Yashwant V Pathak *Editor*

Surface Modification of Nanoparticles for Targeted Drug Delivery

Springer

Surface Modification of Nanoparticles for Targeted Drug Delivery

Yashwant V Pathak
Editor

Surface Modification of Nanoparticles for Targeted Drug Delivery

Springer

Editor
Yashwant V Pathak
College of Pharmacy
University of South Florida
Tampa, FL, USA

Adjunct professor at Faculty of Pharmacy
Airlangga University
Surabaya, Indonesia

ISBN 978-3-030-06114-2 ISBN 978-3-030-06115-9 (eBook)
https://doi.org/10.1007/978-3-030-06115-9

Library of Congress Control Number: 2019932815

This Springer imprint is published by the registered company Springer Nature Switzerland AG
The registered company address is: Gewerbestrasse 11, 6330 Cham, Switzerland

To the loving memories of my parents and Param Pujaniya Dr. Keshav Baliram Hedgewar and Mananiya Madhukar Limaye who gave a proper direction; my wife Seema, who gave a positive meaning; and my son Sarvadaman who gave a golden lining to my life.

Foreword: Nanobiotechnology Convergence for Drug Delivery

Convergence of knowledge offers a new universe of discovery, innovation, and applications. Nanoscale science and engineering field has emerged at the confluence of many disciplines and now is converging with biotechnology, medicine, digital revolution, cognitive sciences, and artificial intelligence. "Converging technologies for improving human performance" (http://www.wtec.org/ConvergingTechnologies/Report/NBIC_report.pdf, 2003) and "Nanotechnology convergence with modern biology and medicine" (Current Opinion in Biotechnology, 2003) have been recognized as most challenging and promising domains in the field from the first years of National Nanotechnology Initiative (www.nano.gov). Now, in 2018, nanobiotechnology and nanomedicine are leading research areas of nanotechnology with large economic and societal implications worldwide.

Targeted drug delivery using nanoparticles has attracted a broad interest from the scientists as well as clinicians as an effective treatment with limited secondary effects. Surface of nanoparticles is key in the recognition of the target and efficient delivery of the drug. Nanoparticulate drug delivery systems are especially promising when using natural and synthetic polymers. Despite significant advances, there are still a lot of open questions and barriers in the safe and efficient implementation of such drug carriers.

The surface characteristics of the nanoparticles play an important in developing efficient delivery systems, in many cases even more important than the nanoparticle core of itself. Nanoparticles for therapeutic applications should be biocompatible, nontoxic, and non-detective by immune systems and induce few side effects. Typically, the size of nanoparticles is defined between 1 and 100 nm, domain that may be extended as a function of the presence of nanoscale properties and phenomena. It was found that if the surface of the nanoparticles is modified by hydrophilic polymer or other means, the respective particles remain in the circulation for longer time and can avoid uptake by reticuloendothelial system. They finally can reach the site of action of specific target organs, tissues, or cells. Many scientists have found that the functionalized nanoparticles with specific ligands which have high affinity to the disease site can be used as site-specific or targeted drug delivery systems. The functional ligand attached to the surface of the nanoparticles can guide the drug

carrier to the desired site such as specific tumor in relation to cancer. The importance of surface modification of nanoparticles for better therapeutic efficacy has been demonstrated in increasing the particle interaction with the targeted tissues and has become a highly promising strategy.

The editor of this volume, Yashwant Pathak, has selected a diverse and representative collection of 25 contributions written by leading authors and scientists in the field of surface modification of nanoparticles. It covers understanding of the surface modification of nanoparticles and its use in the targeted drug delivery, as well as functionalization of lipid nanoparticles for site-specific drug delivery systems. Several chapters are focused on disease-specific targeting such cancer, lung cancer, intracellular infections, and surface modification of liposomes and brain targeting.

The volume presents a diverse collection of opinions and ideas expressed by leading scientists, engineers, medical researchers, and educators in the field. The contributions integrate various perspectives and underline the importance of nano-biomedical convergence. I encourage the readers to explore the interesting points of view, methods, and trends assembled in this well-documented collection of research works.

National Science Foundation, Alexandria, VA, USA Mihail C. Roco
National Nanotechnology Initiative, Alexandria, VA, USA

Preface

Nanotechnology is a very broad interdisciplinary area of science leading to vast areas of research and applications, thousands of new product development and improvement of existing products, and a significant intellectual property development and industrial activity. Nanoparticles are the end product of a wide range of chemical, physical, or magnetic interactions and biological processes leading to very small particles mostly in submicron range and with new definitions by FDA within the range of 1–100 nm.

Nanoparticles provide many advantages especially when used for medical applications. Particle size and surface characteristics can be easily changed to suit the requirements for the delivery of the drugs. The drug release can be controlled and localized and can be easily transported to the site of action. Drugs which are highly hydrophobic in nature can be modified to hydrophilic properties and hydrophilic drug can be made hydrophobic as per the needs and applications. Site-specific targeting can be achieved using appropriate antibodies and targeting ligands to the surface of the nanoparticles. Best part of the nanoparticle application is it can be used for a variety of routes of administration such as intravenous, nasal, oral, and transdermal and so on.

There are limitations in the applications of nano-materials because of their restricted interactions with different solvents and body constituents and compartments when delivered in the living body. Surface modification of nanoparticles can improve the interactive properties of the nano-materials to suit their applications in different scenario.

Surface properties of the nano-materials decide how it will interact with other materials as well as in body systems when delivered in vivo. The main purpose of surface modification of nanoparticles based on its application is to offer hydrophilic, hydrophobic, conductive, or anticorrosive properties to the nanoparticles for specific applications.

Another possibility is to develop multifunctional hybrid coating to the nanoparticles so that these can be used as targeted drug delivery systems. Functionalized nanoparticles have applications in many different areas including engineering, biomedical, nanomedicine, and so on.

Functionalization of nanoparticles also helps in stabilizing the nanoparticles and provides specific size and shape and receptor affinity. It can also prevent the aggregation of colloidal nanocrystals and create many sites on the nano-surface to attach necessary ligands to target the nanoparticles.

This book consists of several chapters addressing various aspects of surface modification of nanoparticles. Chapters 1–4 discuss about understanding the surface characteristics of nanoparticles, surface modification for targeted drug delivery, surface modification of PLGA nanoparticles, and functionalized lipid nanoparticles. Chapters 5–8 discuss various ways of surface modifications for cancer application of nanoparticles using appropriate techniques. Remaining chapters cover various aspects of nanoparticle surface modification for versatile application in targeting the drug to the site of action.

Overall, I expect that this book will be a very good reference source for academicians, industry experts, and more importantly the students of nanomedicine. This book can also be used as a resource for teaching the graduate classes where nanotechnology applications in medicine are discussed.

This book was an effort of many scientists and chapter authors who have submitted the quality work in record time so we could get the book out in very short time. My sincere thanks to all the authors who have contributed to this book.

The Springer group has always been supportive of new projects especially Ms. Carolyn Spenser who has been very appreciative of my efforts to come up with new ideas and book projects. Many people from Springer have worked very hard to make this project happen, and I have no words to express my feelings for their hard work to get the book out.

My university and our Dean Kevin Sneed of the College of Pharmacy, at the University of South Florida, and my colleagues here are very supportive of my adventures in book publishing.

Without the support of my family nothing can happen, as this work is in a way an encroachment on their time, but they have been always very considerate toward my academic activities.

I think we tried our level best to make this a very good resource, but inadvertently if any mistakes left unattended, kindly do inform me immediately so I will be able to update in the second edition.

Tampa, FL, USA Yashwant V Pathak

Contents

Contributors

Samson A. Adeyemi Wits Advanced Drug Delivery Platform Research Unit, Department of Pharmacy and Pharmacology, Faculty of Health Sciences, School of Therapeutic Sciences, University of the Witwatersrand, Johannesburg, South Africa

M. Azam Ali Division of Sciences, Department of Food Science, Centre for Bioengineering and Nanomedicine, University of Otago, Dunedin, New Zealand

António J. Almeida Faculty of Pharmacy, Research Institute for Medicines (iMed. ULisboa), Universidade de Lisboa, Lisbon, Portugal

Umi Athiyah Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia

I. Banerjee School of Nano Sciences, Central University of Gujarat, Gandhi Nagar, Gujarat, India

Priyanka Bhatt College of Pharmacy, University of South Florida, Tampa, FL, USA

Mayank Bhushan Department of Nanotechnology, North Eastern Hill University, Shillong, Meghalaya, India

Raja Biswas Centre for Nanosciences and Molecular Medicine, Amrita Institute of Medical Sciences and Research Centre, Amrita Vishwa Vidyapeetham, Kochi, Kerala, India

Tapash Chakraborty Drug Delivery Research Laboratory, Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh, Assam, India

Nitya R. Chawda School of Nano Sciences, Central University of Gujarat, Gandhi Nagar, Gujarat, India

Tafadzwa Justin Chiome Matrix Nano (A Saveer Group Company), Greater Noida, India

Yahya E. Choonara Wits Advanced Drug Delivery Platform Research Unit, Department of Pharmacy and Pharmacology, Faculty of Health Sciences, School of Therapeutic Sciences, University of the Witwatersrand, Johannesburg, South Africa

Malay K. Das Drug Delivery Research Laboratory, Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh, Assam, India

Sanjoy Das Drug Delivery Research Laboratory, Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh, Assam, India

Trinayan Deka Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh, India

Namdev Dhas Department of Pharmaceutics, Institute of Pharmacy, Nirma University, Ahmedabad, Gujarat, India

Ya Ding Department of Pharmaceutical Analysis, China Pharmaceutical University, Nanjing, China

Lopamudra Dutta Department of Pharmaceutical Technology, Jadavpur University, Kolkata, West Bengal, India

Fernando Luis Esteban Florez Division of Dental Biomaterials, Department of Restorative Sciences, Joint Appointment OUHSC Graduate College, University of Oklahoma College of Dentistry, Oklahoma City, OK, USA

Diana P. Gaspar Faculty of Pharmacy, Research Institute for Medicines (iMed. ULisboa), Universidade de Lisboa, Lisbon, Portugal

Kathleen Halasz Department of Pharmaceutical Sciences, College of Pharmacy, University of South Florida, Tampa, FL, USA

Anas Hanini College of Pharmacy, University of South Florida, Tampa, FL, USA

Dewi Melani Hariyadi College of Pharmacy, University of South Florida, Tampa, FL, USA

Faculty of Public Health, Airlangga University, Surabaya, Indonesia

Yihui Hu Department of Pharmaceutical Analysis, China Pharmaceutical University, Nanjing, China

Shoaib Iqbal Department of Chemical and Biomolecular Engineering, Clemson University, Clemson, SC, USA

Mohammad Tajul Islam Centre for Materials Science and Technology, University of Otago, Dunedin, New Zealand

Pradeep Kumar Wits Advanced Drug Delivery Platform Research Unit, Department of Pharmacy and Pharmacology, Faculty of Health Sciences, School of Therapeutic Sciences, University of the Witwatersrand, Johannesburg, South Africa

Yogesh Kumar Department of Biochemistry and Molecular Biology, Pondicherry University, Puducherry, India

Sunita Lahkar Drug Delivery Research Laboratory, Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh, Assam, India

S. K. Mahapatra Department of Physical Sciences, Central University of Punjab, Bhatinda, Punjab, India

Yu Ma Department of Pharmaceutical Analysis, China Pharmaceutical University, Nanjing, China

Tejal A. Mehta, Ph.D. Department of Pharmaceutics, Institute of Pharmacy, Nirma University, Ahmedabad, Gujarat, India

Nathalia Mercado College of Pharmacy, University of South Florida, Tampa, FL, USA

Doniya Milani Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia

Benson Peter Mugaka Department of Pharmaceutical Analysis, China Pharmaceutical University, Nanjing, China

Biswajit Mukherjee Department of Pharmaceutical Technology, Jadavpur University, Kolkata, West Bengal, India

Vignesh Nagarajan Matrix Nano (A Saveer Group Company), Greater Noida, India

Hiep X. Nguyen Department of Pharmaceutical Sciences, College of Pharmacy, Mercer University, Atlanta, GA, USA

Ashley Oake Morsani College of Medicine, Tampa, FL, USA

Khushali Parekh Department of Pharmaceutics, Institute of Pharmacy, Nirma University, Ahmedabad, Gujarat, India

Komal Parmar ROFEL, Shri G.M. Bilakhia College of Pharmacy, Vapi, Gujarat, India

Anita P. Patel Faculty of Pharmacy, Nootan Pharmacy College, Sankalchand Patel University, Visnagar, Gujarat, India

Dhruv Patel College of Pharmacy, University of South Florida, Tampa, FL, USA

Jayvadan K. Patel Faculty of Pharmacy, Nootan Pharmacy College, Sankalchand Patel University, Visnagar, Gujarat, India

Param Patel College of Pharmacy, University of South Florida, Tampa, FL, USA

Shyam Patel College of Pharmacy, University of South Florida, Tampa, FL, USA

Yashwant V Pathak College of Pharmacy, University of South Florida, Tampa, FL, USA

Adjunct proffessor at Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia

Viness Pillay Wits Advanced Drug Delivery Platform Research Unit, Department of Pharmacy and Pharmacology, Faculty of Health Sciences, School of Therapeutic Sciences, University of the Witwatersrand, Johannesburg, South Africa

Jayakumar Rangasamy Centre for Nanosciences and Molecular Medicine, Amrita Institute of Medical Sciences and Research Centre, Amrita Vishwa Vidyapeetham, Kochi, Kerala, India

Anupam Sarma Drug Delivery Research Laboratory, Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh, Assam, India

Achal Shah College of Pharmacy, University of South Florida, Tampa, FL, USA

Neha Shah Department of Pharmaceutics, Institute of Pharmacy, Nirma University, Ahmedabad, Gujarat, India

Heather Sheardown Department of Chemical Engineering, Faculty of Engineering, McMaster University, Hamilton, ON, Canada

Sanjay Sudan Matrix Nano (A Saveer Group Company), Greater Noida, India

Vijaykumar Sutariya College of Pharmacy, University of South Florida, Tampa, FL, USA

Aparna Viswanathan Centre for Nanosciences and Molecular Medicine, Amrita Institute of Medical Sciences and Research Centre, Amrita Vishwa Vidyapeetham, Kochi, Kerala, India

Viroj Wiwanitkit DY Patil University, Pune, India

Joseph Ayobabalola University, Ilara-Mokin, Nigeria

Faculty of Medicine, University of Nis, Nis, Serbia

Hainan Medical University, Haikou, China

M. Naveed Yasin Department of Chemical Engineering, Faculty of Engineering, McMaster University, Hamilton, ON, Canada

Chapter 1
Understanding Surface Characteristics of Nanoparticles

Ashley Oake, Priyanka Bhatt, and Yashwant V Pathak

Abstract Nanoparticles are widely used in many fields of research due to their versatile nature. They can be defined as a particle that is microscopic in size, around 1–100 nanometers and due to their size possess a set of quantum properties unlike bulk material. Individual spherical nanoparticles would be considered zero dimensional, while nanowires, nanotubes, and nanobelts are categorized as one dimensional. Thin filaments (nanodiscs, nanosheets, nanomembranes, and nanoplates) are composed of nanomaterial and larger compounds, so it is categorized as two dimensional. These particles can be classified through structure analysis (electron microscopes and X-ray diffraction) and property management (X-ray spectroscopy and photoluminescence/fluorescence). Two methods of preparation of nanoparticles include "top-down" and "bottom-up" approaches, which can be further divided into gas, liquid, and solid phase synthesis. Nanoparticles are mainly used in biological applications which include nanosensors, drug delivery, and tissue engineering, but also can be found in cosmetics, electronics, and automotive industry.

Keywords Nanocarrier · Targeted drug delivery · Drug delivery · Surface modification

A. Oake
Morsani College of Medicine, Tampa, FL, USA

P. Bhatt
College of Pharmacy, University of South Florida, Tampa, FL, USA

Y. V Pathak (✉)
College of Pharmacy, University of South Florida, Tampa, FL, USA

Adjunct professor at Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia
e-mail: ypathak1@health.usf.edu

Y. V Pathak (ed.), *Surface Modification of Nanoparticles for Targeted Drug Delivery*, https://doi.org/10.1007/978-3-030-06115-9_1

1 Introduction

The use of nanoparticles in the field of pharmaceutical research has vastly grown due to their versatile nature. Nanoparticles can be defined as a particle that is microscopic in size, around one nanometer, and can be found naturally or manufactured to be used in research. Because they are larger than an atom, but small enough to not be affected by the physical laws they can be manipulated to specifically target unique structures [1]. Examples of particles having size in nano range and found in nature can be metals, proteins, polysaccharides, and viruses that can be formed by natural disasters and environmental events including wildfires and erosion [2]. The use of nanoparticles can be tracked back thousands of years with the use of clay in ceramics or metal nanoparticles for color pigments [2]. Gold and silver nanoparticles were used to make different forms of glass, including the Lycurgus Cup, in Rome as early as the fourth century. Depending on the direction and the angle of the light, the glass produces different optical properties due to the size of the nanoparticles. The gold nanoparticles gave it more of a red tint and silver resulted in a yellow color.

