therefore, it is critical for the drug delivery company to assess both strengths and weaknesses of intended delivery technology and scrutinize the relevant delivery technology for further screening.

22.4.3 Analyzing Therapeutic or Administrative Unfulfilled Needs

One of the main purposes of reformulation is satisfying the unfulfilled needs of the selected drug candidate. The input from clinicians and physicians assists the companies to know what type of reformulation technology would be appropriate to satisfy these needs. For instance, the administration-related unfulfilled need for the asthma led to evolvement of easy-to-operate new inhaler system, whereas development of sustained release products for reducing the dosage frequency resulted in enhanced patience compliance in case of cardiovascular drug therapy [7]. Further, anti-inflammatory drugs have been reformulated as delayed release systems that reduces gastric disturbances which is the major side effect of this category of drugs.

22.4.4 Conducting a Competitive Screen

This is another significant step in selecting a drug candidate to proceed with competing reformulation. In spite of the fact that reformulations of drug candidates going off-patent are prime candidates for significant competitive activity, reformulations of drugs within the same class present a significant competitive threat as well. Analyzing the drugs in the pipeline as well as other new therapies can be employed to complete overall screening [7].

22.4.5 Mapping the Market

Both the pharmaceutical and drug delivery companies should be aware of the market potential of the reformulation product in order to negotiate a commercially successful deal. The components that determine the market size include the relevant patient population, the price of the therapy, penetration of the market, and market share estimates [21]. Although estimates of direct patient populations are available in clinical literature, the relevant "subset" of the patient population is a more difficult estimate. For example, depending upon the drug candidate, the "subset" patient population may be defined as those patients with associated diabetes; or those patients seen as outpatients, or those patients that have contractual insurance. Such patient subsets can be identified with the help of secondary search of the literature and/or patients' databases and records. Moreover, the required price-per-day of the therapy can initially be estimated using available analogs from the product class. Reformulation may also command price premium when realized reasonable, whereas some companies use the pricing strategy that lowers the prices over time and gain market share. The market share estimates usually reflect the degree of competition in the category and the marketing partner's previous success in that category [7]. The market penetration is affected by the degree of fragmentation of the market, suggesting that the marketing campaigns can easily target the physicians and increases the direct sales of the product. To prepare for the final negotiation, analogous deals that have been conducted on similar products can be used as standard criteria to recommend reasonable deal structure.

22.5 Decisive Reformulation Factors

The five decisive factors which are sources of value during successful reformulation are: extended patent life: brands vs generics, reduced patient noncompliance, enhanced therapeutic efficacy, decreased manufacturing costs, and expanded market.

22.5.1 Extended Patent Life: Brands Versus Generics

In today's managed care environment where pricing-power for the pharmaceutical firms are restricted and managed care organizations use drug formularies, the drug reformulations can command premium prices only when they actually deliver value on these products. Reformulating a branded drug creates an improved version over the less expensive generic versions of the original branded product, and may be preferred by clinicians as well as patients. Patents on the reformulated product allow the company to effectively extend the patent life of the drug causing the dramatic rise in the branded drug's market share. In this framework, the company with the branded product may launch a reformulation 1 year before patent expiration to quickly preserve the market share. During this period, generic versions could seize only a small portion of the market, until the patent expires on the reformulated product [17]. For instance, Hoechst Marion Roussel Inc. reformulated Cardizem to Cardizem CD, a once-daily controlled release reformulation of diltiazem. The company was able to retain 86% of sales of diltiazem with the reformulated product after the patent expiration. However, it was markedly different than the market evolution for Tagamet (cimetidine). Further, in the scenario where generic drugs are expected to represent an increasing percentage of the pharmaceutical market, the large number of upcoming patent expirations on blockbuster products must not be neglected as it might be the steering trend for the growth of this industry [7].

Each year, patent expiration may be due to the best-selling blockbuster drugs. In this situation, summarizing the value of these drugs compels the companies toward a strategic move to reformulate these branded drugs using appropriately screened drug delivery technologies. In a similar fashion, generic companies target these drugs as their opportunities to develop premium-priced products. Further, drug companies require to employ a range of delivery technologies to reformulate their products, since each drug represents distinguished technical challenges and different unfulfilled clinical needs. In this context, drug delivery companies tend to apply their technologies across a range of clinical segments despite the fact that they are focused players. This strategy functions as diversified technology platforms so as to elaborate their commercial applicability and viability.

22.5.2 Reduced Patient Noncompliance

Patients' noncompliance with the prescribed medication regimens is one of the most significant obstacles to curing diseases and keeping patients healthy. Noncompliance describes why therapies often demonstrate greater efficacy in closely monitored clinical trials than in routine medical practice. Compliance with prescribed therapy regimens is not a significant issue in the closely supervised hospital setting as it is for the outpatients. Patient compliance becomes a more significant issue when the average length of hospital stays decreases, as it releases the patients in a less sturdy environment. Noncompliance in US healthcare system approximately costs about

$100 billion per annum in direct and indirect costs, significantly exceeding the $30 billion cost of prescriptions themselves according to the study of Center for the Advancement of Health, Washington [7]. Therefore, despite everything, adherence to the prescribed regimen including the correct timing, dosage, method of delivery, and physical status determines the drug's ultimate success. Among factors affecting patient noncompliance, the nature of the disease and disease symptoms, cognitive or functional ability, and financial resources are personal factors which depend on the individual patient. Some other important factors influencing patient compliance include the frequency and mode of administration and the extent of drug-related side effects, which are formulation dependent and can be modified through drug reformulation. Table 22.2 summarizes the factors affecting patient noncompliance, ways to achieve compliance and outstanding reformulations which enhanced both compliance and sales to the companies.

22.5.3 Enhanced Therapeutic Efficacy

Besides patient compliance, enhancing therapeutic efficacy employing better drug delivery technologies could determine the reformulation success. Bioavailability improvements may help drugs to work more efficiently using technologies that allow the release of the drug at specific times [22]. In 1996, Covera-HS, an Alza reformulation of hypertensive drug initially launched by Searle, was designed to deliver peak concentrations when blood pressure and heart rate are at their highest [7]. Recently, nanotechnology-based breakthroughs in the field of anticancer therapy are not only well-established but also an ongoing interest to attain the highest therapeutic outcomes. In the field of diabetes treatment and research, a number of companies worldwide are working on technologies to help diabetic patients maintain stable, close to normal physiologic levels of insulin. This is because closely titrated patients have been subjected to fewer long-term complications from the disease as compared to those with widely fluctuating levels.

22.5.4 Decreased Manufacturing Costs

Decreasing manufacturing cost is an obvious method of increasing profitability. On numerous occasions, oral drug formulation with poor bioavailability is required to be administered in high doses, as only they show dissolution-limited absorption resulting in only a small percentage of the drug absorbed by the body. Reformulations that are designed specifically to improve the bioavailability of the drug need less amount of drug dose to produce an equivalent therapeutic effect, thereby reducing manufacturing costs [23]. In general, the manufacturing cost of a small molecule is low, whereas macromolecular therapeutic agents such as proteins and peptides are very expensive. Although the research efforts for maximizing the bioavailability of macromolecular therapeutics cost millions of dollars, their successful reformulation development will be rewarded in the market.

Table 22.2 Factors and ways to achieve patient noncompliance

Factors	Ways to achieve high compliance	Outcomes and examples
Patient related factors • Nature of the disease and disease symptoms • Cognitive or functional ability • Financial resources	*Support and counseling* • Family, government, and social support • Patient counseling • Family, government, and social support • Raising financial resources, generating health insurance and other additional financial resources, etc.	*Outcome* • Reduced patient noncompliance
Formulation related factors • Mode and frequency of administration	• Developing oral drug delivery/other system as most accepted mode of administration • Developing once-a-day or other appropriate controlled release reformulations	*Outcomes* • Reduced patient noncompliance • Increased sales, net growth, and revenue generation for the pharmaceutical and drug delivery companies *Example* • Bayer AG's Adalat CC product for hypertension reformulated in 1993 from a 3–4/day to 1/day hiked to sales of $1.1 billion worldwide in 1997
• Nature and extent of side effects	• Developing different reformulations to address dynamic patients' needs	*Examples* • TAP pharmaceuticals' Lupron depot for prostate cancer and endometriosis reformulated in 1989 from a daily injection to a once per month injection, climbed to worldwide sales of $990 million in 1997 • Amphotericin B, a powerful antifungal causes severe nephrotoxic side effect profile limiting its use in about 40% of the patients who could not receive the full therapeutic course as a result of the risk of kidney failure. NeXstar (the liposome company) and Sequus developed liposomal formulations that protect the kidney while maintaining equivalent efficacy to the generic formulation. The improved reformulations commanded a 10 times price premium of the generic and displayed 14-fold increased sales

22.5.5 Expanded Market

Due to unique and delicate structural features, the majority of peptides and proteins macromolecules are traditionally delivered via an invasive parenteral route [24]. In those cases, where treatments require periodic physician visits or admission to the hospital, injection therapy does not significantly increase cost or inconvenience to the patient. However, patients simply do not tolerate injections for many chronic conditions. Therefore, the inclusion of more alternative administrative routes and patient-friendly formulations would allow pharmaceutical companies to apply their drugs to a much broader patient population. Additionally, it would open the gates to expand and utilize the range of prospective chemical compounds that may be considered as drugs, to their fullest potential.

22.6 Drug Industry's Need of "New Deliveries" Approach

As discussed in the preceding sections, new formulations that augment bioavailability, optimize drug delivery profiles, or improve patient compliance by reducing dosing frequency and/or improving patient's experience for the usage of medication demonstrate real capability to deliver rapid returns on investments as compared to the development of an entirely new molecular entity. Therefore, strategies that are used to achieve any of these goals are identified as pharma company's productive keystone in today's cost-constrained environment. Therefore, global pharmaceutical companies are intensely looking for new strategies to increase profitability through developing the controlled release reformulations of existing drugs due to short pathway for approval while maintaining their market share. Pharmaceutical companies are increasingly reformulating existing compounds, often using the FDA's NDA 505(b) (2) pathway [25], which is generally faster and more cost-effective than bringing new compounds to market. The pathway can provide patent extension for existing compounds that secure continued market exclusivities for several additional years. As 505(b) (2) product development often targets enhanced bioavailability and/or optimized drug delivery profiles, it can provide reduced pill-burden as well as new indications or specialized formulations for pediatrics [23]. Figure 22.4 shows significant reformulation strategies based on "New Deliveries" approach.