Nanoparticles were first discovered by Richard Feynman where he presented his findings on December 29, 1959, in a lecture titled "There's plenty of room at the bottom." He discussed the possibility of manipulating molecules and atoms at a molecular level. At this scale, gravity would no longer be a factor, but Van der Waals attraction would be of importance. Feynman wanted to create "molecular machines" that could build new molecules at an atomic level. Although this discovery influenced many fields of science, the term nanotechnology was not used or strongly studied until the 1977 when Eric Drexler brought the concept to MIT. The Foresight Institute was established in 1986 to focus on studying transformative technology, specifically nanotechnology. They gave away the "Feynman Prize in Nanotechnology" 1993 to Charles Musgrave of Caltech for his study with hydrogen abstraction tools. This award is now given away every year to individuals who show advancements in nanotechnology towards the creation of these "molecular machines" [3].

Nanoparticles can be very easy to manipulate due to their very high surface area to volume ratio and within this range of size, particles tend to have more atoms on their surface than in their interior. This ratio changes the way they interact with other materials and gives them quantum properties. Diffusion of these particles is very high, especially at higher temperatures, and they tend to have lower melting points. The size of these particles are one nanometer to one thousand nanometers so they are anywhere from the size of an antibody to the size of a virus [3]. Nanoparticles can be classified as hard or soft depending on the material that is used. Hard nanoparticles are composed of materials including titania or silica and soft nanoparticles are composed of lipids. More research has been focused on the use of these particles in medicine due to their ability to improve the efficiency of a drug delivery. Even though less material may be used, the particles tend to be more reactive [4].

Advances in medicine have promoted the use of nanoparticles because of this manipulative ability. Nanocarriers are nanoparticles, usually of polymeric materials, used to carry a drug or a target agent. When these nanocarriers are able to carry multiple agents or drugs they are referred to as a nanovector [3]. Adding different layers can create a multifunctional nanoparticle to target different functions of the body [5]. With the discovery of any new molecular substance that could be used in medicine, there is a long new drug development that must take place to get it approved. First it is evaluated on all of the physical and chemical properties it exhibits to determine its direct absorption in the body. This process can take anywhere from 1 to 2 years where everything from solubility to protein binding are tested and recorded. The toxicity and safety of the drug are both tested in the preclinical evaluation with comparable animals which could take 2 to 3 years to complete. After this process is completed, the investigational new drug application is submitted for approval. With the approval, it is legal to start preforming clinical trials on humans which could take up to 8 years to complete. A new drug application can be submitted so it will officially be on the pharmaceutical market [3].

Depending on the size of the nanoparticles, applications involving medicine or biology are but not limited to: tissue engineering, drug and gene delivery fluorescent biological labels etc.. In order for these actions to happen, a bioinorganic interface needs to be added to the nanoparticle to act as a coating [5]. The distribution of the nanoparticles throughout the body is determined by the size because only nanoparticles with a diameter smaller than 200 nm can pass through blood vessels. With intravenous injections and subcutaneous, different tumors and cancers can be passively targeted. Imparting charge to a particle will also affect the uptake and distribution [2]. Nanoparticles also have many different applications other than medicine including electronics, laser technology, solar energy conversion, and everyday objects including tires. Nanowires are used in molecular electronics to create an improved coating in order to enhance the quantum effects. They also act as inorganic fillers in tires to reinforce the rubber structure [4].

Nanoparticles can be divided into three categories: zero, one, and two dimensional. Individual spherical nanoparticles would be considered zero dimensional and nanowires, nanotubes, and nanobelts are one dimensional. Zero dimensional structures can be a variety of shapes from spheres to cubes and are under 100 nanometers in every measurement. One dimensional structure are long hollow structures with a diameter under 100 nanometers. Thin filaments (nanodiscs, nanosheets, nanomembranes, and nanoplates) are composed of nanomaterial and larger compounds, so it is categorized as two dimensional. Two dimensional structures are flat along a polygon shaped surface with thickness in the nanoscale measurement of 1 to 100 nanometers. Due to the size of every dimension, every category of material has a variation of abilities. The specific properties of the nanomaterial, which could be conductivity, are determined by many aspects of the materials including chemical composition, measurements of the particle, and crystal structure. The electrical activity of these particles can be affected by minuscule changes, which allows the production of single electron devices. Removing or

adding atoms to a nanoparticle can directly impact the amount of conductance channels which change the electrical ability of nanowires. This advancement has led to the applications of nanocrystal biotags, nanocrystal solar cells, and quantum dot lasers [6].

2 Characterization of Nanoparticles

Nanoparticles possess what is referred to as quantum properties due to their very high ratio of atoms on the surface compared to the interior of the particle. They are not governed by the same laws as larger matter, so they are not influenced by gravity, but forces like Van der Waals. Their unique chemical and physical properties cause them to exhibit much lower melting points and have a higher level of reactivity. Smaller nanoparticles tend to produce much lower wavelengths compared to those of greater size. The strength, color, and size of the particle will depend predominantly on the material used during synthesis. Nanoparticles are directly characterized by the physical and chemical properties and this characterization is especially important when dealing with nanomedicine and the classification of a newly formulated drug [3].

Structure analysis is a method of classifying nanoparticles with the use of microscopic techniques to observe surface characteristics. X-ray diffraction, transmission electron microscopes, scanning electron microscopes, and atomic force microscopy can all be used to determine the structures of a nanomaterial being studied. Property measurement is another method that concentrates on analyzing the exact composition and properties of the material through the use of X-ray spectroscopy and photoluminescence/fluorescence. X-Ray spectroscopy can help determine the binding energy, properties of core electrons, bonding information, and oxidation states in metals. Another form of analysis is electron paramagnetic resonance spectroscopy, which study compounds with unpaired electrons and by manipulating the alignment of these electrons a g-factor will give information on the properties of the compound [6].

2.1 Electron Microscope

The first microscope invented that gave scientists the ability to view these particles was the electron microscope, which was developed by physicist Ernst Ruska in 1931. Instead of light beams, the microscope manipulates electrons to create a high-resolution image on a fluorescent screen of their path. Transmission electron microscopes are widely used for many forms of research in this field. The cathode-ray device that was experimented with by Joseph John Thomson in 1897 greatly influenced this discovery. These rays were focused using multiple electron lenses in a vacuum to increase magnification [7]. An illumination system generates a wide

parallel beam of electrons using a thermionic or field emission source. The thermionic emission source heats either tungsten or lanthanum hexaboride compared to the field emission source that uses high voltage to create a beam of electrons. Field emission sources create a much higher density beam due to the voltage and may produce a higher resolution image [3]. Electron microscopes now have a spatial resolution of 50 picometer, which is one trillionth of a meter, compared to the distance between atoms which is 200 picometer [8].

An electromagnetic condenser lens focuses the beam with a very small focal length to bend the path and then it travels into a chamber that is sealed by O-rings. The beam strikes the object by passing through it and then arrives at an objective lens that diffracts the beam to be captured by a projector lens. The projector lens creates an image on a fluorescent screen that is black and white [7]. Multiple projector lenses can be used to magnify an image up to 1.5 million times with a TEM [3]. The lighter areas indicate higher exposure to electrons in a less dense area of the specimen and the darker areas indicate a denser area of the specimen that electrons had a more difficult time passing through [7]. A high degree of resolution in these images is due to the fact that electron waves are 100,000 times smaller than light waves used in compound light microscopes. When preparing a sample to be observed it is important that the electrons have the ability to pass through the object. The material needs to be exceptionally thin for the voltage that is used, which is around 120 and 300 kV with a TEM. To obtain the basic information from the sample, staining agents need to be used to observe many optical properties [3]. Heavy metal salts, including phosphor-tungstic acid and uranyl acetate, are the most efficient agents to use on a carbon support film [9].

Scanning electron microscopes are very similar to transmission electron microscopes but have a much finer beam maintained in a vacuum. The object being magnified gives off secondary electron currents from the surface layer due to the excitation of electrons [7]. Emitted secondary electrons or backscattered electrons are then used to construct an image of the object [8]. Scanning coils allow the microscope to scan the entire image horizontally along an x- or y-axis or vertically along the z-axis, whereas in TEM that is not possibly due to the stationary set up. The beam also has the ability to rotate 360° or be tilted at an angle. The material used, energy of the beam, and incident angle all will determine the depth of the beam in the material [3]. This microscope was not invented until 1965 due to the complexity of transforming these electron currents into a three-dimensional image [7].

2.2 X-Ray Diffraction

X-ray diffraction is mainly used to observe the crystalline structure and atomic arrangements of nanomaterials. They have no contact with the specimen and their wavelength is about 1 Å, which is about 10^{-10} m, so they are nondestructive. A collection of materials is compared to the diffraction pattern produced to determine the specific compounds present in the specimen. Information recorded about time,

pressure, and temperature can be used to calculate properties including phase transitions and solid-state reactions [6]. When observing the material, the back focal plane of the objective lens will show spots or rings referred to as electron diffraction patterns. These patterns are a result of parallel and coherent beams having interference by refracted electron waves from surrounding sources. This pattern can be viewed on a magnified screen or CCD camera with the addition of a selected area electron diffraction aperture to differentiate different regions. Atomic scale order can be determined from the pattern, such as if it is polycrystalline or single-crystalline and the grain morphology. Convergent beam electron diffraction creates wider spots that include a variation bright and dark spots to analyze the thickness of the specimen and chemical bonding [8]. Electrons emitted from the specimen after the initial beam can retain all of their energy and return to the vacuum in a process called back scattering or elastic scattering. Inelastic scattering occurs when these electrons lose their energy and it is transferred to a group of secondary electrons which transport to the vacuum or are absorbed into the specimen [3].

2.3 Infrared/Visible Spectroscopy and Photoluminescence

Spectroscopy is a property measurement method that specifically measures the absorption levels of different nanomaterials. Infrared spectroscopy used infrared light to identify chemical bonds and functional groups, which can indicate structural behavior and properties. This method can be used to measure the amount of CO_2, water, and hydrocarbon contamination due to the formation of carbonate by metal oxides. Visible spectroscopy is also referred to ultraviolet visible spectroscopy due to the fact that it uses a range of visible light on the electromagnetic spectrum. Absorption measures the change in electronic transitions when pi or nonbonding electrons absorb energy, mostly from ground state to excited state. This method can help determine the presence of semiconductor nanostructured materials, the concentration of organic molecules, and protein stability/activity in the solution. Spectroscopy only requires a small amount of sample mixed into a liquid solution, which can be recovered after testing [6]. Quantitative analysis of the concentration of the solution is directly related to the Beer–Lambert law that states that change in light absorption is directly proportional to the change in concentration. If the initial concentration and wavelength of a solution are known before a reaction, the wavelength after the reaction can be used to calculate the final concentration [10].

After the excited electrons from the absorption techniques undergo relaxation, they return to a stable ground state and instead of absorbing energy, they release light energy. These relaxation times vary depending on material used and may last anywhere from fractions of a second to hours. In most cases, the energy that is emitted during this relation is less than the energy that was absorbed during the excitation process. Where this light falls on the spectrum and its intensity indicate the properties exhibited and the quality of the material/impurities can be determined by the emission transitions. This technique is mainly used for determining the exact composition of the material, but also gives some information on energy level coupling [6].

3 Synthesis of Nanoparticles

In order to synthesize nanoparticles with specific functions, they are required to be composed of multiple layers including a surface and internal structure. Unlike most bulk materials, the surface layer of the nanoparticles is most active and can be modified by different compounds to exhibit specific properties. The internal structure is made up of a monolithic matrix and core, which can be made of a multitude of compounds and layers held together by covalent and hydrogen bonding. In certain conditions, these layers can be hollow or just a medium. When the core of the nanoparticle is unfilled it is referred as a hollow particle, but if one of the layers in the matrix is unfilled it is referred to porous. Liposomes are usually filled with an aqueous phase in order to help dissolve drugs in certain pharmaceutical applications [11]. Other hollow materials may be used for catalytic support, thermal insulators, and micro vessels. Core–shell nanoparticles are made up of separate materials, which can be organic or inorganic. These materials may have different properties and can produce multifunctional nanoparticle. Coating specific cores with a shell made of a different material aids in creating a more functional and stable particle [12]. Depending on the application, the nanoparticles have to be permanently connected or may have the ability to be deconstructed to reassemble for a different use [11].

Organic nanoparticles need to be synthesized in a moderate temperature, pressure, and pH compared to inorganic nanoparticles which can be synthesized in harsher conditions if needed [3]. Inorganic particles with inorganic shells mostly contain either metal oxides or silica and are the most widely used form used due to the multiple applications. This category can be used for bio-imaging, biological labeling, information storage, and catalysis. Adding silica to these particles reduces conductivity, increases stability, and does not affect the surface reactions so inorganic material is not difficult to study. This silica coating is usually paired with a silver or gold core through the Stöber method synthesis. A precursor and water are added to an alcohol solution to create a precipitate, which then bonds together to create these particles. Particles with gold metal oxides instead of silica exhibit magnetic properties, have increased stability, increased biocompatibility, and optical properties. Inorganic particles with organic shells mainly use a polymer shell that increase biocompatibility and oxidation stability. With this organic shell, the forces between particles can be controlled to prevent too much clustering. Organic particles are very similar to the former, but the organic center creates a more flexible and strong particle for applications involving paints or microelectronics. This combination can also be used to make hollow particles [12].

Organic particles with organic shells are mainly used for drug delivery, biomaterials, catalysis, bio sensing, and many other biological applications. These particles have a special property referred to as glass transition temperature, which is the temperature at which these particles are stable. When particles fall under this they reach a state where they are too brittle and lack a tough coating, but they acquire a film-forming ability. Specific polymers can be used in drug delivery due to their biodegradable nature and also can determine how fast the drug is released. Inorganic

materials can be placed on these outer coatings for more efficient bonding with adjacent particles [12].

Two methods of preparation of nanoparticles have been presented to the public over the years which include “top-down” and “bottom-up” approaches. The top down approach follows the theory of molecular machines that manipulate smaller amounts of matter to build these nanoparticles, which is just reducing larger particles. These methods are mainly used to make inorganic nanoparticles due to the fact that the product may not be 100% pure [3]. Lithography and ion beams are two of the mainly used application in this category [13]. Bottom up synthesis involves the atoms and molecules coming together to form the particles in a medium. Most of the methods used today to produce such materials follow the bottom up approach and can include chemical synthesis, chemical vapor deposition, microemulsion, hydrolysis, and thermal decomposition [3]. This approach has proven not only to be more cost effective, but also produces smaller, more precise nanoparticles. Depending on the desired product, the top down approach can be used to produce the core and the bottom up approach can be used to coat the particle in a separate material [12]. Synthesis can take place in a liquid, gas, or vapor phase but the gas and vapor phases will produce smaller nanoparticles. The liquid phase will contain milder conditions for the nanoparticles, but the size will depend on the chemical reaction or phase separation of the solution used [3].

3.1 Gas-Phase Synthesis

Gas phase synthesis usually occurs when a gas or solid that has been evaporated reforms on substrate through condensation and then the formation of a solid or semisolid. This vapor is required to be super saturation for the synthesis of these particles, which can be accomplished through many applications. Methods in this category usually happen in very high temperatures and a controlled pressure environment [6].

Physical vapor deposition (PVD) can be categorized into either thermal evaporation, Rf sputtering, and pulsed laser deposition. During thermal evaporation, a source material is heated in an ultra-high vacuum until it starts to evaporate and settles onto a substrate. Rf magnetron sputtering uses capacitive plates and magnetic coils to remove atoms at a lower pressure [6]. This is done by manipulating high energy ions to target the source material and remove the neutral particles and redeposit them on the substrate [14]. There are four categories of this which include: ion-beam sputtering, direct current, radio-frequency, and magnetron [6]. Pulsed laser deposition uses a laser beam to disrupt a target material until it evaporates to form a vapor plume and reforms onto the desired substrate [15]. When this plume interacts with the substrate, it creates a crystalline film [6]. This method has the ability to synthesize films with multiple compounds and create metastable materials, which are materials that are able to transform over time [16].

Chemical vapor deposition (CVD) is very similar to the PVD method with the addition of a gaseous reactant. Compared to other methods it is extremely versatile and precise, so it can be used to make semiconductors and microelectronics [15]. This method can be classified into many types which include thermal CVD, low pressure CVD, plasma enhanced CVD, metal-organic CVD, molecular beam epitaxy, and atomistic deposition [6]. Atomistic deposition provides a high level of control with the material which can lead to create pure materials for coating and nanodevices [15]. It has the ability to create thin films due to the splitting of the reaction into halves [6]. Molecular beam epitaxy uses thermal molecular beams with an ultra-high vacuum to evaporate a source material and deposit it on a substrate to create epitaxial growth [17]. The pure elements being used in this are kept in quasi-Knudsen cells due to their temperature control. The most common materials used in this method are gallium and arsenide. Thermal, low pressure, plasma-enhanced, and metal organic methods are all very similar as they all happen at low pressure and include evaporation. Thermal and low pressure both occur at high temperatures about 900 °C compared to plasma-enhanced which occurs at lower temperatures around 300 °C with the addition of plasma for thick films. Metal-organic precursors can be added to create thin films and one-dimensional nanomaterials [6].

Specialized furnaces can be used to vaporize a source material in the presence of an inert gas. The temperature for this method can reach 1700 °C but may be limited by the containment unit used to hold the material. After reaching such high temperatures, the vapor is condensed with a cooling process to form the desired nanoparticles. Adding the source material to a flame can be used to produce these particles, but this method is not as precise and does not produce pure particles. It is performed at high temperatures up to 2000 °C and leads to oxygenation of most materials used. Coating these particles in different elemental compounds can help prevent this oxygenation and produce pure particles [18].