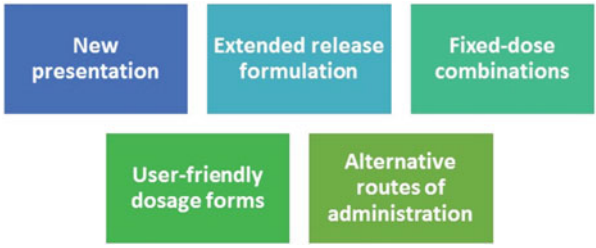

Fig. 22.4 Different reformulation strategies based on "New deliveries" approach

22.6.1 New Presentations

New presentation means changing a physical form of the dosage form [7]. For example, reformulating capsule to a tablet or a softgel, to differentiate it from the original product. Although new presentation is a market-driven reformulation strategy, it must be based on a combination of the target product profile and the characteristics of the API. Target product profile, which is driven by the marketing and clinical considerations for the new presentation, must direct the pharmaceutics team in terms of the specifications required whether it is the dissolution rate for a controlled release formulation or the dosage levels for fixed-dose combinations. For example, a non-steroidal anti-inflammatory drug (NSAID) ibuprofen finds applications in several areas of medicine that is presented from the original conventional ibuprofen tablet to a controlled release version for chronic pain and gastric side effects prevention. In the present time, ibuprofen can be found in many other presentations such as softgels and used in different markets or disease areas [23]. The reason behind softgel presentation of ibuprofen claims to have a faster onset as it dissolves ibuprofen in the softgel matrix presenting a potential advantage for acute pain. Other ibuprofen presentations include pediatric suspensions, fast-melt tablets, topical gels, and combination products with analgesics or decongestants. Also, formulations or presentations and packaging that target specific disease areas such as menstrual pain or headaches are also available for ibuprofen, each having a marketable advantage for its particular market or disease area [23].

22.6.2 Extended-Release Formulations

Pharmaceutical companies often develop extended-release drug products, including oral dosage forms or injectable depots as part of a company's reformulation strategy to differentiate its brand from generic competition, thereby extending market exclusivity. In spite of the fact that extended-release formulations are more challenging to formulate, they provide added value and distinguished merits such as improved pharmacokinetic profiles, prolonged duration of therapeutic effect, lower incidence of adverse reactions, and reduced dosing frequency resulting in improved patient compliance [22]. Since extended-release formulations are able to maintain desired plasma drug concentration below toxic concentrations, this reformulation strategy offers specific benefits in the condition of chronic diseases and complex dosage regimens requirements [26].

Pfizer reformulated its twice daily, immediate-release 5-mg tablet (tofacitinib citrate, Xeljanz) for rheumatoid arthritis into a once-daily, modified-release formulation. Pfizer's NDA for Xeljanz 11 mg once-daily, modified-release tablet was accepted to review by FDA based on its NDA that represented data demonstrating its pharmacokinetic equivalence in key parameters with older Xeljanz 5 mg twice-daily formulation. Roche, in collaboration with GlaxoSmithKline, reformulated the oral bisphosphonate into a once-monthly formulation that was initially marketed as bisphosphonate ibandronate, Boniva, as once-a-day tablet for the prevention and

treatment of postmenopausal osteoporosis [27]. This was the FDA's first-ever approval of the improved formulation for once monthly oral treatment for any chronic disease [28]. Despite its advantage in terms of reduced dosing frequency, this oral bisphosphonate requires a rigid treatment schedule to follow, such as to stay in the standing position without eating, drinking (except water), or taking other medications pre- or post- administration of this medicine. Oral bisphosphonates may not be suitable for some women, either due to other medical conditions or because the patient is unable to remain standing posture for a specific time. To address the need, Roche and GlaxoSmithKline developed an injection of ibandronate (3 mg) available as a prefilled syringe. This injection is intravenously administered over a period of 15- to 30-second once in every 3 months [29].

22.6.3 Fixed-Dose Combinations

Fixed-dose combinations combining two or more drugs in a safe and effective manner are another potential reformulation strategy for pharmaceutical companies striving to maximize the value of their products. Fixed-dose combinations can be used in two ways: first, to combine different actives into one, single-dosage form, and second, to combine an immediate-release with an extended-release formulation for achieving a precise release profile of a specific drug. The advantages of fixed-dose combinations include enhanced efficacy through the synergistic effect of potentially lower doses providing increased therapeutic benefit and reducing the pill-burden, thereby increasing overall patient compliance [26, 30]. Table 22.3 depicts some successful fixed-dose combinations.

22.6.4 User-Friendly Dosage Forms

User-friendly dosage forms are formulations that provide a more positive experience to the user as compared to traditional tablets and capsules. Such dosage forms are designed to circumvent the widespread difficulties related to the swallowing of traditional dosage forms. Examples of user-friendly dosage forms include reformulating large tablets such as paracetamol into effervescent tablet, or aspirin into oral disintegrating granules to avoid swallowing difficulties. Other examples include oral disintegrating tablets or fast dissolving tablets that eliminate the need of water for administration. In this list, Capsugel designed its Coni-Snap consisting of sprinkle capsule feature and innovative closure rendering it easier and safer for both care-givers and patients to open the capsule and administer the medication by sprinkling the contents onto soft food for oral consumption. It is important to note that this reformulation strategy is utilized mainly for instantaneous release, and find limited scope in controlled release formulations, since the primary concern of latter is to achieve and maintain desirable kinetics and release profiles for better therapeutic outcomes which are difficult to achieve while focusing on "user-friendliness" feature of the dosage form. However, microneedles or passive transdermal patches

Table 22.3 Examples of reformulated fixed-dose combinations

Combination	Purpose	Brand Names
Perindopril + Amlodipine Irbesartan + Hydrochlorothiazide Amlodipine besylate + Benazepril HCl	Antihypertensive combinations to provide better blood pressure control	Prestalia® Avalide® Lotrel®
Glibenclamide + Metformin Pioglitazone + Metformin	Antidiabetic combinations for more effective glycemic control	Glucovance® Actoplus Met®
Darunavir + Cobicistat Efavirenz + Emtricitabine + Tenofovir + Disoproxil fumarate	Antiretroviral combinations for the treatment of HIV/AIDS Antiviral combination to treat HIV	Prezcobix® Atripla®
Rifampicin + Isoniazid + Pyrazinamide + Ethambutol	Antitubercular combinations to target bacteria in different ways and make treatment more effective and to reduce possible bacterial resistance	Voractiv®
Artemether + Lumefantrine	Antimalarial combinations to utilize markedly different absorption and elimination parameters to complement and support the product efficacy	Coartem®
Clindamycin + Benzoyl peroxide	Anti-acne topical combination therapy	Onexton gel®

are attractive patient-friendly reformulation options offering unique benefits in terms of patient-friendly features, enabling patients to self-administer and opening new opportunities to move treatments out of the clinic and into the patient's own home [23, 25].

22.6.5 Alternative Routes of Administration

In general, the use of alternative routes of administration is a reformulation strategy that develops delivery systems to be administered by routes other than oral. Two major alternatives to oral delivery are the non-invasive transdermal route and the invasive parenteral route. Many times, an appropriate non-oral route can be recommended to deal with drug's specific problem such as limited oral bioavailability due to first pass metabolism. While considering the reformulation for converting an oral dosage form to a suitable non-oral dosage form, technological performance, opportunity to capture additional market share, and product life cycle management are important factors to be considered at an early phase to avoid delays in project timelines and formulation processing [23, 25, 26].

22.7 Drug Industry's Need of Miniaturization and Nanotechnology: Evolution and Revolution of Nanomedicine

Including patent expiration of several blockbuster drugs, numerous other challenges led to altered drug landscape stimulating the drug companies to focus on alternative drug development technologies. Miniaturization using nanotechnology emerged as a very potential approach and considered as an urgent need for the drug development process to combat many challenges associated with conventional and new deliveries. This also addressed the enormous pressure experienced by US drug companies to deliver superior quality medications to the consumers without adversely affecting the profit margins [31]. Almost five decades before, the study of nanomedicine began with the description of the first lipid vesicles and came up with a great rise in the past two decades [32, 33]. The basic concept of nanotechnology is scaling down the material size to their molecular level. This principle entirely alters and enhances the physicochemical properties of nanomaterials [1, 33]. Therefore, nanomedicines offer enormous promise for advancing the treatments [1, 34, 35]. Further development of nanomedicine should own a thorough understanding of technical and medical aspects along with economic hurdles that hinder their clinical translation to the market.

Since its evolution to till nowadays, there has been tremendous excitement and expectations regarding the potential impact of nanomedicine in the healthcare sector. Although the early phase of the nanomedicine commercialization process is encouraging, there are numerous obstacles as the development process furthers. One such obstacle is the complicated and confused patent claims which emerged from the mushrooming of patent applications, and continued granting of unusually broad-ranging patents by the US Patent and Trademark Office (PTO). In addition, a wide definition of nanotechnology as per US National Nanotechnology Initiative is not adequately accurate and relevant from the nanomedicines' context [31]. These challenges are major causes liable for hindered translation of nanomedicines to clinic and commercialization. Consequently, there will be restrictions to its full exploration to benefit the society. This fact creates a need for a robust patent system to promote development of marketable revolutionary nanomedicines that upgrade patient's overall health and life quality at minimum costs of healthcare. Drug companies' annual R&D investment increased to US$40 billion in 2003, from US $1 billion in 1975 [36]; till 2018, it raised to US$79.6 billion. In spite of this rise over the years, the number of new approvals remained almost the same, i.e. 20–30 drugs per year. Besides, over the past few years, new molecular entities covered only 25% of new product approvals, most of the product approvals belonged to either reformulations or new combinations of existing drugs [37]. This revealed the fact that only 30% of new drugs are capable of recovering their ever-increasing R&D costs. Further, the drug industries are also haunted by issues such as the weakened product pipeline, as well as decreasing numbers of new drug approvals by the USFDA and other foreign drug agencies. However, nanotechnology's potential still impresses governments around the globe allocating research funds in this area.

Issues such as growing international rivalries [38, 39] and political alliances strain the battle lines. In global scenario, US$12.4 billion in 2006, which was 13% higher than 2005, was spent by corporations, governments, and venture capitalists for nanotechnology research [40]. Studies claimed the presence of over 500 nanotechnology-based consumer products in the market worth US$30 billion in 2005 [41, 42]. Although it is hard to estimate its volume and growth, the nanomedicine sector undeniably comprises a billion-dollar market with a rapid rise [43].

Since its evolution, nanomedicines are available in numerous forms such as liposomes, nanoparticles, nanoemulsions, polymer–protein/drug conjugates, self-assemblies, dendrimers, nanocrystals, polymeric vesicles, antibody–drug conjugates, nanogels, nanotubes, gold nanoparticles etc., among which several forms are already approved nanoproducts for diagnostic and/or therapeutic use [44]. In 1995, the first PEGylated liposome delivering doxorubicin (Doxil) was approved by the FDA, following which the nanomedicine research never looked back. However, this field is still not fully exploited to its real promise for revolutionizing the therapies and diagnosis. This is because the development pathway for nanomedicine toward their success has never been absolutely straightforward. It is unarguable that nanomedicines present exceptional merit such as superior efficacy, improved transport across the biological barriers, disease targeting, better absorption and bioavailability, prolonged and higher drug retention, reduced toxicity and immunogenicity, etc. over their conventional counterparts [45]. These merits are attributed to their basic physicochemical properties like nanosize, higher surface-to-volume ratio as well as surface characteristics that have been utilized in developing advanced nanomedicine products [46]. Therefore, a thorough physicochemical characterization of nanomedicines prior to in vivo evaluation is one of the requirements for progressing this field. In general, when research is performed using appropriate standards, controls, and procedures, it gathers important understanding for the whole scientific community; however, the regulatory agencies' guidance is very important for identifying the information needed for NDAs. The diversity and complexity of different nanomedicine approaches obstruct the fulfillment of a general guidance protocol for nanotechnology-based products; however, FDA addresses this point with industry-guidance documents. For instance, the "Liposome Drug Products," provides information about chemistry, manufacturing and control, clinical pharmacokinetics, bioavailability, and labeling requirements for liposomal products [47]. Also, another document "Drug Products, Including Biological Products, that Contain Nanomaterials" [48] provides vital information for the development of new nanomedicine approaches. In addition, one study claimed that the variability of published data with respect to the characterization and experimental details reported is one of the causes of the hardships in the progress of different nano-drug delivery systems employed for various therapeutic applications [49]. This fact suggests a need to harmonize the nanomedicine' characterization using standard protocols and appropriate reporting of the experiments in order to promote comparability between different approaches and reproducibility of the data. The majority of nanomedicines studied were nanoformulations of already approved drugs, wherein nanomaterials used possessed specific physicochemical

properties with safety and toxicity concerns. As nanosystems interact differently with biological components, it is important to examine their toxicities using validated protocols based on the nanomaterial types [50].

Nanomedicine ranges from nanomaterials to biological devices and nanoelectronic biosensors for advancing human health and treatment. Nanomedicines are engineered nanoscale materials whose nanostructure extends unique therapeutic benefits for a range of diseases and conditions. Nanomedicines can be conveniently divided into three major classes: nanopharmaceuticals (i.e. intended for drug delivery), nanodiagnostics (i.e. used for imaging and diagnostics), nanotheranostics (i.e. combined therapeutic and diagnostic), and nanobiomaterials (i.e. medical implants). Nanopharmaceuticals hold 75% of the approved nanomedicines' market share [51]. Initially, the first generation nanopharmaceuticals were developed by reformulating existing drugs for purposes such as solubility enhancement or altered biodistribution, in order to achieve efficient drug delivery at the site of action with reduced organ toxicities [52]. Such reformulated nanomedicines have been mainly employed in cancer treatment for enhanced clinical outcomes [53–55]. The majority of the nanoformulations in development and clinical trials focused on cancer targeting with more than 80% of the overall nanomedicine-based publications during the last two decades [56]. The second generation nanomedicines were produced to achieve better efficiency of therapeutic biomacromolecules and other biotherapeutics to take direct advantage of nanoscale on their therapeutic efficacy [57].

22.8 Challenges in Nanomedicines' Clinical Translation and Commercialization

Inadequate understanding of medical and technical aspects of nanomedicines such as nanomaterials used, processes employed, in vivo fate as well as safety and toxicity are major parameters that hinder clinical translation of nanomedicine to commercial scale. The challenges encountered in nanomedicine development to commercialization is presented in Fig. 22.5. The points discussed below highlight the obstacles in nanomedicine research and development suggesting some remedial measures to foster the commercialization of nanomedicines [58–60].

22.8.1 Biological Barriers

Nanomedicine development has been a formulation-directed approach in which novel delivery systems are firstly engineered and characterized using the physicochemical interpretation. Therefore, the limitations in its clinical translation cannot be recognized until the nanomedicine is attempted to align with a pathological application. The relationship between biology and technology should be understood to identify the impact of disease pathophysiology on nanomedicine in terms of retention and efficacy,

Fig. 22.5 Challenges in nanomedicine development to commercialization

distribution, accumulation. Also, the correlation of biopharmaceutical factors of the delivery system with its in vivo animal or human behavior is crucial during successful nanomedicine development. This leads to applying a disease-directed approach to capacitate utilization of pathophysiological changes in the nanomedicine design and development for augmenting their clinical translation [61]. It is crucial to consider the relationship between disease pathophysiology and disease heterogeneity in humans along with the physicochemical properties of various nanomedicines to overcoming biological barriers. This approach enables enhanced targeting of the drug to the diseased tissues and/or reduced drug accumulation in non-target organs. Significantly less research efforts have been dedicated toward a complete understanding of the interrelationships between nanomedicine performance and patient physiology under particular clinical conditions. Therefore, undertaking a disease-directed approach to nanomedicine development could build extensive in vivo data sets, that at its best can anticipate clinical efficacy in patients to promote clinical translation.

22.8.2 Manufacturing and Scale-up Complexities

Nanoformulations possess physicochemical and structural complexities which diminish its clinical translation. Also, complicated and/or tiresome procedures involved in synthesis can be problematic for large-scale pharmaceutical production which can limit the clinical translation potential [62–66]. In addition, manufacturing is based on overall product quality and cost, whereas quality comprises of the

manufacturing process and stability of the formulation. Potential issues challenging the overall product quality include poor quality control, scale-up complexities, incomplete purification processes, high cost of material and/or manufacturing, low production yield, low batch-to-batch reproducibility, consistency and storage stability, inadequate infrastructure and/or in-house expertise, scarcity of venture funds, and pharmaceutical industry investment [60, 62, 63, 65].

22.8.3 Biocompatibility and Toxicity Issues

In general, the regulatory authorities recommend the sponsor to precisely assess any changes in the drug substance and drug product formulation at any phase of the manufacturing process to determine the direct or indirect effects of any such change on the product safety. Recommended changes affecting product safety during the critical manufacturing control throughout the investigational new drug (IND) process are: changes in the synthesis steps, reagents used to manufacture the drug substance, product, or formulation, changes giving different impurity profiles, changes such as chemical synthesis, fermentation process or derivation from a natural source used in the actual manufacturing method, changes in the source of raw materials, changes in the sterilization method used for the drug substance or drug product, changes in the administration route, changes in the composition and/or type of dosage form, changes in the product manufacturing process that can affect product quality, and changes in the drug product package that can affect product quality, e.g. dose delivery [67]. In addition, specialized toxicology studies can be conducted in animal models to assess both short-term and long-term toxicity, as circulation half-lives and drug retention times are generally considerably raised with nanoencapsulation. Further, an in-depth understanding of the absorption, distribution, metabolism, and excretion of novel nanomaterials in vivo is important for predicting the toxicological responses to nanomedicine [65, 68]. Enough assessment protocols are also needed to monitor various aspects of the nanomedicine drug delivery process including pharmacokinetics, biodistribution, target site accumulation, localization in healthy tissues, kinetics of drug release, and therapeutic efficacy [69]. Additionally, real-time imaging techniques can be incorporated to achieve a better understanding of nanomedicine's interactions with biological organs and tissues, following in vivo administration [68, 70, 71]. Moreover, biocompatibility, immunotoxicological, and inflammatory potential should be assessed with functional outcomes and correlated with tissue uptake mechanisms and clearance [70]. Also, the need to be well-examined based on dose, dosage form, and administration route to establish safe drug limits before clinical trials, specifically when nanomedicines contain any new material that has been used first time for the clinical applications should be addressed [70, 71].

22.8.4 Ineffective Patenting

Over the last few decades, a significant increase has been observed in the number of nanotechnology patent applications, which gives rise to patent review delays, patent thickets, and invalid patent issuance [72–74]. Bawa and co-workers indicated a need for a universal nano-nomenclature on identical or similar nanostructures or nanomaterials, and the use of more refined search tools including commercial databases so that issuance of multiple nanopatents on the same invention can be avoided [72, 74]. Also, the online publication database used by the Patent and Trademark Office should be searchable through worldwide publications including old research that were published before the emergence of online publication databases [65]. Intellectual property and patenting of nanomedicine require improved clarity with the possible implementation of universal regulations and policies.

22.8.5 Inadequate Regulations

In spite of the scientific and regulatory challenges, nanomedicines demonstrate enormous promise to boost up the pharmaceutical market by advancing the treatments for augmenting human health. Nanomedicine manufacturing for commercialization is influenced by regulatory factors such as quality control, safety, and patent protection as proposed by government policies [65, 66, 70]. Further, the inadequacy of straightforward safety and regulatory guidance has affected the timely and effective clinical translation of nanomedicine [65, 66, 70]. This can be exemplified by polymers which have been investigated for nanomedicines; the safety and efficacy of which are dependent on the polymer conjugation chemistry and polydispersity, molecular weight, and molecular structure [31, 75]. At present, there is an increased number of novel polymeric materials employed in the fabrication of complex polymer-based nanoformulations which creates a need for appropriate regulatory framework to assist in their evaluation [31]. Since each polymer-based nanoformulation is different, each one should be considered individually based on dose, dosing frequency, administration route, and proposed clinical indication. Main regulatory authority of respective regions, e.g. Food and Drug Administration (FDA), Therapeutic Goods Administration (TGA), and European Medicines Agency (EMA) are currently engaged for the nanomedicine regulations within the conventional framework.

The first generation nanomedicines were relatively simple, and therefore they passed regulatory approval by complying only with general standards of medicinal compounds. The modern nanomedicines are complex formulations and these general regulations are inadequate to confirm their quality, safety, and efficacy for clinical use [62, 65, 66]. This is because the complex nanomedicine structures exhibit in vivo complexities like unclear interaction with cells and tissues within the human body, the polyfunctional nature of some formulations like the integration of therapeutics with imaging diagnostics (theranostics) and their increased complexity for clinical

use [62, 65, 66]. These parameters collectively indicate that the regulatory protocols should consider nanomedicine complexity, route of administration, pharmacokinetics, pharmacodynamics and safety profile, and bridge both medicine and medical devices regulations for their validation [65]. Ongoing efforts for developing global regulatory standards for nanomedicines by key countries need closer collaboration with academia, industry, and regulatory agencies [63, 70, 76]. Contract manufacturing organizations (CMOs) worldwide that are specialized in producing nanoproducts in compliance with the regulatory standards are limited [63] and can be divided based on their infrastructure capabilities of producing specific nanomedicines, e.g. liposomes, polymeric nanoparticles, dendrimers, drug–polymer conjugates, etc. The nanomedicines produced by CMOs are likely to be marketed in many countries and be governed under the same regulatory standards [63], incorporating industrial standards for both quality control and environmental issues for complete evaluation and documentation of nanomedicine production processes [70]. Manufactured nanomedicines still need to comply with general pharmaceutical standards such as purity, sterility, stability, manufacturing operations, and related industrial control standards [70]. In addition, validated analytical methods for physical evaluation of particle size and size distribution, surface chemistry, morphology, surface area, surface coating, hydrophilicity, porosity, and surface charge density of nanomedicine which can affect in vivo performances are also required [65, 66, 70]. Nanomedicine characterization methods vary depending on the type of nanomaterials employed and nanostructures. Also, the standardized toxicity protocols and testing methods to ensure product safety and efficacy are required.

22.8.6 Financial Resources, Profitability, and Overall Cost-Effectiveness

The nanomedicines' development is mainly dependent on start-ups as well as small- and medium- scale enterprises in spite of their huge R&D costs [77]. Usually, such small- to medium- scale enterprises suffer from insufficient financial resources to utilize and market their inventions, and they are seldom capable of commercializing nanotechnology-based therapeutics [77]. Large-scale pharmaceutical companies may not have a clear incentive to collaborate with such enterprises [77, 78] because their profit with traditional blockbuster drugs can be compromised if they invest in the development of new nanomedicine. This fact leads to low commercial interest to switch to nanomedicine option in spite of their high therapeutic potential. Here, profitability is also endangered due to high purchase costs for patients [77].

22.8.7 Generics Market and Insurance Policies

In theory, brand and generic products differ only in their prices [79]. It is a general tendency of insurance companies and other third-party payers to refund only the cheapest generic products [80]. Current health policies do not include the costs of

"unproven" technology related to experimental therapies for both general and nanomedicines. Cheaper generics drive the overall medicine market growth rather than innovative products. This is indicated from the expected growth of the generics market by 10–15%, as compared to 7–9% growth by the overall pharmaceutical market. Although a profitable generic market may lead to more revenues, it also provides limited progressive medical benefits for patients when compared with advanced nanomedicines [81].