3.2 Liquid-Phase Synthesis

Liquid-phase routes are a useful method to develop inorganic nanoparticles, which include products like gold. A citrate route was discovered by Turkevich which uses gold chloride, sodium citrate, and water. It produces spherical gold nanoparticles as the sodium citrate acts as a reducing and stabilizing agent in a water solvent [2]. The hot injection method produces instantaneous nucleation by injecting an organometallic cold precursor into a hot solvent, which in turn produces CdSe, CdS, and CdTe quantum dots. This method was developed in 1993 by Murray, Norris, and Bawendi in order to produce smaller particles of higher quality that are kept under isothermal conditions. The specific temperature used for this method does not greatly affect the nanocrystal's size due to the short reaction time [19].

A colloidal solution occurs when a substance is dispersed in a solution but is unable to dissolve in that solution [20]. This method is generally used to make inorganic particles with an organic coating or outer shell on them. Versatility is a

main factor on the considerable use of this method as it can be used to synthesize particles for many applications including biomedicine and optoelectronics [6]. A precursor that is primarily organometallic or inorganic salts is added to stabilizing molecules, which are then heated to higher temperatures to dissociate the precursor. This leads to nucleation of the products which are then injected into a separate solution to control the reaction. Surfactants bind to these newly formed nanocrystals and determine the surface characteristic, size and shape. Compared to other methods, colloidal synthesis is not expensive and easy to carry out to produce optically active particles that have the ability to form thin films and other two-dimensional nanomaterial [20].

Sol–gel methods involve the process of my steps to form a cell that can be dehydrated to form metal oxide nanoparticles. Metal precursors are hydrolyzed to form a colloidal solution similar to the method above, but then form wet porous gel. This product is left out until the solvent has completely separated from solid product which is converted to Xerogel or Aerogel. Aqueous sol–gel methods involve the addition of water and are primarily used to create bulk materials. Due to the high reactivity of the metal oxides and water, this method has shown some difficulties with controlling the procedure. Nonaqueous sol–gel methods involve organic solvents instead of water and are more precise in their nature, so this method is favored in the synthesis of smaller nanomaterials and powders [21]. This gel product can undergo high temperatures to be transformed into glass products, ceramic fibers, thin film coatings, and other gel like materials [6].

Water-in-oil microemulsions are synthesized using a hydrocarbon fluid colloidal solution and water, which with the use of surfactant, creates small aggregates of molecules. These micelles are in a reverse form than the ones usually formed in water with their polar groups pointed inwards because they are formed in oil. When these particles are formed, the surfactant polar molecules are pulled towards the core while the hydrocarbon nonpolar chains are on the outside [22]. According to the Brownian motion theory, these particles are constantly moving and colliding in a random fashion. These particles mix and exchange material to form new particles and then dissociate again [23]. The energy triggering method involves creating a reaction though the involvement of reactant precursors which triggers the reaction. A second method is referred to as one microemulsion plus reactant, which instead of a precursor, a reactant itself is just added to the solution with a second reactant. After this, a precipitating agent is added to create the final product [22].

Supercritical hydrothermal synthesis is a method that uses water at the super critical temperature to create nanoparticles [24]. This could also be categorized as a solvothermal synthesis but is more precise in the material it produces [6]. Nanocatalysts are added to solution with a precursor and a reagent to synthesis crystals that undergo a nucleation process. Along with the catalysts already added, water can also act as a catalyst. At this temperature, because water is close to evaporating, the components are more likely to evenly dissolve in the solution [24]. This process is performed in an enclosed unit, like an autoclave, with a stable pressure which determines the density of the product. Most of the products synthesized with this method are one-dimensional structures or further used for ceramics [6].

3.3 Solid-Phase Synthesis

The solid-phase method focuses the grinding and milling of larger matter into smaller particles, which is referred to as ball milling. This matter is placed in a cylindrical vessel that rotates with the help of steel ball grinding mediums. Impact from the balls, vessel, and other particles break the material up into smaller nanoparticles [25]. Materials like metals with a face centered cubic crystal structure are not stable enough to undergo this method under normal conditions, so they must be synthesized with the use of hydrogen to prevent softness [6]. This method produces metallic particles in bulk but does not have the same high quality as other methods do [25]. In mechanical attrition, energy is added to coarse grained powder to reduce the grain size by a factor of 10^3 [26]. The main devices used are attrition mills which spin on a vertical axis, a horizontal mill which spin on a horizontal axis, 1D vibratory mill that shakes up and down with a large steel ball, a planetary mill that includes a spinning plate with small containers that are also spinning in the opposite direction, and a 3D vibratory mill that shakes in all directions [27]. These particles exhibit higher atomic internal strains, enthalpy, and specific heat due to high-angle grain boundaries [26].

4 Applications of Nanoparticles

Due to the versatility of inorganic and organic nanoparticles, there are many applications that these materials are involved in. In medicine, nanoparticles can be involved in protein detection, gene delivery, and biological labels. Nanoparticles are similar size to most protein and have a biological layer that makes them suitable for biological tags. The many biological tags can have functions including fluorescent signaling, shape recognition, biocompatibility, linkers, protective layers, or antigen detection [5]. Ligands including peptides and antibodies are covalently bonded to nanoparticles, so they can actively target cells. This can lead to higher concentrations of the drug being delivered and easier detection of the targeted cells [28]. Tumors can be passively targeted with polymeric micelle nanoparticles that are specifically synthesized for the increased vascular density of the tissue [29].

Nanoparticles can also be used as sensors in the body to detect change in cellular functions. Magnetic biosensors, which are magnetic nanoparticles that contain antibodies, can be used to detect specific pathogens in the body and trigger the body's immune system to react. Using a microfluidic device, these detected molecules can be removed from the body and could be utilized for drug-resistant bacteria [30]. The Weissleder group at MGH Center for Systems Biology has developed a system that uses nucleic acid probes to specifically detect 16S rRNA in bacteria. This is only found in bacteria, so this system is very precise in detecting even trace amounts in a small portion of blood [31]. These sensors can not only detect nucleic acid but differentiate in peptidoglycan walls as well. With the addition of quantum dots, these

metallic sensor nanoparticles can exhibit optical bio-sensing, which is just an amplification of the sensing activity. Quantum dots are small semiconductor particles with high efficiency and optical properties. These nanoparticles can differentiate between two bacteria with thick peptidoglycan walls and double-stranded DNA, which they could not do without the use of quantum dots [30].

4.1 Drug Delivery

Most of the drug delivery research is currently focused on the detection and treatment of cancer cells [30]. Cancer cells can be identified using an immune-magnetic technique which targets a positive epithelial cell adhesion molecule. This cell search system uses iron nanoparticles that are coated with biotin and anti-epithelial cell adhesion molecule to locate the cells. These molecules are coated with a polymer layer to help initiate detection and capture the cells targeted [32, 33]. Nanoparticles used for this treatment are synthesized from many materials including polymers and lipids that carry a drug. Tumors can either be passively or actively targeted through a combination of multiple drugs. During passive transport, the nanoparticles are not carried along and do contain ligands, so they reach the tumor through leaky vessels and intraorgan pressures. Active transport nanoparticles do contain ligands that specifically search and bond to the targeted mass [34].

Through receptor-mediated endocytosis, ligands can increase the concentration of the drug in the tumor and enhance the desired activity. This method can be utilized to attempt to treat multidrug resistance strains. If further treatment is needed in cases, the option of creating a combination therapy drug is a possibility. The only issue faced with this method is the adverse effects the drugs might unknowingly have on each other [30]. Enhanced permeability and retention effect cause nanoparticles of greater size to passively target tumor cells over average tissue cells in the body. Tumors cells proliferate at a much higher rate than normal cells which leads them to release more vascular endothelial growth factors. Blood vessels in tumors are larger, which provides a preferred pathway for larger molecules, similar to nanoparticles, causing them to accumulate in this area. Nanoparticles can also be manipulated to target and release drugs at a higher pH levels or higher temperature, which are usually conditions around tumor cells and tissues [34].

Methods to combat this disease through the use of nanoparticles include magnetic therapy, photodynamic therapy, photothermal therapy, radiotherapy, and ultrasound. Magnetic therapy involves the use of metal nanoparticles that penetrate deep into the targeted tissue and are heated by an electric field to release a desired drug. Perfluorohexane nanoparticles are used in photodynamic therapy to carry photosensitizers to the tumor cells to convert oxygen to cytotoxic reactive singlet oxygen. Photothermal therapy uses gold nanoparticles that convert light energy in order to increase temperature to kill tumor cells and tissues. Nanoparticles with antineoplastic drugs can be combined with radiation therapy or ultrasound techniques to damage the DNA in these cancer cells [34].

Issues that are faced during these applications involve the instability of the carriers and poor oral availability. A study done in 2016 resulted in only 0.7% of the administered dose actually reached the targeted solid tumor [35]. An attempt to overcome the drug resistance is to envelop nanoparticles in endosomes to avoid the *P*-glycoprotein recognition. Research pertaining to these issues is still an ongoing battle for scientists due to the safety and regulatory requirements they have to follow [34].

4.2 Tissue Engineering

Recent advancements in medicine have incorporated nanoparticles in the process of tissue engineering. These nanoparticles create a surface layer across the bone implant to prevent the body from rejecting the unfamiliar surface. It also reduces inflammation while healing and stimulate the production of osteoblasts. Ceramic and other materials have been used for these procedures and more than 90% of implants heal without complications. The only material that shows complications with proper film development is metal due to its nonreactivity with biological features [5]. When targeting tissue development, the one factor that needs to be considered is the relationship with the extracellular matrix. Nanotopographic surfaces have been created to control the release and activation of biological drugs and factors in the body [36]. Nanogratings and nanopits have been synthesized to mimic the layer of tissues specifically for cell interactions through the process of anodization and micelle lithography [34]. This structure directly affects the morphology and differentiation of the cell and has allowed researchers to manipulate these properties [36].

Nanofabricated scaffolds have been designed for the reconstruction of these tissues, including bone, nerve, muscle, and cardiac, due to their similarities to protein nanofibers. Porous PLGA scaffolds are patterns created to increase the roughness of the surface to improve adhesion and growth. Polymeric nanoparticles with growth factors are loaded into these scaffolds to control the release of biological drugs from the extracellular matrix [36]. These nanoparticles are made from biocompatible polymers which form a core–shell micelle to carry a vast array of macromolecules. The use of these particles can also improve the efficacy and safety of the materials leading to improved outcomes for the target. Dendrimers are branched polymeric nanoparticles that can be sensors or carriers. The increased size of these molecules allows them to carry many molecules that are attached to the cores through chemical linkages [30].

4.3 Nonbiological Applications

Many of the uses of nanoparticles have a strong involvement in medicine, but these particles may also be used in cosmetics. Silver nanoparticles makes up 12% of all the nanomaterial used in cosmetics and can be found in makeup, hair care products, toothpaste, shampoo, and even sunscreen. There are many claims that the use of

nanoparticles may cause issues with toxicity in the body, but studies show that silver is naturally removed from the blood stream making it very safe to use in these products. Its main use is to protect against certain skin diseases due to its antibacterial properties as it attacks the respiratory chain in bacteria to cause cell death. These particles can help with wound recovery by targeting the dermis cells and prevent scarring [37]. Along with cosmetics, nanomaterial is widely used in the electronics industry with computers, batteries, and circuits and in the automotive industry with tires and fuel [11].

5 Conclusion

The manipulation of nanoparticles can be used in many applications including the pharmaceutical industry. Zero-dimensional nanomaterials are individual spherical nanoparticles or powders, one-dimensional nanomaterial are nanowires, nanotubes, and nanobelts, and two-dimensional materials are nanomembranes or nanosheets used for larger applications. Due to their size, which is much smaller than bulk material but larger than atoms, they exhibit quantum properties specific to their overall structure. They have a high surface area-to-volume ratio which makes them extremely reactive and easy to manipulate. Electron microscopes use high energy beams of electrons to produce a high-quality magnified image of these particles. X-Ray diffraction is used to view the crystalline structure of materials and can help determine specific properties including phase transitions and solid-state reactions. Infrared–visible spectroscopy uses a liquid solution to measure absorbance of the material, which helps determine chemical bonding, functional groups, concentration of organic molecules, and protein stability/activity in the solution.

Two methods of preparation of nanoparticles “top-down” and “bottom-up” approaches. The top down approach involves mainly solid phase synthesis that breaks larger matter down to synthesize new microscopic particles. Bottom up synthesis involves the atoms and molecules coming together to form the particles in a liquid or gas medium. Physical and chemical vapor deposition methods are categorized as gas-phase synthesis because they use high temperature and pressure to evaporate a source matter in a vacuum. These evaporated molecules are then transported to a substrate to create new microscopic materials and films. Colloidal solutions are used in liquid phase synthesis with a precursor and stabilizing molecules, which are then heated to higher temperatures to dissociate. This solution is injected into another medium to bind to surfactants and create nanocrystals. Sol–gel methods are similar to colloidal solutions with metal precursors to create a gel leading to nanoparticles used for glass products, ceramic fibers, and thin film coatings. Ball milling and mechanical attrition are solid phase synthesis that involve a cylindrical vessel with steel balls to grind up matter into smaller microscopic particles.

There are many applications that nanoparticles are involved in including nanosensors, drug delivery, tissue engineering, cancer treatment, cosmetics, and electronics. Nanosensors can detect changes in biological functions and can be

manipulated to target specific mechanisms. Biological tags and ligands can be added to nanoparticles to control fluorescent signaling, shape recognition, biocompatibility, linkers, protective layers, or antigen detection. Tumor cells can be targeted actively or passively by the use of drug delivery methods. Magnetic therapy, photodynamic therapy, photothermal therapy, radiotherapy, and ultrasound are the main methods being applied to treat this disease. Nanofabricated scaffolds can be used to help reconstruct bone and tissue by manipulating the surface layer and extracellular matrix. Nanoparticles can also be found in many other nonbiological applications including cosmetics, automotive, and electronics.

6 Future Trends

The main focus of current research is drug delivery in cancer treatments and genetic applications. Nanoparticles provide a more efficient, safer method for gene therapy and RNA interference [36–45]. Many of the drugs are difficult to practice with due to their issues involving enzymatic degradation and intracellular entry, so different combinations of polymers and lipids are being tested to create a carrier. Overuse of these nanoparticles has exhibited high toxicity levels, immune response, and instability. Cyclodextrin-containing polymers are currently being researched on its safety and efficiency [46]. Gold particles are now being researched on their possibility of destroying tumor cells. They are very optically active and do not degrade easily over time, so will the addition of light waves they can be heated to high temperatures. This would help destroy tumors using the photothermia method and is completely biocompatible with humans and thus a huge degree of risk or toxicity is avoided [47].

References

1. Sheposh, R. (2016). Nanoparticle. In *Salem press encyclopedia of science.*
2. Heiligtag, F. J., & Niederberger, M. (2013). The fascinating world of nanoparticle research. *Materials Today, 16*(7–8), 262–271.
3. Pande, M., & Bhaskarwar, A. N. (2016). *Nanoparticles: Preparation and characterization*. New York: Momentum Press.
4. Jarvie, H., Dobson, P., & King, S. (2017). Nanoparticle. *Encyclopedia Britannica.*
5. Salata, O. (2004). Applications of nanoparticles in biology and medicine. *Journal of Nanobiotechnology, 2*(3).
6. Kestell, A. E., & DeLorey, G. T. (2010). *Nanoparticles: Properties, classification, characterization, and fabrication. Nanotechnology science and technology*. New York: Nova Science.
7. Cannon, B. D. (2013). Electron microscopy. In *Salem press encyclopedia of science.*
8. Van Huis, M. A., & Friedrich, H. (2014). Chapter 7: Electron microscopy techniques. In *Nanoparticles* (pp. 191–230). https://doi.org/10.1007/978-3-662-44823-6_7.