22.9 New Deliveries and Nanomedicine: Market and Forecast

Among various new deliveries, controlled release drug products are relatively difficult to formulate due to drug substance challenges, including very low solubility, lack of absorption, high dose, gastric fluid degradation, first pass metabolism, and/or rapid clearance rates [22]. Additionally, treatments for patient population where swallowing is difficult, controlled release formulations can be challenging because of high drug loading, inability to crush, excipient amount, and dosage size. To combat these challenges, pharmaceutical companies look forward to use enabled technologies including amorphous or nano-sized particles, multiparticulates, and multilayer tablets [25]. Products requiring high drug loading and low solubility are particularly difficult to formulate without being large in size. Geriatric and pediatric populations have difficulty swallowing large, monolithic controlled release dosage forms [31, 82], whereas multiparticulates including particles, pellets, beads, granules/mini-tablets offer more flexibility in terms of the final dosage forms that can vary from capsules and tablets to orally dissolving tablets, sachets, and sprinkle capsules. Drug incompatibility can be a concern for some fixed-dose controlled release combination products whereby multilayer tablets or multiparticulate products can be employed to maintain stability. Furthermore, the role of polymers in controlled drug delivery development is enormous and polymeric controlled drug delivery using semi-synthetic, synthetic, and biodegradable polymers is highly researched and applied approach in the pharmaceutical field. Medical devices employing biodegradable polymers will drive the worldwide biodegradable polymers market. Increased market trend for controlled and nanoscale drug delivery systems is attributed to the use of technological advancements that can be applied to various formulation approaches. Routes of interest for new deliveries and nanomedicine that attract future pharmaceutical market are represented in Fig. 22.6.

Pharmaceutical market foresees future growth in specialized dosage forms, nanomedicines, monoclonal antibodies, and other functionalized polymer-based medicines for autoimmune, cancer, cardiovascular, neurological, viral, and other disabled disorders. Drug delivery devices such as pen injectors and prefilled syringes will also exhibit rapid growth. Among new deliveries, controlled release formulations will maintain market growth. The overall market potential will be alleviated by patent expirations and generic competition. Controlled drug delivery market predicts overall future growth in oral controlled release segment, retaining the highest market share based on the number of applications for marketing

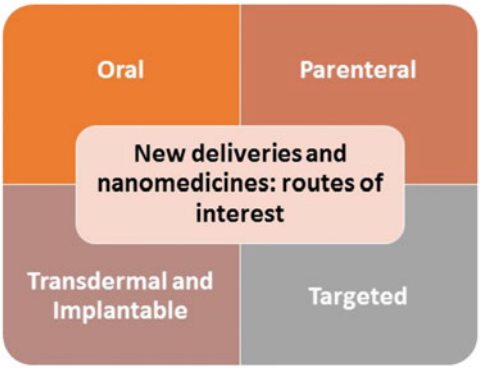

Fig. 22.6 Drug delivery systems of interest for future market

approvals received in 2017. The targeted drug delivery beared the fastest raise during the forecast period [32, 83]. Activation-modulated osmotic delivery is also likely to witness the magnified growth rate. The maximum compound annual growth rate (CAGR) during the forecast period is expected due to higher investments by leading competitors such as India, China, and Brazil as they predicted promising growth during the forecast period. This market growth seems achievable due to increased tendencies for catering specific patient care through improved healthcare infrastructure and medical technologies. According to Grand View Research Inc.'s report, market size is expected to reach US$90.18 billion with a CAGR of 14.0% by 2025 [83]. In addition, increasing demand in novel drug delivery systems for geriatric and pediatric population as well as alternate therapies will come up with a boost to the market [32]. According to the report, the Asia Pacific region will broadcast the largest CAGR but North America will keep the leading edge over the market during the forecast period due to advanced technology and infrastructure, expanded R&D activities, and presence of key players in the region. Meanwhile, the pharmaceutical sector is captured between the pressure of lowering the prices and the raising expenses of successful drug discovery and development. In this situation, some products are being launched and some others are expected to reach the market soon. Approaches using depot, nanoparticulate targeted delivery, transdermal delivery, etc. are showing rising interest and will continue to contribute in the overall market growth, whereas some more sophisticated wearable devices and advanced transdermally controlled drug delivery systems are marketed and some more are in the development pipeline.

As per WHO, cancer is one of the major causes of mortality and morbidity globally, with approximately 14 million cancer-related new cases in 2012 with 8.2 million deaths. These figures demonstrate the increased nanomedicine demand to fight this deadly disease and is expected to lift market progress [84]. Nanomolecules and associated technology-based products are accommodated in potential product pipeline and anticipate a market-drive with powerful growth avenues. This is because about 40% of products in phase II trials are expected to result in some key commercialization over the coming years to contribute revenue generation over the

forecast period. The field of precision medicine for the customized treatments of genetic abnormalities is a substantial option of future medicine. A report by Grand View Research Inc. assesses nanomedicine market to reach US$350.8 billion by 2025. This lucrative growth rate is expected through a rise in number of research grants, higher demand for prevention of deadly diseases, rise in the number of venture capitalists from developing countries as well as higher number of international research collaborations and partnerships to push growth in nanomedicine market during the forecast period [85].

22.10 Concluding Remarks

New drug development process requires considerably higher cost and time as compared to developing a new delivery or reformulation of an existing drug. Modern drug delivery technologies are capable of incorporating improved formulations of drug to fabricate new delivery systems leading to therapeutic and economic benefits. Since drug discovery research is a very costly and tedious affair, many pharmaceutical and drug delivery companies are focusing on repurposing or reformulating existing drugs providing improved efficacy, convenience of drug administration, higher patient compliance, low investment, low failure risk, less time, and high returns as compared to developing a new drug. Reformulating an existing drug as new delivery or nanomedicine after the product's patent protection period may be chosen as value-added strategy since reformulation strategy can provide the benefit of patent protection, and helps companies to retain their sales with the original product even after patent expiration. Developing new deliveries and/or nano-drug delivery system of existing drugs represents a cost-effective way to advance the drug candidates as compared to new drug discovery approach; therefore, it is considered at an earlier stage in the development process. Pharmaceutical companies assess drug-driven, patient-driven, and market-driven needs for reformulating an original product to the new deliveries or nano-drug delivery systems including both technical and commercial objectives, and follow the steps explicitly for a successful reformulation of an existing drug. Modern pharmaceutical and drug delivery market have come far away to witness several breakthroughs providing new hopes in medical treatment and will likely to maintain its therapeutic potential and financial growth at a phenomenal rate in the future. A huge economic prospective of drug delivery market supports the idea of partnering between pharmaceutical companies and academic laboratories. This sort of partnership may encourage nano-drug delivery research, promoting opportunities for clinical translation of promising research findings as enabled nanomedicine for advancing the treatment and enhancing the human health.

Acknowledgement The library supports of Medical Sciences Campus, University of Puerto Rico during the literature search for the preparation of this manuscript is kindly acknowledged.

Conflict of Interest The authors declare no conflict of interest.

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Global Growth of Nanomedicine and What Role it Will Play for Economically Weak Countries

23

Sunita Lahkar and Malay K. Das

Abstract

Nanotechnology related to the health sector, known as Nanomedicine, has benefited scientific ideas to be implemented in the research and development of Nanomedicine. Nanoscale matter is beneficial over traditional therapy for the diagnosis and treatment of several diseases like Cancer, Diabetes, Alzheimer's disease, and Parkinson's disease. Even though Nanomedicines are accepted worldwide and several governmental as well as nongovernmental organizations and industries are promoting Nanomedicine-based projects, the existing global inequity, in terms of socioeconomic status, health, and regulatory policies, retards its proper and successful implication. Thus, the present review discusses several applications, benefits, global market size, challenges to be faced, and the global impact of Nanomedicine.

Keywords

Nanomedicines · Health issues · Regulatory policies · Toxicity · Socioeconomic issues

23.1 Introduction

Nanotechnology is the 'top to bottom' process based on the qualitative and quantitative manipulation of matter at atomic and molecular levels with a scale of 1–100 nm for specific technological use. The word 'Nano' originated from the Greek word meaning "Dwarf", which signifies one-billionth of a metre (10^{-9} m). The term 'Technology' was also derived from the Greek word 'tekne' and 'logos'

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M. K. Das, Y. V Pathak (eds.), *Nano Medicine and Nano Safety*,
https://doi.org/10.1007/978-981-15-6255-6_23

meaning know how to do things [1]. It is a multidisciplinary field to sum up the exploitation necessary for technology development at the nanoscale level involving a collaborative approach of both basic sciences such as chemistry, physics, and biology and applied sciences such as biophysics, molecular biology, and bioengineering [2]. As such, the term Nanotechnology was first coined by 'Norio Taniguchiin' in 1974 who defined it as "the production technology to obtain the extra high accuracy and ultrafine dimensions, of 1 nm". It is processed by separation, consolidation, and deformation of materials by one atom or one molecule [3]. Dated back to history, in 1959, Richard Fennyman, the father of nanotechnology in his lecture "There's Plenty of Room at the Bottom: An Invitation to Enter a New Field of Physics" explained about the manipulation of individual atoms or molecules at the atomic level. He gave the concept of "nanoscale machines" that can arrange the atoms in the way they want [4]. Later on, a technique to deposit single atomic layers (Molecular Beam Epitaxy) was invented in 1968. The concept given by Richard Feynman was more widened after the advent of the Scanning Tunneling Microscope (STM) in 1981 and the Atomic Force Microscope (AFM) in 1986, which paved the way for scientists to visualize and work on the manipulation of matter in the nanoscale range [5]. On the other hand, in the same year, 1986, Eric Drexler, was associated with molecular nanotechnology described in his book "Engine of Creation: The Coming Era of Nanotechnology" that uncontrollable pieces of machinery could be built by molecular nanotechnology as the nanoscale assemblers that could make several replicate copies of themselves described the benefits and risks of nanotechnology [6]. Later on, in 1989, nanotechnology was applied when xenon atoms were used to write the IBM logo on a copper surface. A series of advancements in nanotechnology took place afterward when S. Lijima discovered Carbon Nanotube for the first time in 1991 [7]. Then, in 1999, the first book on Nanomedicine entitled "Nanomedicine" was published by R. Freitas [8]. Also, the National Nanotechnology Initiative was launched in 2000 [9]. Later on, in 2001, Feynman prize in nanotechnology was awarded in the areas of developing nanometer-scale electronic devices and for the synthesis and characterization of carbon nanotubes and nanowires. Feynman Prize in nanotechnology was awarded in 2002, for using DNA in the self-assembly of new structures and molecular machine modeling and in 2003 for the development of nanoscale silicon devices [10]. In 2004, the first policy conference on advanced nanotechnology was held. In 2005–2010, a 3D nanorobotics concept was introduced [11]. Afterward, in 2011, the era of molecular nanotechnology was started [7]. To date, the nanotechnology concept has gained widespread attention worldwide. Researchers throughout the world worked on nanotechnology to explore its applicability in several sectors. Nanotechnology in the size range of 1–100 nm has different inherent properties from bulk materials such as electrical conductance, chemical reactivity, magnetism, optical effects, and physical strength. Small-sized nanoscale matter has a high surface area to volume ratio. Its other advantages are being stronger, lighter, cheaper, durable, precise, and can be mass-produced [12]. Thus, nanomaterials are used as quantum dots in display screens, as nanorobots used to clean the block capillaries allowing efficient surgery, in genetic nanotechnology, molecular nanotechnology,

and tissue engineering [13]. Thus, Nanotechnology-based scientific endeavors received several explorations in different sectors of organic chemistry, surface chemistry, molecular biology, electronics, warfare, health, etc. Health is a crucial sector and nanotechnology used in health and medicine is called Nanomedicine [14]. Nanomedicine is considered to be the promising branch of nanotechnology in the twenty-first century.

23.2 Nanomedicine and its Application

Thus, Nanomedicine can be defined as the branch of nanotechnology consisting of materials in the size range of 1–100 nm which are used for diagnosis, imaging, treatment, and prevention of most of the diseases. The materials that have particle sizes in the range of 1–100 nm are known as nanoparticles [15]. Nanomedicines have a profound impact on the health sector and pharmaceutical industries. Common drawbacks with conventional systemic administration include difficulty with poorly soluble and impermeable drugs, nonspecific targeting, systemic toxicity, difficulty in maintaining drug concentration within the therapeutic window which ultimately reduces the efficacy and availability of the drug at the targeted site. On the other hand, the size of nanoparticles resembles the pore size of our biological membrane and thus nanoparticles can easily penetrate through the biological membrane to deliver the drug at the site of action, i.e. diseased tissue. As small-sized nanoparticles have a large surface area, they can deliver sufficient concentration of the drug to the diseased tissue within the therapeutic concentration. Thus, targeted, site-specific drug delivery is the most important property of nanoparticles [16]. Specific drug targeting to the site of action prevents unwanted accumulation in nonspecific sites causing the reduction of unwanted side effects and enhanced bioavailability of the drug. Nanomedicines are useful for targeted delivery and dissolution of drugs that are poorly water-soluble. Small-sized nanoparticles have a large surface area and thus increase the rate of drug release (Dissolution) which makes an increase in bioavailability at the specific site [17]. Nanomedicines undergo passive targeting of the drug by the enhanced permeability and retention (EPR) mechanism. It can increase the retention of drugs in the body for long periods by increasing the absorbability of drugs [18]. To date, several research studies have been done, and still, research studies are undergoing to explore its implications in several fields. The safety and efficacy of nanodrug delivery over the conventional therapy find faster opportunities in the delivery of anti-cancer drugs, hormones, vaccines, gene and brain-targeted delivery of anti-HIV drugs, anti-Alzheimer drugs, and anti-cancer drugs. Prior to the treatment of diseases, diagnosis is required. Nanomedicines based on non-invasive technology improve the intracellular diagnosis and screening of diseases in our body in a precise and controlled manner. Thus, nanoparticle-tagged fluorescent markers and quantum dots as diagnostic agents for intracellular imaging have advantages due to the requirement of small volumes of samples, reduction of reagent, and fast reaction time [19]. The different types of nanoparticles [20], shown in Fig. 23.1a, have several applications in the medical field in terms of drug delivery,

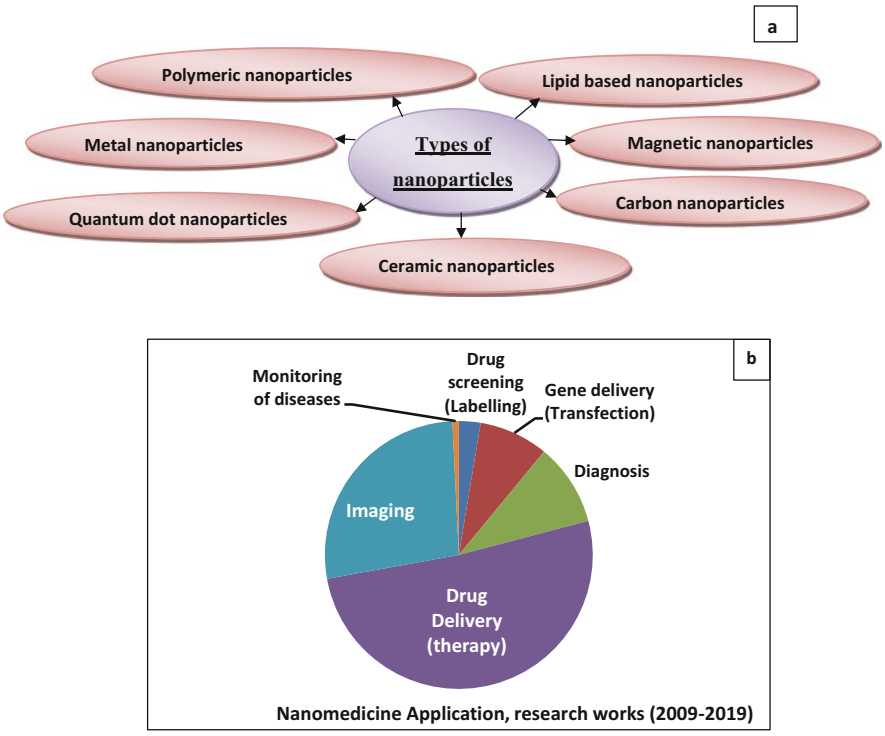

Fig. 23.1 (**a**) Classification of nanoparticles, (**b**) Nanomedicine application (2009–2019) shows the highest Nanomedicine-based research is done in drug delivery to specific diseased sites

gene therapy, image contrast agents, biomarkers, etc. To date, the FDA has approved some of the Nanomedicines [21] for marketing as shown in Table 23.1. The status of research activities from 2009 to 2019 in terms of Nanomedicine applications in several areas such as drug delivery, gene therapy, imaging, and diagnosis and monitoring of diseases is shown in Fig. 23.1b, indicating that the highest number of research studies are done in drug delivery followed by imaging.

23.3 Global Market of Nanomedicine

Nanomedicine is the fastest growing market owing to its potential to diagnose and treat diseases at the same time. With reference to BCC Research, "Nanotechnology in Medical Applications: The Global Market", Nanomedicine market is expected to grow at a compound annual growth rate (CAGR) of 13.5% by 2024 as observed in 2019 [46]. As per the Grand View Research Inc. report (Revenue, USD Billion; 2013–2025), Nanomedicine market value is estimated to be $350.8 billion by 2025 [47]. With reference to this report, the nanoparticles' regional outlook shows that in the Nanomedicine market, North America is the leading country with a revenue

Table 23.1 FDA-approved nanomedicine

Sr. no.	Marketed products	Pharmaceutical companies	Drug incorporated	Application	Ref.
1	Adagen®	Sigma-Tau	Pegademase bovine	Severe combined immunodeficiency disease (SCID)	[22]
2	Cimzia®	UCB	Certolizumab pegol	Crohn's disease, rheumatoid arthritis, psoriatic, arthritis, Ankylosing spondylitis	[23]
3	Copaxone®	Teva	Glatiramer acetate	Multiple sclerosis (MS)	[24]
4	Eligard®	Tolmar	Leuprorelin	Prostate cancer	[25]
5	Macugen®	Bausch & Lomb	Pegaptanib	Macular degeneration, neovascular age-related	[26]
6	Mircera®	Hoffman-La Roche	Methoxy polyethylene glycol-epoetin beta	Anemia associated with chronic kidney disease	[27]
7	Neulasta®	Amgen	Pegfilgrastim	Neutropenia, chemotherapy	[28]
8	Plegridy®	Biogen	Peginterferon beta-1a	Multiple sclerosis	[29]
9	Adynovate	Baxalta	Antihemophilic factor (recombinant), PEGylated	Hemophilia A	[30]
10	Pegasys®	Genentech	Pegylated interferon alfa-2a	Hepatitis B; hepatitis C	[31]
11	PegIntron®	Merck	Pegylated interferon alfa-2b	Hepatitis C	[32]
12	DaunoXome®	Galen	Daunorubicin citrate liposome injection	Kaposi sarcoma	[33]
13	AmBisome®	Gilead Sciences	Amphotericin B	Fungal/protozoal infections	[34]
14	Krystexxa®	Horizon	Pegloticase	Chronic gout	[35]
15	DepoDur®	PaciraPharmaceuticals	Morphine sulfate	Analgesia (postoperative)	[36]
16	Estrasorb®	Novavax	Estradiol	Menopausal therapy	[37]
17	Emend®	Merck	Aprepitant	Antiemetic	[38]
18	Megace ES®	Par pharmaceuticals	Megestrol acetate	Antianorexic	[39]
19	Avinza®	Pfizer	Morphine sulfate	Psychostimulant	[40]
20	Zanaflex®	Acorda	Tizanidine	Muscle relaxant	[41]
21	Tricor®	Lupin Atlantis	Fenofibrate	Hyperlipidemia	[42]

(continued)

Table 23.1 (continued)

Sr. no.	Marketed products	Pharmaceutical companies	Drug incorporated	Application	Ref.
22	NanOss®	Rti surgical	Hydroxyapatite	Bone substitute	[43]
23	Rapamune®	Wyeth pharmaceuticals	Sirolimus	Immunosuppressant	[44]
24	OsSatura®	IsoTis Orthobiologics	80% hydroxyapatite and 20% ss-tricalcium phosphate	Bone substitute	[45]

share of 42% [47]. Europe is the second largest revenue shareholder followed by Asia-Pacific, Latin America, and the Middle East and Africa (MEA) [47]. The tremendous growth of these countries is due to the presence of patented Nanomedicine products (U.S. patent), Table 23.2, [48], highly developed healthcare systems, the presence of several Nanomedicine-based manufacturing companies, and the involvement of government for the upliftment of several healthcare development programs [47]. In the year 2000, the National Nanotechnology Initiative (NNI) was initiated in the United States, to look after nanotechnology-related activities. Several agencies are working under NNI to work in collaboration with companies and universities for the treatment of several cancer and infectious diseases using Nanomedicines [49]. Nano-manufacturing in Small Business Innovation Research (SBIR) programs is such an example [50]. In a recent strategic plan presented by the NNI in 2016, several programs were identified to further advance the research and development programs, over the forecast period. The Nanomedicine market is expected to grow at a faster rate in the Asia-Pacific region due to its rising aging population, increasing international research collaborations, rising nanotechnology R&D expenditure, and rapidly growing healthcare industry. Similarly, in the Nanomedicine product outlook report, high revenue shares are expected in therapeutics, regenerative medicines, in-vitro diagnostics, in-vivo diagnostics, and vaccines [47]. The Nanomedicine market is dominated by six Pharmaceutical industries which attributed to 65–70% of nanotechnology-based products for technology improvement, drug bioavailability improvement as well as administration. These companies are Stryker Corporation (U.S.), 3 M Company (U.S.), St. Jude Medical, Inc. (U.S.), Smith & Nephew, Inc. (U.K.), Affymetrix, Inc. (U.S.), and Perkin Elmer, Inc. (U.S.) [51]. Other pharmaceutical companies are Combimatrix Corp, Ablynx NV, Abraxis Bioscience Inc., Celgene Corporation, Teva Pharmaceutical Industries Ltd., Arrowhead Research, GE Healthcare, Merck & Co. Inc., Pfizer Inc., and Nanosphere, Inc. [52]. In February 2017, the European Commission approved the first drug, REVLIMID (Lenalidomide), for marketing in Europe for the monotherapy of multiple myeloma [53]. The existence of several other companies in the market increases competition among the existing major companies. The positive effect is that these companies are adopting several new strategies to develop Nanomedicine leading to innovation so as to solve challenges in the healthcare system. As per the Nanomedicine market survey for diseases, significant progress is found in Nanomedicine based cancer chemotherapy during these years [54]. Also, several drawbacks linked with oral administration of drugs for a range of neurological or brain disorders lead to significant research in Nanomedicine-based treatment of neurological disorders like Alzheimer's disease, Parkinson's disease, brain cancer, and neuro-AIDS. The advantage of Nanomedicine is that it can easily cross the blood–brain barrier (BBB) owing to its small size ($\leq$100 nm) and low molecular weight. Thus, a sufficient concentration of the drug within its therapeutic range can be delivered across the BBB [55]. Some of the nanoparticles, such as Gold nanoparticles, Lipid nanoparticles, Polymeric nanoparticles, and Chitosan nanoparticles, are promising for drug targeting to the brain. Cancer is also such disease responsible for mortality and morbidity of millions