9. De Carlo, S., & Harris, J. R. (2011). Negative staining and cryo-negative staining of macromolecules and viruses for TEM. *Micron, 42*(2), 117–131. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2978762/.
10. Kocsis, L., Herman, P., & Eke, A. (2006). The modified beer-Lambert law revisited. *Physics in Medicine and Biology, 51*(5), N91–N98.
11. Bhatt, P., Lalani, R., Vhora, I., Patil, S., Amrutiya, J., Misra, A., & Mashru, R. (2018). Liposomes encapsulating native and cyclodextrin enclosed paclitaxel: Enhanced loading efficiency and its pharmacokinetic evaluation. *International Journal of Pharmaceutics, 536*(2018), 95–107.
12. Ghosh Chaudhuri, R., & Paria, S. (2012). Core/Shell nanoparticles: Classes, properties, synthesis mechanisms, characterization, and applications. *Chemical Reviews, 112*, 2373–2433.
13. De Mello Donegá, C. (2014). Chapter 1: The nanoscience paradigm: "Size matters!". In *Nanoparticles* (pp. 1–12). https://doi.org/10.1007/978-3-662-44823-6_1.
14. Este, G. O., & Westwood, W. D. (1998). AC and RF reactive sputtering. In *Handbook of thin film process technology*.
15. Choy, K. L. (2003). Chemical vapour deposition of coatings. *Progress in Materials Science, 48*(2), 57–170.
16. Juhasz, J. A., & Best, S. M. (2011). Surface modification of biomaterials by calcium phosphate deposition. *Surface modification of biomaterials* pp. 143–169.
17. Cho, A. Y., & Arthur, J. R. (1975). Molecular beam epitaxy. *Progress in Solid State Chemistry, 10*(3), 157–191.
18. Kruis, F. E., Peled, A., & Fissan, H. J. (1998). Synthesis of nanoparticles in the gas phase for electronic, optical and magnetic applications. *Journal of Aerosol Science, 29*(5), 511–535. https://doi.org/10.1016/S0021-8502(97)10032-5.
19. Williams, J. V., Kotov, N. A., & Savage, P. E. (2009). A rapid hot-injection method for the improved hydrothermal synthesis of CdSe nanoparticles. *Industrial & Engineering Chemistry Research, 48*, 4316–4321.
20. Murray, C. B., Sun, S., Gaschler, W., Doyle, H., Betley, T. A., & Kagan, C. R. (2001). Colloidal synthesis of nanocrystals and nanocrystal superlattices. *IBM Journal of Research and Development, 45*, 47–56. https://doi.org/10.1147/rd.451.0047.
21. Rao, B. G., Mukherjee, D., & Reddy, B. M. (2017). Novel approached for preparation of nanoparticles. In D. Ficai & A. Grumezescu (Eds.), *Nanostructures for novel therapy* (pp. 1–36). Amsterdam: Elsevier. https://doi.org/10.1016/B978-0-323-46142-9.00001-3.
22. Malik, M. A., Wani, M. Y., & Hashim, M. A. (2012). Microemulsion method: A novel route to synthesize organic and inorganic nanomaterials: 1st Nano update. *Arabian Journal of Chemistry, 5*(4), 397–417.
23. Floyd, K. A., Eberly, A. R., & Hadjifrangiskou, M. (2017). Adhesion of bacteria to surfaces and biofilm formation on medical devices. In Y. Deng & W. Lv (Eds.), *Biofilms and implantable medical devices* (pp. 47–95). Oxford: Woodhead Publishing.
24. Yoko, A., Aida, T., Aoki, N., Hojo, D., Koshimizu, M., Ohara, S., Seong, G., Takami, S., Togashi, T., Tomai, T., Tsukada, T., & Adschiri, T. (2018). Supercritical hydrothermal synthesis of nanoparticles. In M. Naito, T. Yokoyama, K. Hosokawa, & K. Nogi (Eds.), *Nanoparticle technology handbook* (Vol. 3, pp. 683–689). Amsterdam: Elsevier.
25. Alves, A. K., Bergmann, C. P., & Berutti, F. A. (2013). High-energy milling. In *Novel synthesis and characterization of nanostructured materials* (pp. 77–85).
26. Fecht, H. J. (1995). Nanostructure formation by mechanical attrition. *Nanostructured Materials, 6*(1–4), 33–42.
27. De Castro, C. L., & Mitchell, B. S. (2002). Nanoparticles from mechanical attrition. In M.-I. Baraton (Ed.), *Synthesis, functionalization and surface treatment of nanoparticles* (pp. 1–9). Stevenson Ranch, CA: American Scientific Publishers.
28. Wang, A. Z., Gu, F., Zhang, L., Chan, J. M., Radovic-Moreno, A., Shaikh, M. R., & Farokhzad, O. C. (2008). Biofunctionalized targeted nanoparticles for therapeutic applications. *Expert Opinion on Biological Therapy, 8*(8), 1063–1070. https://doi.org/10.1517/14712598.8.8.1063.

29. Jain, R. K. (2010). Delivering nanomedicine to solid tumors. *Nature Reviews. Clinical Oncology, 7*(11), 653–664.
30. Wang, E. C., & Wang, A. Z. (2014). Nanoparticles and their applications in cell and molecular biology. *Integrative Biology, 6*(1), 9–26.
31. Chung, H. J., Castro, C. M., Im, H., Lee, H., & Weissleder, R. (2013). A magneto-DNA nanoparticle system for rapid detection and phenotyping of bacteria. *Nature Nanotechnology, 8*, 369–375.
32. Patel, J., Amrutiya, J., Bhatt, P., Javia, A., Jain, M., & Misra, A. (2018). Targeted delivery of monoclonal antibody conjugated docetaxel loaded PLGA nanoparticles into EGFR overexpressed lung tumor cells. *Journal of Microencapsulation, 35*(2), 204–217.
33. Xin, Y., Yin, M., Zhao, L., Meng, F., & Luo, L. (2017). Recent progress on nanoparticle-based drug delivery systems for cancer therapy. *Cancer Biology and Medicine, 14*(3), 228–241. https://doi.org/10.20892/j.issn.2095-3941.2017.0052.
34. Wilhelm, S., Tavares, A. J., Dai, Q., Ohta, S., Audet, J., Dvorak, J. A., & Chan, W. C. W. (2016). Analysis of nanoparticle delivery to tumours. *Nature Reviews Materials, 1*, 16014.
35. Shi, J., Votruba, A. R., Farokhzad, O. C., & Langer, R. (2010). Nanotechnology in drug delivery and tissue engineering: From discovery to applications. *Nano Letter, 10*(9), 3223–3230. https://doi.org/10.1021/nl102184c.
36. Yewale, C., Baradia, D., Patil, S., Bhatt, P., Amrutiya, J., Gandhi, R., & Kore, G. (2018). Docetaxel loaded immunonanoparticles delivery in EGFR overexpressed breast carcinoma cells. *Journal of Drug Delivery Science and Technology, 45*, 334–345.
37. Bhatt, P., Lalani, R., Mashru, R., & Misra, A. (2016). Abstract 2065: Anti-FSHR antibody fab' fragment conjugated immunoliposomes loaded with cyclodextrin-paclitaxel complex for improved efficacy on ovarian cancer cells. *Cancer Research, 76*(14 Supplement), 2065.
38. Patil, S., Bhatt, P., Lalani, R., Amrutiya, J., Vhora, I., Kolte, A., & Misra, A. (2016). Low molecular weight chitosan-protamine conjugate for siRNA delivery with enhanced stability and transfection efficiency. *RSC Advances, 6*(112), 110951–110963.
39. Bhatt, P., Khatri, N., Kumar, M., Baradia, D., & Misra, A. (2015). Microbeads mediated oral plasmid DNA delivery using polymethacrylate vectors: An effectual groundwork for colorectal cancer. *Drug Delivery, 22*(6), 849–861.
40. Bhatt, P., Vhora, I., Patil, S., Amrutiya, J., Bhattacharya, C., Misra, A., & Mashru, R. (2016). Role of antibodies in diagnosis and treatment of ovarian cancer: Basic approach and clinical status. *Journal of Controlled Release, 226*, 148–167.
41. Lalani, R., Amrutiya, J., Patel, H., Bhatt, P., Patil, S., & Misra, A. (2016). Approaches and recent trends in gene delivery for treatment of atherosclerosis. *Recent Patents on Drug Delivery and Formulations, 10*(2), 141–155.
42. Vhora, I., Patil, S., Bhatt, P., Gandhi, R., Baradia, D., & Misra, A. (2014). Receptor-targeted drug delivery: Current perspective and challenges. *Therapeutic Delivery, 5*(9), 1007–1024.
43. Vhora, I., Patil, S., Bhatt, P., & Misra, A. (2015). Chapter one—Protein—And peptide–drug conjugates: An emerging drug delivery technology. In D. Rossen (Ed.), *Advances in protein chemistry and structural biology* (Vol. 98, pp. 1–55). Amsterdam: Elsevier.
44. Tandel, H., Bhatt, P., Jain, K., Shahiwala, A., & Misra, A. (2018). In-vitro and in-vivo tools in emerging drug delivery scenario: Challenges and updates. In A. Misra & A. Shahiwala (Eds.), *In-vitro and in-vivo tools in drug delivery research for optimum clinical outcomes* (pp. 19–42). Boca Raton: CRC Press.
45. Gajbhiye, S., & Sakharwade, S. (2016). Silver nanoparticles in cosmetics. *Journal of Cosmetics, Dermatological Sciences and Applications, 6*(1), 48–53. https://doi.org/10.4236/jcdsa.2016.61007.
46. Thota, A., Megaji, S., & Badigeru, R. (2015). Nanoparticles—The current research. *Research and Reviews: Journal of Botanical Sciences, 4*(1), 69–78.
47. Dong, Y., Chen, Y. C., & Feldmann, C. (2015). Polyol synthesis of nanoparticles: Status and options regarding metals, oxides, chalcogenides, and non-metal elements. *Green Chemistry*, (8).

Chapter 2
Surface Modification of Nanoparticles for Targeted Drug Delivery

Param Patel, Anas Hanini, Achal Shah, Dhruv Patel, Shyam Patel, Priyanka Bhatt, and Yashwant V Pathak

Abstract Over the course of recent years, nanoparticles have been the center of attention used to treat many health related diseases. Nanoparticles are used due to it being efficient and having the ability to overcome certain biological barrier such as tumor, malignant melanoma, and treating HIV. Nanoparticles are known to have many different manipulating structures and characteristics which gives these particles a huge advantage in treating cancer. Nanoparticles are also used in tumor suppression due to their extraordinary ability of modifying their cell surface. One of the other great advantages of nanoparticles is to treat malignant melanoma. Two of the main components used in malignant melanoma therapy is poly(ε-caprolactone) (PCL) and poly(ethylene glycol) (PEG). Both components being FDA approved, have extraordinary effects in drug delivery through nanotechnology if used in a conjugated manner. One of the barriers faced in malignant melanoma therapy is losing the ability to encapsulate and retain a drug if ligands on the surface adjust the chemical properties of the polymer, which can be overcome by the use of dopamine. Nanoparticles have been greatly advantageous in breaking through barrier of successful HIV therapy. To treat this retroviral disease, the use of solid lipid nanoparticles is made due to it being able to improve the long-term stability of colloidal nanoparticles.

Keywords Nanoparticles · Surface modification · Tumor specific delivery Quantum dots · Metallic nanoparticles

P. Patel · A. Hanini · A. Shah · D. Patel · S. Patel · P. Bhatt
College of Pharmacy, University of South Florida, Tampa, FL, USA

Y. V Pathak (✉)
College of Pharmacy, University of South Florida, Tampa, FL, USA

Adjunct professor at Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia
e-mail: ypathak1@health.usf.edu

Y. V Pathak (ed.), *Surface Modification of Nanoparticles for Targeted Drug Delivery*, https://doi.org/10.1007/978-3-030-06115-9_2

1 Introduction

The utilization of nanoparticles with multifaceted properties is done in a wide array of biological applications. This encompasses gene and drug delivery for diagnostic and therapeutic purposes. Nanoparticles have shown to be very promising in the field of advanced drug delivery to specifically and rapidly target cells. Folic acid conjugated drugs target folate receptors-positive cancer cells. Research indicates that folate receptors upregulate 90% of ovarian carcinomas, also high to moderate levels in the brain, lung, kidney, and breast carcinomas. Folic acid is classified as a vitamin B which makes it imperative for cell survival as it has a role in the biosynthesis of nucleic acids. Folic acid has a high affinity ligand which complements conjugated anticancer drugs' differential specificity for folate receptors [1].

The extracellular matrix of tumors poses a biological barrier to the diffusion of advanced drug delivery systems and therapeutics. To overcome this obstacle, the surface of bromelain can be modified to increase the diffusion of silica nanoparticles across the tumor extracellular matrix. This is an ideal delivery method for cancer therapy as it is able to travel through the bloodstream and target the tumor site [2].

Therapeutic effects of chemotherapy on malignant melanoma can be enhanced using paclitaxel (PTX)-loaded methoxy poly(ethylene glycol)-b-poly(ε-caprolactone) nanoparticles (MPEG-b-PCL NPs) with modified surfaces contain polydopamine as a drug delivery carrier. This is a new therapy for treating malignant melanomas with anticancer drug loaded nanocarriers. This strategy has attracted much interest due to its high permeability to drugs, biocompatibility, non-cytotoxicity, and thermal products [3]. Similarly, PEGylated liposomes of PTX has also been developed with potential efficacy for treatment of ovarian cancer [4].

The preparation, characterization, and modification of lipid nanoparticles is essential for targeted cellular delivery methods. Previous research indicates that uncoated lipid nanoparticles of stavudine are effective in the treatment of HIV. Stavudine lipid nanoparticles with surface modification can serve as a drug delivery system for anti-HIV chemotherapy. This can be done both in vivo and in vitro [5]. This selective surface coating of lipid nanoparticles has proven to be very effective for specific targeting of infectious diseases. This technique helps overcome obstacles to HIV therapy at specific targets in the human body such the brain, spleen, bone marrow, etc. [6].

2 Surface Modification Using Cobalt Oxide Nanoparticles for Targeted Drug Delivery in Anticancer Treatments

Nanoparticles are solid and ranges in size from 1 to 100 nm. Manipulating the structure and characteristics of nanoparticles can affect their function introducing new techniques and technologies [7–9]. Nanoparticles (Table 2.1) vary to a great extent based on the properties of shapes, spatial arrangement, electronic configuration,

Table 2.1 Nanocarriers, constituents, and applications [12]

Nanoparticles	Definition
Liposomes	Spherical vesicles containing bilayered structure that can reassemble itself in aqueous systems. Few advantages of this nanoparticle is its ability to protect biomolecules along with their biodegradability and biocompatibility
Albumin-bound	Nanoparticle known for carrying hydrophobic molecules into the bloodstream. An advantage of using these nanoparticles include its ability to avoid solvent-based toxicities for therapeutics due to it naturally binding to hydrophobic molecules
Polymeric	Nanoparticles made from biodegradable and biocompatible polymers. In an aqueous solution, it has the ability to assemble itself into a core–shell micelle formation. The wide use of these nanoparticles is due to it having the ability to improve the safety and efficacy of the drugs they carry
Iron oxide	Nanoparticles known for being superparamagnetic. These molecules are very stable because they have an iron oxide core with hydrophilic coat of dextran. These nanoparticles are greatly advantageous because of its decreased toxicity and increased imaging sensitivity and specificity
Quantum dot	Nanoparticles that display optical and size dependent electronic properties. Quantum dots are known to be stable against photobleaching, have high efficiency, long lifetime, and emit bright colors. They have different biochemical specificities which can be excited and detected
Gold	Nanoparticles known to offer facile surface modification, biocompatibility, and many size and shape dependent optical and chemical properties. Due to these nanoparticles free electrons having the ability to interact with light, it enhances their light absorption, fluorescence, scattering, and surface-enhanced Raman scattering. It has great potential for infrared phototherapy because it can transfer absorbed light into heat

energetics, chemical reactivity, phase changes, catalytic capabilities, and assembly. Modifying the surface of nanoparticles for advanced drug delivery methods can alter its properties to target specific receptors in molecules on various types of cells [10, 11]. Nanoparticles are successful in biomedical and biopharmaceutical applications when their surface is synthesized and modified properly. In the solid phase, nanoparticles can be semicrystalline, amorphous, grain, or a mixture of the three. They can also be inorganic, organic, or a combination of the two. Additionally, the nanoparticles' structure can be comprised of multiple layers of different materials and when combined the structure is termed as nanocomposites. The creation of these nanocomposites can create a large number of nanoparticles that previously did not exist in nature. There are three types of nanoparticles that are created; nanoparticle composites, nanoparticle bulk composites, and composite nanofilms. These composite materials have a very successful track record in aerospace, sports, transportation, and defense. Nanocomposites are even used by developed nations to generate raw materials. There are even nanocomposites that are nanopolymers, inorganic–organic, or inorganic–inorganic. This variety allows for so many combinations to be made. The nanoparticles' method of preparation can be determined by its function, either chemical or physical. These methods can be further divided into liquid phase, gas phase, and solid phase methods based on the mechanism of the reaction.

Table 2.2 Anticancer treatments [6]

Anticancer drugs	Description
Chemotherapies	Known to kill cancer cells during their initial periods of division. This therapy is given through tablets or intravenous drip. Use of this therapy has many side effects, biggest being hair loss
Hormone therapies	Therapy used to stop the growth of cancer cells that are sensitive to hormones. Examples of this drug are: Tamoxifen and aromatase inhibitor. Few side effects related to this drug include: Bone pain, headaches, thinner hair, and nausea
Biological therapies	Known to target and kill specific types of cancer cells. Trastuzumab is known to be the biggest class of drugs in this category known to prevent breast cancer. Many side effects to this therapy are loss of appetite, hot flashes, skin changes, etc

Cobalt nanoparticles have catalytic, magnetic, and optical properties [13]. Cobalt oxide is mainly used as a biopolymer or organometal. These cobalt oxide nanoparticles have anticancer properties after surface modification. The cobalt oxide nanoparticles have great solubility in aqueous solutions and a decent hydrodynamic size. These nanoparticles have excellent uptake by cancer cells when combined with amide. Cobalt oxide nanoparticles are prepared by a thermal decomposition method after which surface modification of phosphonomethyl iminodiacetic acid (PMIDA) takes place [14]. These PMIDA-coated cobalt oxide nanoparticles can be coupled with folic acid. Methotrexate and doxorubicin are anticancer drugs that have been attached to folic acid nanoparticles that induce apoptosis in cancer cells. The above-mentioned drugs fall in class of chemotherapeutics. Other therapeutic classes used in cancer are hormones and biological therapies (Table 2.2).