Table 23.2 List of patented nanomedicines, US Patent

Sr. no.	Title	Application	Patent number
1	Nanoparticulate systems prepared from sorbitan esters	Medicines or medical devices, in tissue engineering or regenerative medicine, for cosmetic, hygienic or nutritional uses, and in surface coatings	9,861,588
2	Preparation of extremely small and uniform sized, iron oxide-based paramagnetic, or pseudo-paramagnetic nanoparticles and MRI T1 contrast agents using the same	MRI contrast agent	9,861,712
3	Mucoadhesive nanoparticle composition comprising an immunosuppressant and methods of use thereof	Delivery of immunosuppressant, cyclosporine A to the mucosal site	9,878,000
4	Nanofiber scaffolds for biological structures	Transplantation	9,884,027
5	Low-density lipoprotein nanocarriers for targeted delivery of omega-3 polyunsaturated fatty acids to cancer	Anticancer activity to malignant liver cells	9,889,092
6	Nanoparticles, composed of sterol and saponin from Quillaja Saponaria Molina for use in pharmaceutical compositions	Cancer treatment	9,907,846
7	Polyvalent-functionalized nanoparticle-based in vivo diagnostic system	Diagnonistic agent	9,910,035
8	Immune-modifying nanoparticles for the treatment of inflammatory diseases	Ameliorate inflammatory immune responses	9,913,883
9	Nanoparticles/theranostic vehicles	Facilitates diagnoses, treatment, and targeting of amyloid deposits	9,950,002
10	Carbon nanotube-based anticancer agent capable of suppressing drug resistance	The present invention provides an anticancer agent comprising a multiwalled carbon nanotube and an anticancer drug covalently attached to the surface of the multiwalled carbon nanotube, with the anticancer agent capable of solving the drug resistance problem	9,981,042
11	Stable liposomal formulations for ocular drug delivery	Treatment of ocular disorders	9,956,195
12	Method of treating diabetic wounds using biosynthesized nanoparticles	Treatment of diabetes	9,974,749

(continued)

Table 23.2 (continued)

Sr. no.	Title	Application	Patent number
13	Targeted self-assembly of functionalized carbon nanotubes on tumors	Treatment of cancer	9,976,137
14	Tolerogenic synthetic nanocarriers for antigen-specific deletion of T effector cells	Tolerogenic immune responses (e.g., antigen-specific T effector cell deletion)	9,987,354
15	Nanospheres encapsulating bioactive material and method for formulation of Nanospheres	Transdermal delivery of a vaccine	10,004,790
14	Liposomes active in vivo on neurodegenerative diseases	In-vivo reduction of the amyloid plaque in the central nervous system	10,010,505
15	Compositions and methods of tumor treatment utilizing nanoparticles	Treatment of cancer after intraperitoneal administration	10,016,365
16	Multifunctional metal nanoparticles having a polydopamine-based surface and methods of making and using the same	Treatment of cancer or bacterial infections, and for use in diagnostic imaging	10,016,499
17	Porous nanoparticle-supported lipid bilayers (protocells) for targeted delivery and methods of using the same	Treatment of hepatocellular cancer	10,022,327
18	Multimodal silica-based nanoparticles	Detection, characterization, monitoring, and treatment of a disease such as cancer	10,039,847
19	Paramagnetic solid lipid nanoparticles (pSLNs) containing metal amphiphilic complexes for MRI	Imaging	10,039,843
20	Nanoparticles drug delivery	Treatment of chronic obstructive pulmonary disease, bronchial asthma, cystic fibrosis, chlorine inhalation, influenza, and acute myocardial infarction	10,034,837
21	Nucleic acid nanostructure barcode probes	Detectable labels for probes	10,024,796
22	Silica-based antibacterial and antifungal nanoformulation	Treatment of citrus canker, inhibit the growth of mold and mildew, and add nutrients to soil used for agricultural purposes	10,085,444
23	Nested particles and the use thereof for the coordinated delivery of active agents	Facilitates sequence-specific drug release.	10,076,509
24	Assembly of micelle aggregates of surfactant micelles and silver nanoparticles and use as antibacterial agents	A product comprising such assemblies for use in treating or preventing bacterial infections	10,064,891

(continued)

Table 23.2 (continued)

Sr. no.	Title	Application	Patent number
25	Methods of treating pulmonary disorders with liposomal amikacin formulations	Treatment of pulmonary disorders	10,064,882
26	Nanoparticulate compositions for targeted delivery of acid-labile, lipophilic prodrugs of cancer chemotherapeutics and their preparation	Treatment of cancer by targeting LDL receptors	10,064,823
27	Nano-enhanced wound dressing	Dermal drug delivery	10,058,455
28	Chemically activated nanocapsid functionalized for cancer targeting	Treatment of cancer	10,053,494
29	Photoactivatable lipid-based nanoparticles as vehicles for dual agent delivery	Incorporation of two drugs for dual treatment	10,117,942
30	Hyaluronidase and a low-density second PEG layer on the surface of therapeutic-encapsulated nanoparticles to enhance nanoparticle diffusion and circulation	Improve penetration in tumour cells and increase the blood circulation of therapeutic agents	10,117,886
31	Methods of assessing amyloid-beta peptides in the central nervous system by blood-brain barrier permeable peptide compositions comprising a vab domain of a camelid single domain heavy chain antibody against an anti-amyloid-beta peptide	Treatment of Alzheimer disease	10,112,988
32	Nanoparticle drug conjugates	Diagnosis, treatment of cancer	10,111,963
33	Magnetic nanoparticles	Highly sensitive detection as well as diminished non-specific aggregation of nanoparticles	10,111,971
34	Particle formulations of all-trans retinoic acid and transforming growth factor beta for the treatment of type 1 diabetes mellitus	Treatment of an autoimmune disease, such as diabetes, or an inflammatory disease	10,105,334
35	Nanoparticles, the process for preparation and use thereof as carriers for amphipathic and hydrophobic molecules in fields of medicine including cancer treatment and food-related compounds	Treatment of cancer	10,100,078
36	Nanoparticulate composition containing antibiotics for intramammary administration in animals	Therapy for mastitis in cows, avoiding the inconvenience of the use of high doses of drugs used in conventional formulations, thus contributing to an improvement in milk quality	10,098,840

(continued)

Table 23.2 (continued)

Sr. no.	Title	Application	Patent number
37	Polycation-functionalized nanoporous silicon carrier for systemic delivery of gene silencing agents	Diagnostic and/or therapeutic regimens for delivery of genetic constructs to one or more cells, tissues, and/or organs of interest and treatment of cancer	10,087,442
38	Rod-shaped plant virus nanoparticles as imaging agent platforms	Detection of tumor or atherosclerotic tissue	10,086,095
39	Therapeutic nanoparticles having EGFR ligands and methods of making and using same	Therapeutic purpose	10,137,088
40	Polymeric nanogels with degradable backbones and from gas components, and compositions and methods thereof	Drug delivery, diagnostics, and specialty materials	10,131,745
41	Monodisperse glycogen and phytoglycogen nanoparticles and use thereof as additives in cosmetics, pharmaceuticals, and food products	Rheological modifiers (including modulation of thixotropic behavior), stabilizers of organic and biological materials, and photostabilizers in sunscreens	10,172,946
42	Cell-targeting nanoparticles comprising polynucleotide agents and uses thereof	Delivery of polynucleotide to targeted cell	10,179,113
43	Method of delivering therapeutics and imaging agents to the brain by nanoparticles that cross the blood–brain barrier	Delivery of therapeutic agents to the brain	10,182,986
44	Surface modified polymeric nanofiber substrates by plasma treatment and fabrication process for the same	Tissue regeneration	10,184,211
45	Biosensor comprising metal nanoparticles	Biosensor for visual detection of an analyte, based on the light to heat conversion properties of metal nanoparticles	10,197,566
46	Iron garnet nanoparticles for cancer radiotherapy and chemotherapy	Radiation treatment of skin lesion for cancer or psoriasis	10,195,297
47	Drug carrier for tumor-specific targeted drug delivery and use thereof	Tumor targeted drug delivery	10,195,155
48	Expansile crosslinked polymersome for pH-sensitive delivery of anticancer drugs	Cancer treatment	10,188,606
49	Polymeric nanoparticles useful in theranostics	Controlled drug delivery	10,233,277
50	Functionalized magnetic nanoparticles and use in imaging amyloid deposits and neurofibrillary tangles	Imaging beta.-amyloid deposits and neurofibrillary tangles	10,232,059

(continued)

Table 23.2 (continued)

Sr. no.	Title	Application	Patent number
51	Liposomal composition comprising a sterol-modified lipid and a purified mycobacterial lipid cell wall component and its use in the diagnosis of tuberculosis	Diagnosis of tuberculosis	10,228,371
52	Nanofiber-based graft for heart valve replacement and methods of using the same	Wound repair and tissue replacement, particularly during heart valve transplant	10,219,895
53	Combinational liposome compositions for cancer therapy	Cancer therapy	10,213,385
54	Fluorescent solid lipid nanoparticles composition and preparation thereof	These nanoparticles allow a prolonged blood circulation half-life, show enhanced photostability and improved fluorescence signals	10,251,960
55	Nanocapsule containing a bioactive compound, and a method of reducing toxicity resulting from cancer therapy	Nanocapsules of bioactive compounds showed synergy in the treatment of cancer therapy-induced toxicity	10,251,842
56	Stereospecific lipids for locoregional therapy with long-term circulating stimuli-sensitive nanocarrier systems	Thermosensitive liposome for treating tumors, especially urinary bladder tumors and other localized tumors	10,251,838
57	Lymph node-targeting nanoparticles	Modulation of immune response by delivery of nanoparticles comprising heparin, chitosan, and at least one immunomodulatory agent, e.g. a cytokine	10,245,319
58	Lipid nanoparticle compositions and methods for MRNA delivery	Treatment of diseases associated with protein/enzyme deficiency	10,238,754
59	Antibody-albumin nanoparticle complexes comprising albumin, bevacizumab, and paclitaxel, and methods of making and using the same	Treatment of cancer	10,279,036
60	Nanoparticle-stabilized microcapsules, dispersions comprising nanoparticle-stabilized microcapsules, and method for the treatment of bacterial biofilms	The microcapsules and dispersions can be particularly useful for treating a bacterial biofilm	10,272,126
61	Rapid diffusion of large polymeric nanoparticles in the mammalian brain	Gene and drug delivery to the brain	10,307,372
62	Multifunctional RNA nanoparticles and methods of use	Therapy and diagnosis of several diseases	10,301,621
63	Delivery of bioactive, nanoencapsulated antioxidants	Delivery of Leutin or other antioxidants for treating or preventing conditions such as age-related macular degeneration or cataracts	10,292,943
64	Crystalline forms of tenofovir alafenamide	Treatment of viral infection using tenofovir alafenamide	10,287,307