Folate receptors are researched for tumor specific drug delivery. This research encourages preclinical and in vitro studies of tumors and therapeutic approaches on them. Myelogenous leukemia is a cancer that is now being treated using targeted folate receptor therapy [15]. Folic acid, which is an essential vitamin B, plays a vital role in anticancer drug targeting for folate receptors. Folic acid-conjugated drugs internalize folate conjugated compounds and bound folates through receptor mediated endocytosis. Folate receptor density increases indicate cancer. A variety of anticancer drugs have been evaluated based on their biological activity. In-spite of cobalt's role as a cofactor of vitamin B12, it can be used for anticancer treatment through surface modification. Research indicates that folate receptors appear to have important aspects of human medicine and physiology [16].

3 Use of Bromelain for Surface Modification of Silica Nanoparticles for Drug Delivery in the Tumor Extracellular Matrix

Bromelain is a digestive protein obtained from the stem or fruit of pineapple. The stem, fruit, and ananain–bromelain extracts are prepared differently and contain different compositions [17]. Bromelain has been used for ancient and modern

medicinal purposes to create various botanical preparations. Bromelain is mainly extracted from the stem. Its composition is a mixture of different components like phosphatase, peroxidase, glucosidase, cellulase, thiol endopeptidase, escharase, and other protease inhibitors. In vivo and in vitro research indicates that bromelain exhibits a multitude of antithrombotic, antiedematous, fibrinolytic, and anti-inflammatory activities. Bromelain is absorbable by the human body without losing proteolytic activity and major side effects. Bromelain has many therapeutic benefits and can be used in treatments to diagnose bronchitis, angina, sinusitis, pectoris, surgical trauma, wound debridement, thrombophlebitis, and increased absorption of antibiotics. Bromelain additionally possesses anticancer properties influences apoptotic cell death [18]. Even though the mechanism of bromelain is not known completely, bromelain has been accepted universally as a therapeutic agent because it is safe to use has minimal side effects. Bromelain has a high therapeutic value due to its pharmacological and biochemical properties. Bromelain has also been used for the treatment of a variety of health issues and is also a nutritional supplement. The human body is able to absorb a massive 12 g per day of bromelain without any major side effects. Bromelain is absorbed through gastrointestinal tract of humans and has a half-life of 6–9 hours. Bromelain produces side effects of a variety of antibiotics and increases bioavailability. These properties of bromelain show promising capabilities for anticancer drugs and therapeutic strategies [19]. Bromelain contains a mixture of cysteine proteases which proteolytically blocks activation of extracellular regulated kinase-2 in T cells of intracellular signal transduction pathways [20]. The product that is extracted from stem bromelain is taken from the pineapple juice and cooled using ultrafiltration, centrifugation, and lyophilization. The rest of the extract is available commercially to the public as a cream, capsule, powder, and tablet. The extract is available in its purest form or through multi enzyme combinations such as Phlogenzym, traumanase, and debridase [21]. In recent research it has been shown that bromelain is able to modulate key pathways that aid in malignancy. New research also indicates that anticancer activity of bromelain has a direct impact on cancer cells and their microenvironment. Properties of bromelain are mainly due to its protease components. Bromelain extracts have proteolytic enzymes which are called glycoprotein along with many minerals, protease inhibitors, organic solvents, colored pigments, and organic acids. Proteinases are the most active components from the bromelain extract and account for about 2% of the proteins. The pH range of these extracts spans from 4.5 to 9.5. During the various stages of cancer development, it has been discovered that inflammation is a common side effect. Research shows that when the inflammation from cancer progression is reduced, then the cancer itself is reduced and inhibited from progressing further. Bromelain has also been shown to downregulate PGE-2 and COX-2 expression in murine microglial cells along with leukemia cells. Bromelain is also found to enhance the function of cells that are responsible for inflammation suppression in the body. They do this by upregulating beta interleukins, alpha tumor necrosis factors, and gamma interferons of the peripheral blood mononuclear cells in humans. These results show that bromelain intensifies the immune response and helps with cellular stress during cancer formation. CD44 expression is greatly enhanced during

cancer cell spreading however bromelain usage has shown to reduce CD44 on tumor cells in humans. It also further benefits humans as it stops inflammation from migrating to other parts of the body. Bromelain also activates natural killer cells to further reduce inflammation caused by cancerous cells. Bromelain treatments greatly reduce the growth of carcinoma in the gastrointestinal tract and reduce cancer cell survival. The human body is able to absorb a massive 12 g per day of bromelain without any major side effects. Bromelain is absorbed in a highly potent form in the gastrointestinal tract and is around 40% of the total bromelain consumed. Research also indicates that bromelain is stable in the highly acidic stomach juice of humans even after 4 full hours. There are pathways that bromelain activates for malignant tumors. The bromelain targets inflammatory, immune, and hemostatic systems during cancer formation and progression stages. Bromelain extracts used for treatment on adenocarcinoma, lung carcinoma, and tumor cells are extremely efficient because they tumors showed signs of regression in just 24 hours. Bromelain overall has much potential and has already proven effective for cancer prevention and cancer regression.

In contrast to other nanocarriers, silica particles possess their own properties to deliver drugs to a range of organic systems, specific organs, and target sites. Silica particles offer a more stable, stealthy, and biocompatible alternative. A range of drugs can be readily encapsulated within the silica particle by a method of fusing sol-gel polymerization through emulsion chemistry or spray-drying. Sol-gel emulsions allow for the synthesis of nanocarriers that are necessary for handling biological material, allow ample temperature processing, and distribution of homogenous drugs. In contrast spray-drying poses challenges including size limitations, low yields, and segregation of the carriers' surface [22]. Silica nanoparticles can be synthesized and tailored to accommodate anticancer drugs. Silica nanoparticles are a popular form of nanoparticles used for targeted cancer therapy due to their simple surface modification through bromelain. Surface ligands similar to antibodies, folate acid, and polypeptides can selectively recognize tumor cell surface markers differentiating them from normal cells [23]. In an experiment, folate was used to deliver albumin with tamoxifen to a tumor target site through an in vivo method with a silica nanocarrier. The results indicated a significant reduction in the tumors volume compared to an uncoated particle [24]. Silica nanoparticles have been proven to have higher penetration within the tumoural tissues [25].

The preferred drug delivery for cancer treatment is prepared to efficiently circulate through the bloodstream, target the tumor site, and release into the subendothelial space [26]. This is a fundamental technique to achieve effective diffusion of the therapeutic drug to the internal matrix of the tumor and maximize efficiency of treatment. Aside from tumor vascularity there are other biological barriers which prolong the diffusion of nanocarriers that induce therapeutic effects. The tumor extracellular matrix (ECM) consists of laminin, collagen, proteoglycans, elastin, hyaluronic acid, and other structural proteins that inhibit effective diffusion of drugs to a great extent. However, particles with unique properties have shown to successfully

navigate through the tumor ECM with the assistance of surface modification. Taking into account that the tumor ECM is susceptible to protease action, research has led to the development of surface coating a synthetic nanocarrier with proteolytic properties. The effective nanocarrier is a mesoporous silica nanoparticle coated with bromelain, cysteine, and sulfhydryl proteases. This concoction enhanced diffusion ability upon contact with the tumor ECM and increased the therapeutic efficiency of the nanoparticle.

4 Surface Modification of MPEG-B-PCL Nanoparticles for Malignant Melanoma Therapy

There are two major issues when discussing the construction of drug delivery devices from amphiphilic block copolymers: biodegradability and biocompatibility. These two factors must be considered when developing these drug carriers. Two components used in the development of polymeric carriers are poly(ε-caprolactone) (PCL) and poly(ethylene glycol) (PEG). Hydrophobic PCL has been approved by the United States Food and Drug Administration (FDA) for medical uses is highly useful in biomedical research because of its high permeability to many drugs, nontoxicity, and biodegradability. On the other hand, PEG is a nontoxic, hydrophilic polymer, which has also been approved by the FDA for medical uses. This component is known for its low cell adhesion, low protein absorption, and other biomedical properties, most of which lead to the prevention of premature elimination of carriers from the bloodstream and increased systemic circulation times. These copolymers, when used together, have remarkable effects in drug delivery through nanotechnology [27].

Modifying the surface of a membrane is very effective in a way which improves antifouling performance. By increasing the hydrophilicity of a surface, we are able to observe improvement in the antifouling ability of the membrane especially because many natural substances have hydrophobic properties. Essentially, the increasingly hydrophilic surface acts as a buffer layer to prevent substances from disrupting the membrane. There have been a number of experiments to find a proper hydrophilic polymer, but recently a new one has surfaced. Polydopamine coating has drawn attention from a large number of scientists because of its extensive properties. It's self-polymerization, special recognition, and high anchoring ability add to the surface hydrophilicity while also keeping a thin layer on the membrane [28]. This polydopamine-mediated surface modification is highly important because the versatility and effectiveness of the coat allow for chemically functional substrates to be used in clinical applications [29].

Polymerization is incredibly efficient at functionalizing the NP surfaces but using dopamine has its added advantages compared to others. Most processes include coupling agents, reactive linkers, or prefunctionalization of the polymer, all

of which can lower the efficiency of the NP. Prefunctionalized polymers run the risk of losing the ability to encapsulate and retain a drug if ligands on the surface adjust the chemical properties of the polymer itself. Dopamine alleviates this issue because it is a relatively simple surface modification method and can be applied to a multitude of drug carriers [30].

Oxidative self-polymerization is a useful method that can be used to form thin polymer films, especially when modifying surfaces with dopamine. This specific method can be used to create a variety of polydopamine-coated planar substrates.

Oxidative self-polymerization of silicon with dopamine to form the PDA capsule was shown by Postma et al. ([31], Fig. 1 of this reference); they showed the oxidative self-polymerization of silicon with dopamine to form the PDA capsule. PDA capsules that surround chemical surfaces are vital to the application of NPs in medicine because they help decrease toxicity toward cells in the body. The fact that the PDA coated capsules benefitted the cells makes the method a promising one for drug delivery applications in medicine. As a result of its directness, efficiency, and generalizability, the PDA method can be expected to become common in application of nanoparticle delivery systems [31].

Malignant melanoma is a combative type of skin cancer that has been found to be more prevalent today than ever before. Throughout the past two decades alone, the occurrence and mortality rate have risen dramatically, well above other types of cancer. Interestingly, this skin cancer can be treated surgically when it is in its primary form but once metastasis occurs, it becomes much more difficult for surgery to have its full therapeutic effect. For this reason, there is an increased demand for higher efficiency in chemotherapy methods for malignant melanoma.

Cancer nanotherapeutics are advancing in the right direction in order to solve severe problems such as nonspecific biodistribution and targeting, lack of water solubility, poor oral bioavailability, and low therapeutic indices. These nanoparticles (NPs) are able to distribute their respective drugs directly to cancer cells by using the unique pathophysiology of tumors (Table. Along with this passive system, there is an active targeting system in place which includes antibodies directed at specific tumor targets in order to increase the selectivity of the NPs [32]. The use of NPs is very advantageous because they have high encapsulation efficiency, high drug loading capacity, minor drug leakage, and sustained release. Some drugs that can be delivered using NPs are docetaxel, puerarin, PTX, and doxorubicin. Although PTX is said to have significant effects in the treatment of malignant melanoma on paper, its extremely poor water-soluble property, low bioavailability, and high toxicity have drastically lowered its use in clinical settings. The drug Taxol® is a formulation of PTX that is prescribed in malignant melanoma therapy but there are many negative side effects that come with its use as a result of the drug vehicle Cremophor-EL. There are detrimental increases in cardiotoxicity, nephrotoxicity, and hypersensitivity in patients who take this drug. In order to reduce side effects, efforts have been escalated to replace this carrier for PTX delivery.

5 Nanoparticles for Cellular-Based Applications of Stavudine

Nanoparticles helped a major barrier to successful HIV therapy but also poses a challenge of delivering the drug at target sites such as the spleen, brain, and bone marrow. Nanoparticles that are based on solid lipids have shown to greatly improve solubility and bioavailability of therapeutic molecules by penetrating through to cellular viral reservoirs. Although surface characteristics and sizes may be the major determinants of the clearance kinetics and bio distribution of colloidal lipid particles; chemical coupling of such ligands is usually difficult, due to the absence of reactive groups at the surface of carriers. Nanoparticles that have selective retention are (10–250 nm) in size. In lipophilic composition, feasibility of production of ultrafine size nanoparticles, solid lipid nanoparticles (SLNs) can be used as carriers for delivering antiretroviral drugs. There is a variety of nanocarriers such as bioconjugates, dendrimers, liposomes, and nanoparticles have been evaluated as potential targeted drug delivery systems by numerous scientist [33]. The size-flow-filtration phenomenon that is usually limited to only tumors, the reticuloendothelial system, and possibly lymph nodes like mentioned early is a passive target of nanoscale carriers. Certain HIV receptors such as CD4, coreceptors (CCR5 and CXCR4), and other receptors are relatively specific for macrophages that provide potential valuable surface targets for drug delivery to all susceptible cells in patients that are diagnosed with HIV [34].

In previous research it has already been demonstrated that lectins play an important role in biological recognition events and can bind to intestinal mucosa and facilitate as well as transport across cellular barriers. In other findings, polysaccharide coatings have been considered an alternative to the other coatings. To be added, they have specific receptors in certain cells or tissues that are involved in tissues addressing and transport mechanism. Research on drug delivery systems that help enhance drug bioavailability by prolonged residence at the site of absorption owing to increase epithelial contact [34]. Bioadhesive that have been found to date are synthetic macromolecules, in the form of micronanoparticles or macronanoparticles that interact with mucosal surface and are hence referred to as mucoadhesive. The adhesion for this reason of a mucoadhesive polymer to some mucosal tissue will therefore be limited, by the time of turnover by the mucus gel layer to only a few hours. To solve this problem, polymeric drug carriers can be attached to certain cytoadhesive ligands that then bind to epithelial surfaces through only specific receptor mediated interactions. To help comprise a structurally diverse class of proteins that are found in organism ranging from all types of viruses and plants to humans are comprised from lectins [35]. Solid lipid nanoparticles (SLN) are an alternative colloidal drug delivery system to polymer nanoparticles. SLNs are usually produced by high-pressure melt emulsification. If a formula contains shear and temperature sensitive compounds, then the harsh productions process is not applicable. For this reason, subsequent adsorptive SLN loading might be a promising alternative. To improve the long-term stability of colloidal nanoparticles, scientists

have been using a method called free-drying that has been considered an advanced and reliable technique. SLNs that are found suitable for parental administration are then converted into dry products.

Stavudine falls in a class of medications called nuclear reverse transcriptase inhibitors. This works by decreasing the amount of HIV in blood. While this medication is used for HIV, it still can cause serious or life threatening lactic acidosis meaning that a build-up of acid in the blood that needs to be treated in a hospital. This is seen more commonly in women, or if a woman is pregnant the risk of taking this medication is higher. Interactions with other HIV treatments require dosage modifications but are relatively rate with NRTIs. On the flip side, concomitant use of either didanosine or lamivudine with stavudine can exacerbate mitochondrial toxicities or otherwise result in diminished antiviral activity. Combinations of stavudine and zidovudine have shown to be antagonistic in previous clinical studies.

6 Toxicopharmacological Aspects

Stavudine, which is a thymidine nucleoside analog, in which vitro exhibits an antiretroviral activity against both HIV-1 and HIV-2 (Table 2.3). Stavudine triphosphate exerts antiviral activity when stavudine is phosphorylated by cellular kinases. HIV replication that is inhibited by stavudine triphosphate takes place by the two following mechanisms:

1. Inhibition of HIV reverse transcriptase by competing with the natural substrate, thymidine triphosphate.
2. It is inhibited by viral DNA synthesis that causes DNA chain termination. In addition, stavudine triphosphate may inhibit cellular DNA polymerases, particularly mitochondrial DNA polymerase γ.

Table 2.3 Aspects of stavudine

Stavudine applications	Stavudine is an oral medication, used for the treatment of infections with HIV. It belongs to the class of drugs called reverse transcriptase inhibitors
Dosage of stavudine	Recommended dose for adults is 40 mg every 12 hours for those weighing 60 kg
	Newborns up to 13 years of age should take 0.5 mg/kg every 12 hours
Side effects of stavudine	A decrease in blood cells
	Muscle pain (myopathy)
	Pancreatitis
	Liver failure
	Metabolic disturbance (lactic acidosis)
	Also damages nerves and can cause a severe peripheral neuropathy
Generic name for stavudine	Zerit

As far as toxicology is concerned, most doses are given orally. A single oral dose of stavudine that is up to approximately 500 times the recommended human dose did not show severe toxicities in animal studies. Rather, there are some long-term effects of stavudine administration which includes decreased red blood cell count, sometimes accompanied by decreased hemoglobin and hematocrit and hepatic alterations (liver enlargement with centrilobular hepatocellular hypertrophy), are clearly indicated in these studies. Although there may be liver enlargement seen in the studies, stavudine does not have any inducing effect on cytochrome P450, and thus the increase in liver weight is likely due to induction of some other protein or enzyme system. Carcinogenicity studies conducted over 24 months that were performed in mice and rats. On the basis of the results from both studies that were conducted, the liver was identified as the main target organ for the development of neoplasm lesions.

7 Conclusion

The use of nanoparticles in applications of drug delivery has become widespread because of various types of surface modifications. As seen in malignant melanoma therapy, surface-modified PTX-loaded NPs are capable of giving off less side effects and a higher therapeutic efficiency. The same can be said of NPs modified with stavudine or bromelain as well. These surface modifications lower the toxicity of the NP, increase the permeability of a membrane to drugs, and increase biodegradability. Moreover, drug delivery methods are looking more promising as we develop stronger methods to increase nanotechnology and its uses in the world of medicine.

References

1. Chattopadhyay, S., Dash, S. K., Ghosh, T., Das, D., Pramanik, P., & Roy, S. (2013). Surface modification of cobalt oxide nanoparticles using phosphonomethyl iminodiacetic acid followed by folic acid: A biocompatible vehicle for targeted anticancer drug delivery. *Cancer Nanotechnology, 4*, 103–116.
2. Parodi, A., Haddix, S. G., Taghipour, N., Scaria, S., Taraballi, F., Cevenini, A., Yazdi, I. K., Corbo, C., Palomba, R., Khaled, S. Z., et al. (2014). Bromelain surface modification increases the diffusion of silica nanoparticles in the tumor extracellular matrix. *ACS Nano, 8*, 9874–9883.
3. Xiong, W., Peng, L., Chen, H., & Li, Q. (2015). Surface modification of mpeg-b-pcl-based nanoparticles via oxidative self-polymerization of dopamine for malignant melanoma therapy. *International Journal of Nanomedicine, 10*, 2985–2996.
4. Bhatt, P., Lalani, R., Vhora, I., Patil, S., Amrutiya, J., Misra, A., & Mashru, R. (2018). Liposomes encapsulating native and cyclodextrin enclosed paclitaxel: Enhanced loading efficiency and its pharmacokinetic evaluation. *International Journal of Pharmaceutics, 536*, 95–107.

5. Hemal Tandel, P. B., Jain, K., Shahiwala, A., & Misra, A. (2019). Vitro and in-vivo tools in emerging drug delivery scenario: Challenges and updates. In A. E. Misra & A. Shahiwala (Eds.), *In-vitro and in-vivo tools in drug delivery research for optimum clinical outcomes*. Boca Raton: CRC.
6. Shegokar, R., & Singh, K. K. (2012). Preparation, characterization and cell based delivery of stavudine surface modified lipid nanoparticles. *Journal of Nanomedicine and Biotherapeutic Discovery, 2*, 105. https://doi.org/10.4172/2155-983X.1000105.
7. Patel, J., Amrutiya, J., Bhatt, P., Javia, A., Jain, M., & Misra, A. (2018). Targeted delivery of monoclonal antibody conjugated docetaxel loaded plga nanoparticles into egfr overexpressed lung tumour cells. *Journal of Microencapsulation, 35*, 204–217.
8. Yewale, C., Baradia, D., Patil, S., Bhatt, P., Amrutiya, J., Gandhi, R., Kore, G., & Misra, A. (2018). Docetaxel loaded immunonanoparticles delivery in EGFR overexpressed breast carcinoma cells. *Journal of Drug Delivery Science and Technology, 45*, 334–345.
9. Bhatt, P., Lalani, R., Mashru, R., & Misra, A. (2016). Abstract 2065: Anti-FSHR antibody fab' fragment conjugated immunoliposomes loaded with cyclodextrin-paclitaxel complex for improved *in vitro* efficacy on ovarian cancer cells. *Cancer Research, 76*, 2065.
10. Bhatt, P., Vhora, I., Patil, S., Amrutiya, J., Bhattacharya, C., Misra, A., & Mashru, R. (2016). Role of antibodies in diagnosis and treatment of ovarian cancer: Basic approach and clinical status. *Journal of Controlled Release: Official Journal of the Controlled Release Society, 226*, 148–167.
11. Ahmad, I. Z., Kuddus, M., Tabassum, H., Ahmad, A., & Mabood, A. (2017). Advancements in applications of surface modified nanomaterials for cancer theranostics. *Current Drug Metabolism, 18*, 983–999.
12. Knop, K., Stumpf, S., & Schubert, U. S. (2013). Drugs as matrix to detect their own drug delivery system of peg-b-pcl block copolymers in matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Rapid Communications in Mass Spectrometry: RCM, 27*, 2201–2212.
13. Morcos, B., Lecante, P., Morel, R., Haumesser, P.-H., & Santini, C. C. (2018). Magnetic, structural, and chemical properties of cobalt nanoparticles synthesized in ionic liquids. *Langmuir, 34*, 7086–7095.
14. Chattopadhyay, S., Chakraborty, S. P., Laha, D., Baral, R., Pramanik, P., & Roy, S. (2012). Surface-modified cobalt oxide nanoparticles: New opportunities for anti-cancer drug development. *Cancer Nanotechnology, 3*, 13–23.
15. Salazar, M. D., & Ratnam, M. (2007). The folate receptor: What does it promise in tissue-targeted therapeutics? *Cancer Metastasis Reviews, 26*, 141–152.
16. Antony, A. C. (1996). Folate receptors. *Annual Review of Nutrition, 16*, 501–521.
17. Chobotova, K., Vernallis, A. B., & Majid, F. A. (2010). Bromelain's activity and potential as an anti-cancer agent: Current evidence and perspectives. *Cancer Letters, 290*, 148–156.
18. Pavan, R., Jain, S., Shraddha, & Kumar, A. (2012). Properties and therapeutic application of bromelain: A review. *Biotechnology Research International, 2012*, 6.
19. Rathnavelu, V., Alitheen, N. B., Sohila, S., Kanagesan, S., & Ramesh, R. (2016). Potential role of bromelain in clinical and therapeutic applications. *Biomedical Reports, 5*, 283–288.
20. Mynott, T. L., Ladhams, A., Scarmato, P., & Engwerda, C. R. B. (1999). From pineapple stems, proteolytically blocks activation of extracellular regulated kinase-2 in t cells. *Journal of Immunology, 163*, 2568–2575.
21. Orsini, R. A. (2006). Bromelain. *Plastic and Reconstructive Surgery, 118*, 1640–1644.
22. Barbé, C., Bartlett, J., Kong, L., Finnie, K., Lin, H. Q., Larkin, M., Calleja, S., Bush, A., & Calleja, G. (1959-1966). Silica particles: A novel drug-delivery system. *Advanced Materials, 2004*, 16.
23. Ma, B., He, L., You, Y., Mo, J., & Chen, T. (2018). Controlled synthesis and size effects of multifunctional mesoporous silica nanosystem for precise cancer therapy. *Drug Delivery, 25*, 293–306.

24. Kanapathipillai, M., Brock, A., & Ingber, D. E. (2014). Nanoparticle targeting of anti-cancer drugs that alter intracellular signaling or influence the tumor microenvironment. *Advanced Drug Delivery Reviews, 79-80*, 107–118.
25. Villegas, M. R., Baeza, A., & Vallet-Regí, M. (2015). Hybrid collagenase nanocapsules for enhanced nanocarrier penetration in tumoral tissues. *ACS Applied Materials and Interfaces, 7*, 24075–24081.
26. Vhora, I., Patil, S., Bhatt, P., Gandhi, R., Baradia, D., & Misra, A. (2014). Receptor-targeted drug delivery: Current perspective and challenges. *Therapeutic Delivery, 5*, 1007–1024.
27. Zhang, W., He, J., Liu, Z., Ni, P., & Zhu, X. (2010). Biocompatible and ph-responsive triblock copolymer mpeg-b-pcl-b-pdmaema: Synthesis, self-assembly, and application. *Journal of Polymer Science Part A: Polymer Chemistry, 48*, 1079–1091.
28. Li, F., Meng, J., Ye, J., Yang, B., Tian, Q., & Deng, C. (2014). Surface modification of pes ultrafiltration membrane by polydopamine coating and poly(ethylene glycol) grafting: Morphology, stability, and anti-fouling. *Desalination, 344*, 422–430.
29. Yang, K., Lee, J. S., Kim, J., Lee, Y. B., Shin, H., Um, S. H., Kim, J. B., Park, K. I., Lee, H., & Cho, S. W. (2012). Polydopamine-mediated surface modification of scaffold materials for human neural stem cell engineering. *Biomaterials, 33*, 6952–6964.
30. Park, J., Brust, T. F., Lee, H. J., Lee, S. C., Watts, V. J., & Yeo, Y. (2014). Polydopamine-based simple and versatile surface modification of polymeric nano drug carriers. *ACS Nano, 8*, 3347–3356.
31. Postma, A., Yan, Y., Wang, Y., Zelikin, A. N., Tjipto, E., & Caruso, F. (2009). Self-polymerization of dopamine as a versatile and robust technique to prepare polymer capsules. *Chemistry of Materials, 21*, 3042–3044.
32. Cho, K., Wang, X., Nie, S., Chen, Z. G., & Shin, D. M. (2008). Therapeutic nanoparticles for drug delivery in cancer. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research, 14*, 1310–1316.
33. Heiati, H., Tawashi, R., Shivers, R. R., & Phillips, N. C. (1997). Solid lipid nanoparticles as drug carriers. I. Incorporation and retention of the lipophilic prodrug 3′-azido-3′-deoxythymidine palmitate. *International Journal of Pharmaceutics, 146*, 123–131.
34. Gunaseelan, S., Gunaseelan, K., Deshmukh, M., Zhang, X., & Sinko, P. J. (2010). Surface modifications of nanocarriers for effective intracellular delivery of anti-HIV drugs. *Advanced Drug Delivery Reviews, 62*, 518–531.
35. Sharma, A., Sharma, S., & Khuller, G. K. (2004). Lectin-functionalized poly (lactide-co-glycolide) nanoparticles as oral/aerosolized antitubercular drug carriers for treatment of tuberculosis. *The Journal of Antimicrobial Chemotherapy, 54*, 761–766.

Chapter 3
Surface-Modified PLGA Nanoparticles for Targeted Drug Delivery to Neurons

Tejal A. Mehta, Neha Shah, Khushali Parekh, Namdev Dhas, and Jayvadan K. Patel

Abstract The major challenge for the treatment of neuronal diseases is the inability of therapeutic moieties to cross the blood–brain barrier (BBB) and nasal mucosal barrier. The therapeutic moieties for the treatment of brain diseases lack targeting due to its non-specificity toward receptors located at BBB and Pgp efflux mechanism. This results in impeding its ability to reach to maximum effective concentration. Many of these therapeutic moieties possess dose-limiting systemic side effects which along with complex dosage regimens hinder patient compliance and result in discontinuation of treatment. A number of drug delivery and drug-targeting systems have been investigated to increase drug bioavailability and the fraction of the drug accumulated in the targeted area, in order to minimize drug degradation and loss, as well as to reduce harmful side effects. Among all, PLGA NPs have been achieved fascinating properties as a carrier system owing to its biodegradable, biocompatible, and easy functionalization properties. Most importantly PLGA is available in various ratios which can be helpful for tuning the entrapment/loading of therapeutic moieties in NPs. Intranasal delivery has come to the forefront as a method that can bypass the BBB and target drugs directly to the brain as an alternative to invasive methods. The objective of this chapter is to provide a broad overview on current strategies for brain drug delivery and its applications. It is hoped that this chapter could inspire readers to discover possible approaches to deliver drugs into the brain. After an initial overview of the BBB and intranasal route in both healthy and pathological conditions, this chapter revisits, according to recent publications, some questions that are controversial, such as whether nanoparticles by themselves could cross the BBB and whether drugs are specifically transferred to the brain by actively targeted nanoparticles. Furthermore, in this chapter, various conjugation strategies for attaching targeting moieties to the surface of nanocarrier have been included. Current non-nanoparticle strategies are also

T. A. Mehta (✉) · N. Shah · K. Parekh · N. Dhas
Department of Pharmaceutics, Institute of Pharmacy, Nirma University, Ahmedabad, Gujarat, India

J. K. Patel
Nootan Pharmacy College, Faculty of Pharmacy, Sankalchand Patel University, Visnagar, Gujarat, India

Y. V Pathak (ed.), *Surface Modification of Nanoparticles for Targeted Drug Delivery*, https://doi.org/10.1007/978-3-030-06115-9_3

reviewed, such as delivery of drugs through the permeable BBB under pathological conditions and using noninvasive techniques to enhance brain drug uptake.

Keywords Surface-modified nanoparticles · Neurons · CNS diseases · Nasal drug delivery · Targeting strategies

1 Introduction

Despite the tremendous advancement in the field of medicine and pharmacology, there is rise in the prevalence of several central nervous system (CNS) diseases. In the recent years, the treatments available for the CNS diseases are symptomatic and are usually unable to generate quality of life and alter or repair damage. Recently, a vast amount of research took place in the development of drugs for CNS diseases. The progress in the various brain disease treatments have developed new avenues in the form of targeted delivery of the prepared formulation for the specific delivery to the neurons. The average time utilized to develop an innovative therapeutic moiety ranges from 12 to 16 years.

1.1 Neuronal Diseases

The brain, spinal cord, and nerves make up the nervous system. Together they control all the workings of the body. When something goes wrong with a part of this system, symptoms are observed like trouble moving, speaking, swallowing, breathing, or learning. There are more than 600 neurologic diseases. Major types include [1] the following.

Etiology	Examples of disease
Diseases caused by faulty genes	Huntington's disease, muscular dystrophy
Faulty development of nervous system	Spina bifida
Neuro degenerative diseases (nerve cells are damaged or die)	Parkinson's disease, Alzheimer's disease
Diseases of the blood vessels that supply the brain	Stroke
Seizure disorders	Epilepsy
Cancer	Brain tumors
Infections	Meningitis

Neurological disorders affect millions of people worldwide. Statistics says that there are more than six million people who die due to brain stroke each year; approx. 80% of these deaths take place in low- and middle-income countries. It is estimated that >50 million people have epilepsy worldwide. Globally, there are more than 47.5 million people with dementia. With 7.7 million new cases every year, Alzheimer's

disease stands on the top among the most common causes of dementia and may contribute to 60–70% of cases [2].

1.2 Introduction to Various Neuronal Diseases

1.2.1 Alzheimer's Disease (AD)

AD is one of the most common forms of dementia, with more than 24 million cases worldwide [3]. The main characteristics of this disease are memory loss, decreased cognitive functions, and thereby physical functions also. It eventually leads to the death of patient due to loss of neuronal cells. The disease can manifest itself in two forms: (1) familial AD, which is caused by genetic causes usually, and (2) sporadic AD which is caused by environmental factors. The major areas of the brain affected by AD are the neocortex, which is principally involved in the processing of sensory information, and limbic system, which plays a key role in the control of emotions, instinctive behavior, learning, and short-term memory (Fig. 3.1).

The basis of disease is still unclear, but its main assurance is accumulation of beta-amyloid (Aβ peptide) plaque [4]. Over the years, studies on various animal models have provided valuable information in understating the pathogenic mechanism of AD. The pathology of AD is better explained by dividing it into three major segments, viz., positive lesions (lesions related to accumulation), negative lesions (due to losses), and inflammatory lesions (the ones due to the reactive processes). Other important histopathological characteristic of AD covers neurofibrillary tangles (NFTs). NFTs are found inside neurons and are made up of paired helical filaments of hyperphosphorylated microtubule-associated protein tau (MAPT). Intracellular accumulation of NFTs may cause dysfunction of the normal cytoskeletal structure of neurons with consequent death. Distribution of plaques (Aβ) and neurofibrillary tangles (NFTs) is not even across the brain during AD but are confined to disposed neural systems [4].

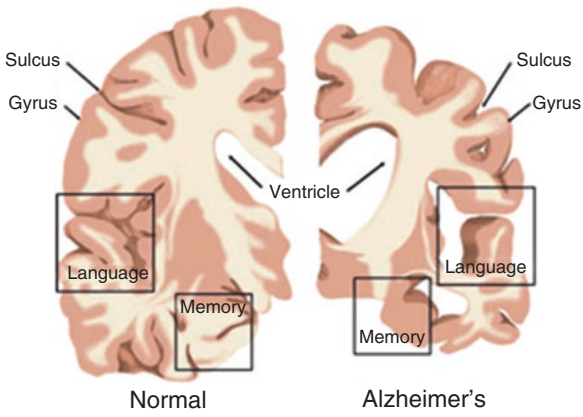


Fig. 3.1 Structure of brain in normal and Alzheimer's disease condition (Source: mybrainx.com)

The roles of important protein and its precursor peptide, amyloid-β protein precursor, have been widely investigated because of the presence of beta-amyloid in the senile plaques [5]. Currently, all the treatments for AD are only able to improve symptoms but cannot lead to the regression of the disease or to its complete cure. Many new treatment regimens for AD have been proposed, but the development of specific drug delivery system which can target the brain and also inhibit the neurodegeneration is still required.

1.2.2 Parkinson's Disease (PD)

PD is one of the neurodegenerative diseases of CNS which weakens motor skills, cognitive processes, and other functions too. Clinical symptoms of PD include bradykinesia, hypokinesia, and rigidity kind of motor and non-motor symptoms. These symptoms are known as Parkinsonism, which is essential for clinical diagnosis in PD. Various studies reflect that major PD symptoms are due to nigral dopamine deficiency-related dysfunction of the basal ganglia [6]. The exact cause of the disease is unknown. Great reduction in the activity of dopamine-secreting cells in pars-compacta region of substantia nigra (Fig. 3.2) is observed which is responsible for the symptoms of PD [7].