(continued)

Table 23.2 (continued)

Sr. no.	Title	Application	Patent number
65	Nanoparticle complexes of paclitaxel, cetuximab, and albumin	Treatment of cancer, in particular cancer that expresses EGFR	10,307,482
66	Lipid nanoparticle compositions for antisense oligonucleotides delivery	Treatment of cancer	10,307,490
67	Nanoparticulate titanium dioxide nanomaterial modified with functional groups and with citric extracts adsorbed on the surface, for the removal of a wide range of microorganisms	Anti-microbial properties with high disinfectant and antiseptic power, removing bacteria, fungi, mycobacteria, spores, mycobacteria, protozoa, and viruses	10,342,840
68	Methods providing a therapeutic macromolecule and synthetic nanocarriers comprising immunosuppressant locally and concomitantly to reduce both type I and type IV hypersensitivity	Comprises of immunosuppressants and therapeutic macromolecules for reducing type I and type IV hypersensitivity	10,357,482
69	Stable oxaliplatin-encapsulating liposome aqueous dispersion and method for stabilizing same	Improve stability of oxaliplatin	10,383,822
70	Conjugated porphyrin carbon quantum dots for targeted photodynamic therapy	Bioimaging and/or photodynamic therapy	10,369,221
71	Dendrimer compositions and their use in the treatment of diseases of the eye	Treatment of bacterial fungal endophthalmitis	10,369,124
72	Synthesis of ursolic acid nanoparticles	The ursolic acid nanoparticles exhibit greater anticancer activity than conventional-size particles, and that the nanoparticles exhibit antimicrobial effect against gram-positive and gram-negative bacteria, as well as fungi	10,442,833
73	Synergistic liposomal formulation for the treatment of cancer	The synergistic liposomal formulation comprising, phophatidylcholine, stearylamine, and anticancer drugs for the treatment of cancer	10,426,728
74	Tolerogenic synthetic nanocarriers for antigen-specific deletion of T effector cells	Nanocarriers comprising administering immunosuppressants and MHC class I-restricted and/or MHC class II-restricted epitopes that can generate tolerogenic immune responses (e.g., antigen-specific T effector cell deletion)	10,420,835
75	Multivalent delivery of immune modulators by liposomal spherical nucleic acids for prophylactic or therapeutic applications	The liposomal spherical nucleic acids of the invention are useful prophylactic and therapeutic applications as well as research and diagnostic indications	10,434,064

of people with 14 million cancer suffered cases in 2012, of which 8.2 million people died as per WHO report [56]. Such cases increase the chance of application of Nanomedicine due to their specific targeting ability, nonaccumulation of the drug in nonspecific organs, and benefits over conventional treatment. Thus, several research studies related to Cancer and other diseases like cardiovascular diseases, orthopedic diseases, and infectious diseases pave the way for better innovative strategies in drug combination using Nanomedicines in the coming years. Another application is Nanorobotics, which is used for specific drug targeting in cancer therapy, is under progress through to 2025. The application of DNA origami has also attributed to further growth [57].

23.4 Nanomedicine: A Disruptive Innovation

The industrial impact of Nanomedicine on the existing conventional drug delivery system causes pharmaceutical companies to think about their existing technology. Not only Nanomedicine but also nanotechnology gain a wide scope and implications in other sectors like electronics, food industry, energy, and agriculture. Small-sized ($\leq$100 nm) nanoparticles based carriers are targeting drugs efficiently and effectively at a faster rate than the conventional injection, tablets, and suspension. Unlike existing drug delivery, Nanomedicine can cross the biological barriers within the body. As reported by the "Transparency Market Research", the global market of Nanomedicine is to reach $177.6 billion by the end of this year due to increasing incidence of chronic diseases like cancer and diabetes [58]. Some of the factors might restrict the growth of Nanomedicines like high cost required for manufacturing and lack of regulatory affairs [59]. On the other hand, the increasing rate of acute and chronic diseases and the rise in the aging population are the factors that demand the application of Nanomedicine in the health sector which enhances the opportunities of Pharmaceutical companies to invest in the Nanomedicine market. In nanotechnology, the manipulation of particles occurs at the atomic level known as Atomic Precise Manufacturing (APM). APM might capture the existing manufacturing technology as researchers worldwide are engaged in several studies related to APM. Some examples are the use of scanning probe instruments for imaging; placing or joining individual molecules; the use of computer-aided design software in protein engineering to design small structural components and functional devices within the nanoscale range; the emergence of structural DNA nanotechnology to fabricate millions of frameworks in the nanometer range; the use of quantum dots for modeling and molecular engineering; and inclusion of molecular mechanics in chemistry [60]. Thus, the emergence of nanotechnology and its deployment in sectors such as health may replace conventional treatment causing a huge impact on the socioeconomic levels of several countries at the ground level.

23.5 Challenges in Global Growth of Nanomedicine

In spite of its huge potential in treating diseases, Nanomedicine faces challenges for its development from laboratory innovation to market or clinics. The challenges faced by Nanomedicine is effective and efficient drug delivery to the specific site, maintenance of therapeutic concentration of a drug with fewer side effects and maximum clinical benefit, improvement of drug biological system interaction, reduction of unwanted accumulation in nonspecific organs, and reduction in toxicity [61]. It is reported that the synthesis of Nanomedicine is costly over conventional physicochemical methods. The requirement of expensive chemicals in Nanomedicine synthesis may be toxic creating environmental hazards and thus safe disposal is essential. Also, complex engineering techniques are needed for manufacturing Nanomedicine, compared to conventional bulk products causing their production and storage more expensive than conventional medicines [62]. Long duration is required to pass stages of drug discovery in the laboratory to the market place involving filing of patents in order to protect intellectual properties of inventor and pharmaceutical companies, clinical trials of drug and time taken to gain regulatory approval of drug diminishes the time available for companies to make a profit [63]. Large pharmaceutical companies can invest fairly to progress a drug from the laboratory to the market place as they are well equipped with, scientists to conduct research, engineers for the manufacturing process, lawyers for filing and defending patents, shareholders, and existing profit availability from marketed drugs. While, for small scale industries, limited budget and insufficient resources may not permit these companies to start up with expensive Nanomedicine projects, ending up with other less expensive opportunities and projects. As per Tufts centre report for the study of drug development, it was observed that the whole phases of Nanomedicines from the laboratory to commercialization which include initial ideas, preclinical studies on animals, industrial development, clinical trials on human volunteers, gaining regulatory approval, commercialization, and marketing requires $2870 million, of which the phase of clinical trials cost about $1012–1744 million [64]. This high cost in the development of Nanomedicine and failure in gaining regulatory approvals lead to an increase in the project failure rate. Unclear guidelines for the assessment of the safety profile and toxic effects of nanomaterials (both to patients, manufacturing persons, and the environment) further prohibit its application. Regarding regulations for approving Nanomedicines or Nanopharmaceuticals, the FDA has not published any specific guidelines except that in 2016, the documents related to general regulations for all nanomaterials related to cosmetics, food ingredients, and animal feedstock were issued. Research studies are going on to prove that Nanomedicines behave differently in a physiological environment in terms of biodistribution, toxicity, pharmacokinetics, and excretion profiles, but clear findings report on these findings is awaited [65]. As discussed, in 2000, NNI discussed challenges related to its aim in advanced healthcare, therapeutic, and diagnostic sectors. Their aim was to use nanotechnology-based biosensors and new imaging technologies for earlier detection of cancer and other diseases; gene and drug targeting systems; the use of rapid

gene sequencing for more effective, less expensive diagnostics and therapeutics; the use of novel biocompatible materials; the use of vision and hearing aids; treatment based on tiny "smart" medical devices to minimize collateral damage of human tissues [9]. Although significant efforts were made in these areas, positive outcomes in fulfilling these aims are still lagging behind. Ex-vivo challenges include the implementation of fully automated pieces of machinery for processing, analysis, and readout data; the use of hundreds of biomarkers in molecular fingerprinting in diseases for preclinical research and clinical diagnostics. Regarding in vivo studies, major challenges are the requirement of appropriate data collection and systematic modeling methods for the identification and prediction of interaction of nanoparticles with biological organs; analysis of nanoparticles in complex tissues; the use of facile and reproducible synthesis of large nanoparticle libraries to enable systematic screening of optimal physicochemical parameters and problems for large scale manufacturing of nanoparticles [66, 67]. Despite several challenges, several Nanomedicines in the form of nanocrystals, liposomes, polymeric nanoparticles, and lipid nanoparticles are in the market (Table 23.1) and still pharmaceutical companies are engaged in high-grade research to apply nanotechnology for efficient treatment of different acute and chronic diseases.

23.6 Impact on Economically Weak Countries: Pros and Cons

Not only the developed nations but also developing nations are attracted towards the opportunities of nanotechnologies in the development of water purification systems, energy systems, medicine and pharmaceuticals, food production and nutrition, and information and communication technologies. In this aspect, many developing countries, such as Costa Rica, Chile, Bangladesh, Thailand, and Malaysia, are investing substantial amounts of resources in the research and development of nanotechnologies. Budding economies like Brazil, China, India, and South Africa are also spending a lot on R and D, which are reflected in their number of scientific publications in journals. However, still, these nations are lagging behind in terms of the financial assistance required for the upliftment of scientific and institutional capacity for necessary infrastructure, regulation of human health, worker safety, machinery, trained personals, and environmental protection [68].

On the other hand, the growth of nanotechnologies has both positive and negative impacts on the export of some countries [69]. The economy of some developing countries is dependent on the production and export of natural products such as rubber, cotton, coffee, and tea. Due to the replacement of natural products by nanoproducts affect the livelihoods of farmers in such countries, for example, the production and export of natural rubber. With the advent of nanotechnology, it is found that the incorporation of nanoscale materials enhances the strength and durability of rubber, which might lead to a decrease in demand for natural rubber, whereas the use of nanoscale titanium oxides, such as titanium dioxide nanotubes (to produce and store hydrogen) increases the demand for its production and export, which in turn enhances the economy of some countries. An increase in the economy

leads to the growth of nanotechnology-based companies which in turn increases job opportunities. As only trained personnel are employed to work in this field, proper education and training should be given. It is found that the world's educational system is lagging behind in preparing students for the "Nanotech Age" [70].