Pathologically, the disease is characterized by the accumulation of alpha-synuclein protein forming inclusions called Lewy bodies [8]. Braak's group had proposed "pathogen theory" which is reasonable somewhat for considering the major gastrointestinal (GI) dysfunctions during PD and prevalence of the same in vegetarians [9]. According to this theory, assumption was made that an "unknown

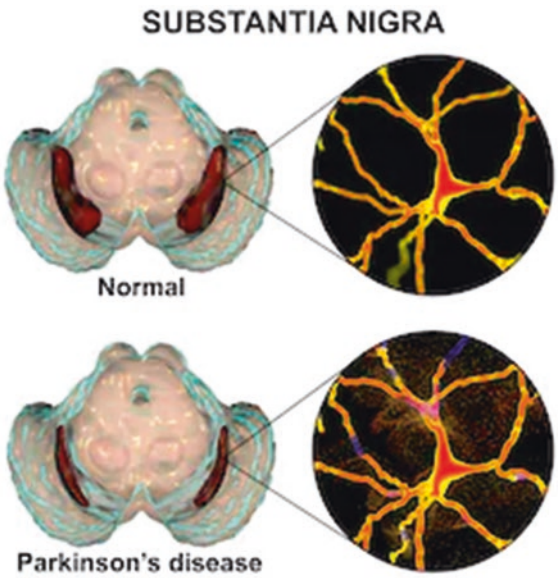


Fig. 3.2 Substantia nigra during normal and Parkinson's disease condition (Source: shutterstock.com—1049278997)

pathogen" pierces the gut wall and enters the central nervous system (CNS) by retrograde transport through the vagus nerve to cause PD [10]. Use of levodopa (L-DOPA) has made current treatments effective for management of motor symptoms. Dopamine agonists and monoamine oxidase B (MAO-B) inhibitors are also useful as dopamine cannot cross blood–brain barrier (BBB) [11]. Drug delivery to the brain remains limited because of the blood–brain barrier (BBB). For example, out of the total L-DOPA administered, only 5–10% is able to cross the blood–brain barrier. Various enzymes metabolize the remaining dopamine, causing side effects, such as nausea, dyskinesias, and stiffness [12].

1.2.3 Brain Cancer/Brain Tumors

Brain tumors can be divided into two major groups: (a) primary brain tumors, which originated from brain tissues, and (b) secondary brain tumors, which result from the metastatic spreading of cancer cells and originated in other regions. Examples of primary brain tumors are gliomas, central nervous system lymphomas (both of which originate from brain parenchyma), meningiomas, and pituitary adenomas (extraparenchymal tumors) [13]. Gliomas can originate from neural stem cells, progenitor cells, or dedifferentiated mature neural cells transformed into cancer stem cells. This classification is based on both their origins in astrocytomas, oligodendroglioma, and mixed oligoastrocytoma. It is also based on their aggressiveness in grades ranging from 2 to 4.

Currently, the use of surgical approaches and/or radiotherapy is most common for brain tumors that not only present a large amount of risk for the patient but are also unable to competently treat more hostile form of gliomas [14]. Such pharmacological treatments should be developed which are able to target cancer cells and destroy brain tumor stem cells as well, in order to prevent a repopulation effect. This will help in preventing all the processes involved in tumor metastatic spreading, including angiogenesis and cell extravasation (Fig. 3.3).

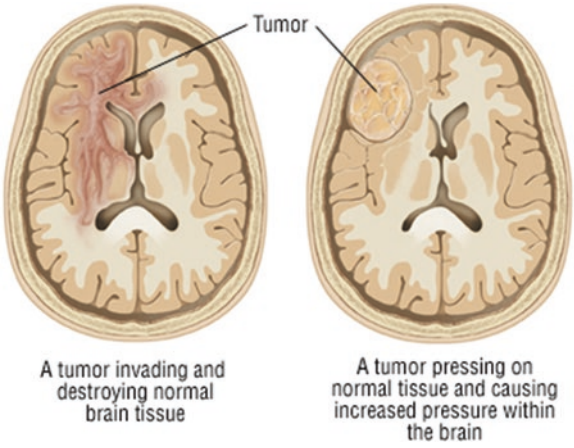


Fig. 3.3 Various types of brain tumors (Source: drugs.com)

Accomplishing successful treatment for CNS diseases remains a vast avenue for research. Till date, a variety of clinical medications for CNS diseases give just constrained change in results and are regularly joined by extreme symptoms. For example, patients determined to have glioblastoma just have a middle survival time of 14 months following surgical resection, radiation, or associative chemotherapy. The trouble in accomplishing enhanced result for CNS disorders originates from the failure to convey therapeutically relevant doses of the therapeutic to diseased cells or regions. The blood–brain barrier (BBB) and nasal mucosa act like the main impediment that keeps most deliberately administrated drugs from entering the CNS. Therefore, before starting with various approaches for targeting drugs to CNS, we need to discuss various obstacles which may hinder the drug to reach CNS to specific sites.

1.3 Blood–Brain Barrier (BBB)

The term "histohematic barrier" was first introduced by LS Stern in 1929. The term "blood–brain barrier" ("Blut-Hirn-Schranke") was coined by Lewandowsky in 1900 [15]. BBB is the subtle network of blood vessels having tightly packed endothelial cells which separates the brain from the circulatory system. BBB is also pericytes, vascular smooth muscle cells (VSMC), neurons, astrocytes, and perivascular macrophages [16]. It is an extremely selective obstacle between blood flow and CNS which plays a crucial role in the homeostasis of the brain by regulating the way of various substances [17]. By virtue of its ability to exclude the passage of certain compounds, the BBB defines compounds as either centrally or peripherally acting [18]. This barrier has less affinity for hydrophilic substances, charged molecules, proteins, and peptides; hence, they are unable to cross the barrier, whereas lipophilic drugs like antidepressants, anxiolytics, and many hormones can cross the barrier with ease.

Besides the brain capillaries, peripheral capillaries also exist. The main difference is the lack of fenestration and intercellular pores, due to the presence of interendothelial tight and adherens junctions, limiting the transport via paracellular pathways [16]. The physical barrier is because of the presence of adherens junctions (AJs) and tight junctions (TJs) between neighboring endothelial cells. TJs comprise of transmembrane proteins located within the paracellular space, preventing paracellular transport by virtually filling the space. Cytoplasmic proteins (zonula occludens [ZO] 1, 2, and 3 and cingulin) are also present which are bound to the actin cytoskeleton [19, 20].

Molecules like proteins, enzymes, and nutrients can travel in and out of the CNS through various pathways. Patients suffering from neurological disorders required prolonged dosing, leading to side effects in nontargeted organs. Majority of drugs which are useful in the treatment of neurological disorders cannot cross the BBB, and hence their potential is reduced as effective therapeutic agent. This left the society with limited treatment options for the patients. Therefore, the development of

noninvasive transport of drug to the brain is highly needed for neurological disorders and brain tumors requiring chronic therapy.

The rate-limiting factors of BBB include the presence of reticuloendothelial system across the BBB which causes protein opsonization. Several approaches have been engaged to improve the drug delivery across the BBB. Nanoparticles (NPs), being of the range from 1 to 1000 nm, are the solid colloidal particles utilized as carrier for drug delivery. Currently NPs are found to have a tremendous advantage over the other methods available for drug delivery across the BBB. Because of its size and functionalization characteristics, nanoparticles are able to penetrate and facilitate the drug delivery through the barrier. Nanomaterials in combination with therapeutic agent can be modified using various mechanisms and strategies for targeted use. Examples of delivery systems are liposomes, polymeric nanoparticles, non-viral vectors, etc. [21]. Nanomaterials are efficacious enough to improve the safety and efficacy of drug delivery devices in brain targeting. Nano-engineered devices are found to be delivering the drugs at cellular levels through nano-fluidic canals. Use of nanotechnology may reduce the need of invasive procedures for delivery of therapeutics to the CNS.

1.4 Nasal Drug Delivery to Brain

Nasal route is an alternative route for those drugs which are difficult to administer orally. This route is being explored by various researchers and proven promising for brain targeting. This route has gained potential attraction for delivering the neurotherapeutics by evading the blood circulation through IN route. Delivering drug by IN route reduces the systemic exposure and hepatic/renal clearance [22, 23]. Olfactory and trigeminal nerves are involved in this pathway, and it has achieved an importance as it can deliver a large range of therapeutic agents [23, 24].

The probable mechanism by which drugs are transported from the nose to the brain is not yet clear, but olfactory pathway plays a vital role. This pathway consists of olfactory epithelium, lamina propria, and olfactory bulb. Entry of drugs to caudal and rostral parts of the brain is promoted in IN delivery as trigeminal nerve enters to the brainstem through pons by innervating the nasal cavity, whereas it enters to the forebrain through the cribriform plate. Drug is delivered to the rostral area of the brain by olfactory pathway where in case of trigeminal pathway, rostral but caudal, both areas of the brain are targeted. Hence, it is difficult to differentiate that intranasally administered drug is translocated to rostral area by olfactory or trigeminal pathway.

Olfactory pathway is a reliable alternative to achieve desired therapeutic effects at lower doses for treating chronic diseases while minimizing the side effects. Direct IN drug transportation to the brain is referred by transmucosal delivery of drug via olfactory or trigeminal pathway. The only route by which the brain is connected with the outside environment is intranasal route. This neural connection has gained consideration for delivery of wide variety of drug molecules by the nanoformulations. Such formulation systems for nucleotides, peptides, and proteins can be

developed to deliver them to the brain by preventing enzymatic degradation and augmenting the pharmacological properties without systemic absorption and toxicity. It was investigated in animal and human studies that different nose to brain drug delivery systems resulted successful by enhancing the nasal permeability, improving adhesion to mucous membrane, providing continuous or measured release of drug, or increasing deposition at olfactory epithelium.

Nanotechnology is a more promising drug delivery system among the advanced technologies of targeting and controlled release systems [25].

There are various strategies to target the brain which includes invasive as well as noninvasive techniques.

2 Nanoparticles as Drug Delivery Vehicles

Merits of nanoparticles as drug delivery system are controlled release of drug, reduced toxicity, improved bioavailability, and enhanced therapeutic efficacy and bio-distribution [26]. Structure of nanoparticles enables them to protect the drugs from environmental degradation due to factors like stomach acid and enzymes [27]. Size range for polymeric nanoparticles is about 10–1000 nm [28] and can be modified with different ligands such as antibodies to create a smart targeting delivery system. To cross the BBB, surfactant-coated polymeric nanoparticles of drug should be of $\leq$300 nm [29].

Food and Drug Administration (FDA)-approved therapeutic devices use poly(lactic-co-glycolic acid) (PLGA). Random ring-opening and copolymerization of two different monomers form PLGA polymer. It is a biodegradable polymer, and its hydrolysis of PLGA produces original monomers, lactic acid and glycolic acid, in the body.

PLGA shows controlled degradation by the altering the ratio of the two monomers: the lower the content of glycolide units, the more time is required. 50:50 monomer ratio in the copolymer exhibits the fastest degradation. Minimal toxicity and ability of controlling the drug release make PLGA the most suitable for drug delivery [30]. Biocompatibility for targeting drug at cellular level shows versatility of PLGA nanoparticles among various nanoparticulate systems [31]. The degradation of polymer matrix of PLGA nanoparticles releases encapsulated L-DOPA. Hence, undesirable effects observed with the conventional oral administration can be reduced [32]. Despite having various advantages of PLGA as a drug carrier, it has been found that nontargeted PLGA NPs could not possibly target to specific site in the brain as compared to targeted PLGA NPs. Taking into consideration aforementioned statement, there are various targeting ligands and various approaches of targeting to specific receptors which get overexpressed in a specific disease. In the following section, we will discuss various targeting strategies and different types of ligands which help to target specific receptors to show significant therapeutic response. Furthermore, we have also discussed various types of surface modification/conjugation strategies of ligands to PLGA NPs.

3 Dual-/Multi-targeting Strategies

Intranasal mucosa and BBB are still a barrier in the treatment of brain diseases. The vast research has been carried out for the development of targeting delivery system for brain diseases (Fig. 3.4). Nevertheless, the developed formulations were not capable enough for the treatment of diseases of the brain due to several reasons: (1) brain diseases usually occur in a particular area of the brain; thus, the conveyance of the therapeutic moiety into the brain is a critical matter when it penetrates the BBB and nasal mucosa and enters the brain. The poor targeting efficiency to the particular diseased area leads to low concentration of drug in the particular diseased area [33, 34]. (2) The dissemination of therapeutic moiety in healthy neuronal cells, which may eventually lead to neurotoxicity [35]. To reduce this difficulty, dual-/multi-targeting nanocarrier system strategies were proposed. Initially, dual-/multi-targeting strategy was employed to overcome two or more barriers present in the brain which may retard the delivery system to reach the specific diseased area. It is generalized that dual-targeting nanocarrier systems should be able to help to pass via nasal mucosa and/or BBB and then precisely bind to cells of the diseased brain. However, certain nanocarriers were also termed as "dual-targeting system" when nanocarriers were formulated for targeting two different locations (i.e., two distinct cells namely neo-vasculature and tumor cells) and in another case targeting the same cell type but at different receptors (i.e., transferrin and folate receptor on cancer cells) [35, 36]. Depending on the target site, one can particularly classify nanocarriers into various applications:

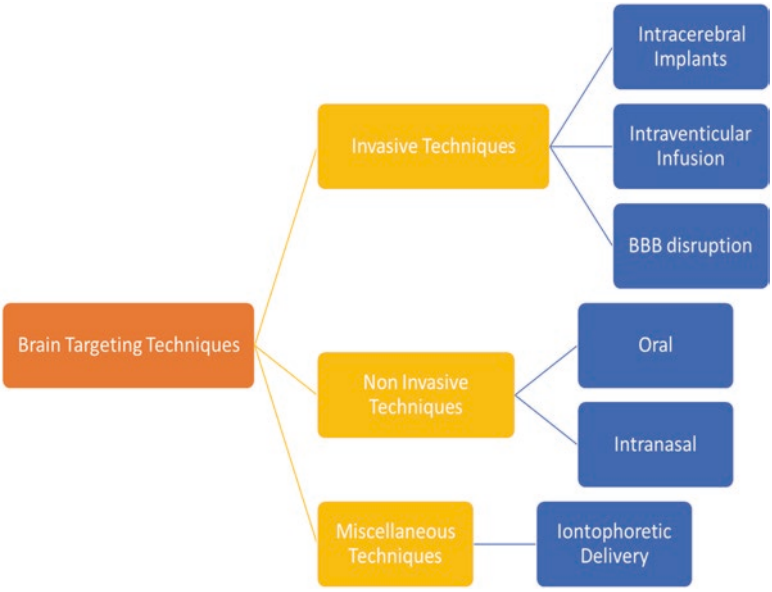


Fig. 3.4 Various approaches to target neurons

1. Targeting nasal mucosa/BBB and diseased cells utilizing unlike targeting moieties: as stated above, the earlier perception of dual-targeting delivery was passing nanocarrier via nasal mucosa/BBB and then specifically targeting to the diseased cells. To overcome this constraint, two targeting moieties were often used for constructing targeting delivery systems, one for penetration of the nasal mucosa/BBB and the other for targeting diseased cells. In the above context, Yin et al. developed sialic acid-modified selenium NPs coated with B6 peptide (B6-SA-SeNPs). Peptide B6 is known for its BBB permeability, and sialic acid has showed significance in the development of cognition and enhancement in learning and memory performance, whereas selenium NPs show synergistic action in AD in the form of antioxidants. In vitro BBB model (bEnd.3 cells) study demonstrated that B6-SA-SeNPs were efficiently crossing the BBB and were getting absorbed by PC12 cells. Furthermore, B6-SA-SeNPs not only inhibited Aβ aggregation but also disaggregated preformed Aβ fibrils into nontoxic amorphous oligomers [37]. In a similar manner, Zhang et al. developed TGN/QSH dual-conjugated H102-loaded PEG-PLA NPs (TGN/QSH-H102-PEG-PLA NPs) as a dual-targeting platform in the treatment of AD. Two targeting peptides, QSH and TGN, were conjugated to the surface of NPs for Aβ targeting and BBB transport, respectively. The maximum concentration of H102 was obtained in the hippocampi of the TGN/QSH-H102-PEG-PLA group mice 1 h after administration, which were 1.86 and 2.62 times the level of TGN-H102-PEG-PLA NPs and non-modified H102-PEG-PLA NPs, respectively [38].
2. Targeting two cell types in the diseased cells: as there can be many targeting sites for specific diseased neuronal cells, which may be responsible for pathological conditions, for example, AD has various targeting sites such as Aβ plaques, mitochondrial targeting, and tau aggregates. This indicates a researcher can target Aβ which can avoid Aβ agglomeration or disaggregate the form; Aβ plaques and other targeting moieties can be attached to target mitochondria which will help in reducing oxidative stress. As a result, the synergistic action may ameliorate the cognitive deficit. Furthermore, in case of brain tumor, there can be various sites for targeting such as blood vessels (angiogenesis) which are present in tumor and receptors present on tumor cells. In this context, targeting moiety which reduces the growth of blood vessel (anti-angiogenesis) treatment can be simultaneously be delivered with another targeting moiety which targets tumor cells which reduces tumor growth [39]. To address the abovementioned requirement, Gao et al. developed dual-functional interleukin-13 peptide (IL-13)/RGD-conjugated PEG-PCL NPs. The selection of the targeting was done with the aim of dual targeting to brain tumor. In this context, RGD would target avβ3 on neovasculature, and IL-13 would target IL13Rα2 on glioblastoma cells. The receptor labeling study demonstrated that IL13Rα2 could mediate the internalization of IL-13-modified NPs, and avβ3 could mediate the internalization of RGD-modified NPs. Furthermore, in vivo study displayed that IL-13/RGD-PEG-PLA NPs also showed high penetration ability than monomodified NPs. Additionally, CD-31 staining study demonstrated that IL-13/RGD-PEG-PLA NPs could target both neovasculature and glioblastoma cells [40].