Health Issues Nanomedicines are effective in treating most of the diseases worldwide such as cancer, diabetes, Alzheimer's disease, and Parkinson's disease as compared with traditional medicines. The lifespan of human beings will increase with Nanomedicine [71]. The initial step before disease treatment is diagnosis; nanotechnology makes the diagnosis of the disease easier due to the presence of a nanosensor that can be placed within the body. It can pass through a patient's bloodstream which can analyze a large number of components in less time [72]. Similarly, Quantum dots are also effective diagnostic tools [73]. Secondly, surface-functionalized nanoparticles can improve drug delivery to the specific diseased site with reduced side effects. Thirdly, the coating of nanoparticles increases the durability and adhesion of implants within the body. However, there are a few factors that obstruct the development of Nanomedicine in the health sector. It is an expensive technique with a lack of technically trained personnel [74]. No doubt that developed countries like the US and European countries can make some efforts, but developing countries are still lagging behind. According to the World Health Organization (WHO), by 2002, 80% of the Nanomedicine market was located in North America, Europe, and Japan, a geographic area which is inhibited by only 19% of the world population lives. However, 90% of the population suffering from the disease is located in poor countries. In these countries, patients are economically weak to buy medicine. It is reported that 18 million people died of communicable diseases in 2001 because of insufficient money and poor medicinal infrastructure [75]. Sophisticated nanotechnology-based medicines and nanodevices failed to proliferate in such countries. Nanomedicine is a knowledge-based technology that is known to few well-trained personnel like doctors, engineers, scientists, and researchers, whereas traditional medicine is known to all sections of people worldwide. This knowledge gap creates insufficient treatment of diseases. Also, the lack of toxicity assessment of Nanomedicines is an obstacle. Nanotechnology is a technical term whether in terms of Nanomedicines or nanodevices and its application in the health sector might convert the art of execution of diagnosis and treatment of diseases to a team and technology service [76]. Thus, the lack of trained personals might cause an improper use of Nanomedicines or nanodevices which in turn might lose the trust of patients in physicians [77]. On the other hand, the National Science Foundation and the Environmental Protection Agency are concerned about the waste disposal of Nanomedicines in industries. The impact of waste in the environment and health is of major concern [78]. The major ways of disposal are air and water. The small-sized nanoparticles remain suspended in air for long periods. Upon inhaling, it might cause harmful respiratory disorders [79]. Thus, a proper waste disposal system is to be developed. In this aspect, developing countries are lagging behind due to insufficient funds and trained personals.

Societal Issues The economic development of any country depends on some criteria such as gross national products, political stability, and equality between citizens. When compared with developed countries, developing countries are lagging behind in some basic necessities such as potable water, education, and health services. The lack of basic necessities shows obvious reasons for reduced research and development. No doubt that few countries like China are affordable in research and development progression. The whole process of progression of Nanomedicines from the R and D sector to commercialization consists of several steps—the creation of research ideas and development of the process for its progression; manufacturing, marketing, and utilization of products; disposal of waste materials [80]. The initial step is a key factor in development. In the case of a wrong decision, it may lead to unnecessary expenses, in terms of money and labour. These expenses might not be bearable by developing countries, while developed countries can easily overcome the expenses and start new research, due to a fair profitable margin from the marketing of available nanoproducts than developing nations [81]. In the case of production and export of minerals, it is observed that most of the mineral deposits are located in developing countries and these countries rely on metal trade for economic development. With the inclusion of nanotechnology, more resistant and light metals can be produced having advantages than available metals. This might decrease the metal trade of developing countries, causing a negative impact on economy. This increases the gap between developed and developing countries [82]. On the other hand, demandable raw materials required for nanotechnology increase the production and export of such countries leading to economic development [83]. No doubt that development of Nanomedicine based pharmaceutical industries can increase employment, but at the same time due to lack of proper infrastructure for Nanomedicine development in research and development sectors, the researcher engaged, workers involved in research related activities might suffer from the effect of harmful toxic materials produced during manufacturing and testing procedures [84]. Nanomedicines consist of very small particles ($\leq$100 nm). Small nanoparticles have a large surface area which might aggregate the short term and long term toxicity in human beings. Thus biocompatibility and toxicity of nanomaterials should be studied during preclinical and clinical studies to ensure the safety and efficacy of Nanomedicines in the human body [85]. Other problems in developing weak countries include poor regulatory procedures and poor buying capacity for Nanomedicines. Still, in some countries like India, Brazil, and South Africa, investment in Nanomedicine research is undertaken for years to inculcate new methods in traditional medicines. Even if Nanomedicines are launched in the market, intellectual property (IP) rights are with pharmaceutical companies and for commercialization, the price will be higher which economically poor people cannot afford causing a deficit in the effective treatment for diseases [86]. Thus, to develop nanotechnology in a socially responsible manner, global equality between developed and developing nations is important. On the other hand, it is reported that improvement in nanotechnologies may improve the living condition of people as support by government, international funding agencies and increase in competition between pharmaceutical companies may improve the

economic condition of developing and weak developing countries making Nanomedicines accessible at an affordable price to all sections of people.

23.7 Future Prospects of Nanomedicines

Nanomedicines have wide future scope over conventional therapy for the treatment of diseases like Cancer, Diabetes, AIDS, and neurological disorders like Alzheimer's disease and Parkinson's disease. Not only treatment, it can also be used for diagnosis and imaging of diseased tissue. There are several FDA-approved Nanomedicines and patented Nanomedicines, and still few Nanomedicines are in clinical trial phases. The developed countries like the USA and European countries have captured the Nanomedicine market worldwide because they have sufficient funds, technologies, and infrastructures. Comparatively, developing nations like India and China are lagging behind because of insufficient funds, infrastructure, and poor economic conditions as in Middle East African (MEA) nations. In developing countries, research-related studies have been carried out during recent years for the development of Nanomedicine and application in clinical practice. Some of the Nanomedicine research perspectives with developing countries in Asia during recent years are listed below:

Regarding Cancer therapy, the Chinese Academy of Sciences reported the application of fullerene derivatives for the specific targeting of cancer stem cells. While in Japan, polymeric micelles were developed for drug delivery in cancer cells. Similarly, the Korean Society for Nanomedicine had established Korean research experts published on DNA imaging (in vitro), multiplex surface-enhanced Raman spectroscopic (SERS) imaging (endoscopic or pathologic tissue imaging), nanoparticle fluorescence imaging (both in vitro and in vivo), and theranostics based on graphene oxide nanoparticles (in vitro and in vivo). The Seoul National University reported on automated, single-cell morphological analysis on bacterial antibiotic sensitivity testing proven to have a great impact on clinical practice. Also, Singapore-based research work reported that nano-sized particles may pass through adherens junctions between endothelial cells to remove vascular leakiness both in vitro and in vivo in a lung metastasis model [87]. It is reported that in India, several organizations both private and government are providing funds for several projects based on the promotion of Nanomedicine research and development such as the Department of Science and Technology (DST) launched Nanoscience and Technology (Nanomission) program in 2007. Similarly, the DBT, Ministry of Science and Technology, Government of India, promotes the development of Nanomedicine-based drug delivery, development, and toxicity testing. The Indian Council of Medical Research (ICMR), Department of Health Research, Ministry of Health and Family Welfare, also promotes the research and development of Nanomedicine. Also, different academic and research organizations and pharmaceutical industries have taken initiative measures for Nanomedicine promotion and development, for example, the Indian Institutes of Technology (IITs), CSIR institutes, medical colleges, and pharmacy colleges. Joint collaboration of academic industries—the

Nano Functional Materials Centre, established by the Indian Institute of Technology Madras in partnership with Orchid Pharma, the University of Hyderabad in collaboration with Dr. Reddy's Labs, Centre for Pharmaceutical Nanotechnology at the National Institute of Pharmaceutical Education and Research (NIPER), is also in collaboration with a pharmaceutical company. Other than these, several Indian academic institutions and industries have collaborated with different foreign countries like USA, United Kingdom, Russia, European Countries—Department of Science and Technology have collaborated with Portugal to work on the project 'Novel targeted chitosan-based therapeutic polymeric Nanomedicines for lung cancer application'. Moreover, a constant rate of increase in Nanomedicine research-based publication shows the growth of Nanomedicine research in India. Also, several Nanomedicine-based products have captured the Indian market as shown in Table 23.3. These include pain management, antimicrobials, cancer treatment, ayurvedic nanoformulation, and biomaterials. For example, Volini, launched by Ranbaxy Laboratories Ltd, Gurgaon, is used for pain relief, which contains Diclofenac sodium entrapped in nanoparticles. Its advantage is smaller-sized particles with increased surface area and enhanced penetration through skin tissue than conventional medicine [88]. Other pharmaceutical industries like *Shasun*

Table 23.3 Nanomedicine products marketed in India

Sr. no	Name of product	Drug	Name of company	Application
1	Nanoxel	Paclitaxel	Fresenius Kabi Oncology Ltd. (erstwhile Dabur Pharmaceuticals Ltd.)	Cancer therapy
2	Genexol-PM	Paclitaxel	Lupin Ltd.	Cancer therapy. It reduces Cremophor EL-related toxicities and increases therapeutic efficacy
3	Paclitax Nab	Paclitaxel	Cipla Ltd	Breast cancer therapy
4	Taxedol	Docetaxel	Venus Remedies Ltd	Treatment of breast cancer, prostate cancer, gastric adenocarcinoma, non-small cell lung cancer, head and neck cancer, and ovarian cancer
5	Trois	Natural ingredients	Venus Remedies Ltd	Treatment of pain and inflammation associated with rheumatoid arthritis, ankylosing spondylitis, osteoarthritis, gouty arthritis, juvenile idiopathic arthritis, backache, sprain psoriatic arthritis, and fibromyalgia
6	Oxalgin Nanogel	Diclofenac sodium	Cadila Healthcare Ltd	Pain management therapy (arthritis, backache, joint pain, etc.)

Pharmaceuticals Ltd., worked in collaboration with Nanoparticle M/s. Biochem, Inc., USA, to develop gold nanoparticles-enabled radioactive medicine to treat prostate cancer. *Cadila Pharmaceuticals Ltd.* developed an alliance with M/s. Novavax Inc., USA, for developing, manufacturing, and selling nanotechnology-based novel therapeutic and prophylactic vaccines, biological therapeutics, and diagnostics in India. On the other hand, nanomedicine developed by *Sun Pharma Advanced Research Centre* is in clinical trials, namely, 'Paclitaxel Injection Concentrate for Nanodispersion (PICN)' and 'Docetaxel Injection Concentrate for Nanodispersion (DICN)' [89]. The Indian Institute of Technology (IIT), Bombay, reported on gold nanostructures, for cancer treatment is in clinical trial phase II [90]. NIPER, Mohali, Chandigarh, NanoCrySP, a nanocrystalline solid dispersion, to improve the aqueous solubility of poorly soluble drugs [91]. Even though significant progress in Nanomedicine based research and development has taken place in developing countries like China, South Korea, and India, but still, proper guidelines on regulatory policies for safety and toxicity of Nanomedicines are not developed in these countries.

23.8 Conclusion

Nanomedicine is the most rewarding sector of nanomaterials due to its worldwide demand and acceptance ratio. The constant rate of increase in the number of research publications over recent years is proof. Still, the existing lacuna in health issues, safety and toxicity issues, socioeconomic status, as well as regulatory policies causes a gap between countries like the USA, European countries, and developing countries. So, global equity, fund provision for research and development, government support, industry support, training of students/personals, increase in Nanomedicine-based collaborative projects with developed countries, and implementation of regulatory policies are the cornerstone for the exploitation of Nanomedicine.

Conflict of Interest The authors declare that there is no conflict of interest. All the tables and figures are original and self-made.

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