3. Dual targeting with one ligand: there are some ligands that could directly target both nasal mucosa/BBB and diseased neuronal cells due to their receptors or transporters that are overexpressed on both nasal mucosa/BBB and diseased neuronal cell. In the above context, Ruan et al. developed DOX-loaded antigiopep-2-conjugated gold NPs as a dual-targeting nanoplatform in the treatment of brain disease. Angiopep-2 is the member of peptide family named angiopep, which are derived from the Kunitz domain of aprotinin. Additionally, angiopep-2 has high LRP binding affinity; thus, it was candidate for the dual-targeting ligand for NPs to penetrate the BBB and target the brain tumor. It was found that the release of DOX was pH sensitive owing to the hydrazine bonding between the gold NPs and DOX. Furthermore the release study demonstrated that at pH 7.4, the 48 h cumulative release was found to be 21.9%, while at pH 5 it was elevated to 88.3%. Above study revealed that the combination of therapeutic activity and dual-targeting property of angiopep-2 peptide can efficiently enhance the survival time of brain tumor-bearing mice from 19 days to 55 days [41].

3.1 Lectins

Lectins are sugar-binding proteins that are exceptionally particular for attachment to the sugar moiety. Lectins are found all through nature, in plants, i.e., seeds, and different nourishments, for example, dairy substance, and the human body [42–44]. On the cell surface of tissues, starches going about as basic parts of the human body are broadly appropriated. They are engaged with different organic procedures, for example, cell bond, irritation, and cell actuation [42, 43]. Different kinds of lectins are being bound by specific sugar moieties present of these carbohydrates. Lectins are robust proteins; they are resistant to gastric acid and digestive enzymes. They have ability to bind to stomach wall, change permeability, damage the epithelial cells, pass through the gut, and enter the blood with other proteins, which may lead to certain adverse immunological responses [45]. Taking into consideration the above statement, lectins originated from plants and animals may cause several adverse reactions to the human body. Lectins have particular targeting capability which marks them as safe and appropriate to be utilized as a prodrug agent. Recently, researchers are utilizing lectin as a targeting moiety to be targeted to specific region of disease, for example, neurological diseases. Furthermore, lectins can be conjugated to the therapeutic moiety, or it can be conjugated to nanocarrier in the form of surface functionalization. So, considering the targeting approach to the brain, lectins can overcome the BBB as well as intranasal route barrier and subsequently increase the efficiency of nanocarrier platform loaded with therapeutic moiety [43]. Wen et al. developed odorranalectin (OL)-conjugated PLGA-PEG NPs to enhance the intranasal delivery of the therapeutic moiety in the treatment of CNS diseases [46]. Recently various lectins have been approved in pharmaceutical world which includes wheat germ agglutinin (WGA) and peanut agglutinin. Among them WGA has been widely utilized in research, more specifically as a targeting moiety for nose

to brain delivery. WGA is obtained from *Triticum vulgaris*, and which has particular affinity toward sialic acid and *N*-Acetyl-glucosamine (GlcNAc). Most of the study concluded that WGA can be safely used for intranasal delivery as it does not produce any toxicity to the normal cells.

3.2 Cell-Penetrating Peptides (CPPs)

As of late, cell-penetrating peptides (CPPs) have been broadly contemplated regarding their utility in drug delivery. This makes it difficult to have a general definition covering the attributes of the distinctive CPPs found. Up until now, one can state that CPPs are short peptides (generally not surpassing 30 amino acids) that have the ability to pervasively cross cell membranes with extremely constrained toxicity, by means of energy-independent (direct membrane translocation) and/or energy-dependent (endocytosis pathways such as clathrin-mediated endocytosis, caveolae/lipid raft-mediated endocytosis and macropinocytosis) mechanisms, without the need of a chiral recognition by particular receptors [47, 48]. Albeit a few unique criteria have been proposed for the classification of CPPs, in view of their sequences, origin, function, or mechanism of uptake, no unified scientific classification of these peptides directly exists. Depending on the origin, CPPs can be categorized as natural and artificial peptides. Additionally, based on the bonding between CPP and cargoes/nanomaterial, CPPs can be classified as non-covalent (may consist of electrostatic interactions/hydrophobic interactions) and covalent (may consist of disulfide or thioester bond). Furthermore, as per their physicochemical properties, they can be simply sorted into three fundamental classes, that is, amphiphilic, hydrophobic, and cationic peptides [49]. Cellular uptake of CPP can be affected by various factors such as concentration of CPP/cargo/nanomaterial-CPP conjugates and size. Additionally CPPs can be decorated over the nanocarriers to achieve target-specific delivery. In this context, vast amount of research has been carried out on CPP as a brain-targeting agent following intranasal route. Additionally, as far as intranasal route is concerned, CPPs, when decorated over the NPs, can efficiently transfer the therapeutic moiety across nasal mucosa followed by brain targeting. They endorse transport of therapeutic moiety via olfactory pathway to the brain [50–52]. Among CPPs, low molecular weight protamine (LMWP), Tat protein, and penetratin are therapeutically important in transmucosal delivery of loaded therapeutic agent. LMWP is considered to be safer as compared to protamine as it is nonantigenic and has low toxicity [53, 54]. The LMWP-CPPs could be used to improve IN transport of therapeutic moiety to CNS. It was considered that conjugates of LMWP-NPs were successfully transported to the brain by noninvasive IN administration. In this context, Xia et al. developed a coumarin-6-loaded LMWP-conjugated PEG-PLA NPs. The in vivo results revealed that intranasally administered LMWP-PEG NPs were detected in the rat olfactory tract, cerebrum, cerebellum, and olfactory bulb and were found to be 2.68-, 2.03-, 2.55-, and 2.82-fold, respectively, as compared to that of PEG-PLA NPs. Biodistribution study also revealed that LMWP-PEG-PLA

NPs after intranasal administration could be delivered to the CNS along both trigeminal nerve and olfactory nerve pathways [55]. Furthermore, Yan et al. developed insulin-loaded Tat-conjugated PLGA NPs to overcome the barriers like nasal mucosa penetration, intracellular transport along the olfactory neurons, and diffusion across the heterogeneous brain compartments. The results demonstrated that Tat-conjugated PLGA NPs can efficiently deliver the insulin to the brain following intranasal route, showing the total brain delivery efficiency of 6% [56]. Additionally, Yang et al. developed rivastigmine-loaded CPP-conjugated liposomes for intranasal administration for brain targeting. It was found that the concentration of rivastigmine loaded with liposomes was higher as compared to free rivastigmine [57]. Kamei et al. hypothesized that CPP could be used to enhance the drug delivery to the brain. For the same, the researcher developed an insulin solution co-administered with penetratin and was intranasally administered to mice. The results demonstrated that insulin co-administered with D- and L-penetratin reached the distal region of the brain from the nasal cavity, including the cerebellum, cerebral cortex, and brainstem. In particular, D-penetratin could intranasally deliver insulin to the brain with a reduced risk of systemic insulin exposure [58]. In addition to intranasal route, CPP can be used as a targeting moiety to cross blood–brain barrier (BBB) and bypass effectively the Pgp in the BBB. For example, they have enhanced doxorubicin transport into the rat brain up to 30-fold. This approach is efficient on the grounds that CPPs use adsorptive-mediated endocytosis to enter the brain. It is triggered by electrostatic interactions between the negatively charged cell membranes of brain endothelial cells and positively charged moieties of the protein. In this context, Lu et al. developed CPP-conjugated BSA NPs to evaluate brain-targeting efficiency. It was found that the permeability of CPP-BSA NPs was 7.76-fold higher than that of BSA NPs [59]. Additionally, Qin et al. developed doxorubicin-loaded Tat-conjugated liposomes for glioma therapy [60].

3.2.1 Glutathione (GSH)

GSH is a natural, hydrophilic, tripeptide molecule which performs antioxidant function against reactive oxygen species and toxic metabolites of the cell [61]. The idea of glutathione-coupled nanoparticles rose up out of the way that the glutathione is the most abundant antioxidant agent existing in the human body; apart from that it additionally acts as an endogenous ligand for glutamate receptors, i.e., *N*-methyl-D-aspartate (NMDA) and 2-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptors which are abundantly expressed in the brain, and consequently it experiences receptor-mediated endocytosis [62]. Additionally, GSH has the ability to interact with transmembrane proteins located in the brain that are involved in the active transport of various materials across BBB [63]. Additionally, glutathione also regulates the permeability of BBB through its action on tight junction proteins (occludin and claudin) of BBB [64, 65]. Among these, Pgp is involved in ATP-coupled reactions in transporting materials conjugated to GSH across membranes. Henceforth, glutathione-coupled nanocarriers establish a promising drug delivery

system for transport of various neurotherapeutics to the brain by surpassing BBB [66]. In this context, Gaillard et al. developed methylprednisolone-loaded GSH-conjugated liposomes for enhanced brain delivery [67]. Results demonstrated that treatment with GSH-PEG liposomes was significantly more effective compared to PEG liposomes. Furthermore, DOX-GSH-PEG liposomes are in clinical trials for the pharmacokinetics, tolerability, and safety in the treatment of brain metastasis or recurrent malignant glioma and solid tumors, which clearly demonstrated clinical significance of GSH-PEG liposomes as a nanocarrier for brain-targeted drug delivery. Furthermore, Patel et al. developed hydrophilic fluorescent marker-loaded GSH-conjugated BSA NPs for brain-specific drug delivery. The study revealed that the permeability of GSH-BSA NPs across the monolayer of MDCK-MDR1 endothelial tight junction was shown significantly higher as compared to BSA NPs and fluorescein sodium solution. Additionally, GSH-BSA NPs exhibited higher uptake by neuroglial cells as compared to BSA NPs and fluorescein sodium solution. Intravenously administered GSH-BSA NPs showed threefold higher fluorescein sodium carried to the brain as compared to BSA NPs [62].

4 Strategies for Targeted Nanoparticles

Various methods/chemistries have been employed to link ligands with reactive groups of the surface of the nanocarriers, and the methods can be classified into covalent and non-covalent conjugations. Common covalent coupling among the other methods involve chemistries such as (1) carbodiimide chemistry, (2) Michael addition, and (3) “click” chemistry.

4.1 Carbodiimide Chemistry

This technique is the most utilized for the conjugation of nanocarrier with the targeting moiety. The merit of this chemistry depends on its simplicity, and the required functional groups present on the targeting moiety and nanocarrier (e.g., amine group, carboxylic acid, and so on), to accomplish the coupling. This results in low possibility to lose ligand-specific activity [68]. Nevertheless, the presence of multiple functional groups in the targeting moiety can put forth a drawback trying to confine multi-site attachment or to control the targeting moiety alignment at the surface of the nanocarrier [69]. The amide bond formation takes place in two subsequent steps. Amid the first, the activation of carboxylic acid groups present on the surface of nanocarrier is carried out by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC). This reagent is generally referred as carbodiimide which can shape

diverse chemical structures. EDC has great solubility in water which empowers direct utilization of EDC in aqueous solutions without addition of any organic compounds. These conditions are appropriate for the attachment of bioactive particles to the carrier surface. The o-acylisourea intermediate formed during the actuation of the carboxylic acid is vulnerable to quick hydrolysis [70]. Thusly, an excess amount of carbodiimide must be utilized to finish the reaction, which can modify the colloidal stability of the subsequent nanocarriers because of the poor solubility of the o-acylisourea. This can be fathomed utilizing *N*-hydroxysuccinimide (NHS) or *N*-hydroxysulfosuccinimide (sulfo-NHS). The ester intermediate of the latter has a better solubility. Albeit still susceptible to hydrolysis (which is anyway slower than o-acylisourea middle of the road), NHS esters as a rule prompt higher coupling efficiency [69].

The above-described information was connected with the activation of the carboxylic group on the surface of nanocarrier. Alternative method is associated with the activation of primary amine of nanocarrier. This process takes place when homo-bifunctional dithio-bis(succinimidyl propionate) (DSP) is used [71]. The DSP was used in the synthesis of drug carrier which was applied in the targeted breast cancer therapy [72]. The DSP activates the amine groups of nanocarriers. Additionally, application of NHS allows the formation of ester during the reaction with monoclonal antibody (trastuzumab). This methodology was very efficient and selective throughout cancer gene therapy [73].

4.2 Michael Addition

Michael addition is the second widely applied approach for conjugating targeting moiety and nanocarrier which involves in particular thiol-maleimide coupling strategy. Maleimides used in Michael addition exhibit slower hydrolysis rate than o-acylisourea intermediates in carbodiimide chemistry. Besides, at near neutral pH, maleimides react with thiols in a highly selective and efficient way resulting into stable thioether bond [68, 69]. The reaction runs quickly and under mild conditions, at the room temperature, and in aqueous solution. Formed thioether bond is stable within 24 h in human serum even in the presence of reducing agent, for example, DTT. To circumvent this limitation, thiol group can be obtained either by reducing existing disulfide bonds (which can be however detrimental for ternary structures of certain peptides/proteins) or by using hetero-bifunctional cross-linking agents such as *N*-succinimidyl-*S*-acetylthioacetate (SATA) or *N*-succinimidyl-3-(2-pyridyldithio) propionate (SPDP) [74]. Application of the system containing the thioether bond guarantees the high selectivity of delivery and the long time of distribution.

4.3 The "Click Chemistry"

The term click chemistry (CC) was developed by Kolb's group and describes the coupling of small molecules and heteroatoms in the form of R-X-R [75]. Reactions belonging to "click chemistry" group share several very important features: (1) a very high efficiency in terms of both conversion and selectivity; (2) mild experimental conditions; (3) a simple workup; (4) little or no by-products; (5) readily available reagents; and (6) easily removed solvent, for example, water [71]. The main method of the purification of final product is crystallization or distillation [75]. Within CC, one can find four different types [75, 76]: (1) cycloaddition, for example, Huisgen catalytic cycloaddition; (2) nucleophilic substitution chemistry, for example, ring opening of heterocyclic electrophiles; (3) carbonyl chemistry of the "nonaldol" type, for example, formation of ureas, thioureas, and hydrazones; and (4) addition to carbon–carbon multiple bonds, for example, epoxilation and dihydroxylation. The major type of "click chemistry" reaction is the cycloaddition which has received remarkable attention in many fields such as bioconjugations and polymer functionalization. This process creates 1,2,3-triazole by the Huisgen 1,3-dipolar cycloaddition reaction between an azides and terminal alkynes (CuAAC) in the presence of the catalyst, copper (I) [71, 77, 78]. The principle drawback of the CuAAC reaction is the necessary removal of the Cu-based catalyst after the coupling. The use of Cu ligands and organic scavengers is however possible to remove most of the catalyst.

5 Work Explored by Various Researchers for Targeting of PLGA NPs to Brain in Different Neuronal Diseases

Various researchers have worked on PLGA nanoparticles for brain targeting. The details are compiled in the Table 3.1.

6 Patents and Clinical Trial Status

Nanotechnology is being explored and has shown very promising results these days. Many nano-based products like curcumin, EGFR, ferumoxytol, etc. are under clinical trials, and initial phase studies are already completed. PLGA nanoparticles are also very well explored in research for neuronal targeting, and it is expected that they too will pass the clinical trial phase and products will be there in the market in the near future. With the enhancement in targeted NP-related research, a lot of attention has been given for commercialization of such nanoparticles which led to the filing of patents related to these NPs. Various types of patents have been filed related to these NPs by different agencies including independent researchers and pharmaceutical companies. The filed patents have been summarized in Table 3.2.

Table 3.1 PLGA NPs for neuron targeting

Architecture	Targeting ligand	Therapeutic moiety	Preparation method	Particle size	Neuronal disease/ disorder	In vitro/In vivo model	Application	Outcome	Ref.
Teniposide-PLGA NPs	–	Teniposide	Single emulsion solvent evaporation	121 nm	Neuroblastoma	U87MG cells	Uptake mechanisms of NPs	• Cytotoxicity study demonstrated that drug-loaded PLGA NPs showed significant cytotoxicity, whereas blank NPs showed no cytotoxicity within 24 h • Endocytosis inhibition test showed that NP internalization within cells took place via clathrin-mediated endocytosis and macropinocytosis	[79]
Carbamazepine (CBZ)/verapamil-PLGA/poloxamer 188 NPs	–	CBZ, verapamil	HPH followed by solvent evaporation	130–150 nm	Epilepsy	Male Wistar rats	The effect of Pgp inhibitor (verapamil) on anticonvulsant effect of CBZ and its nanoformulation in the rat model	• It was found that verapamil significantly increased the anticonvulsant effect of CBZ and reduced its effective dose by at least 30% • CBZ-loaded poloxamer 188-coated PLGA NPs enabled 30-fold increase of its anticonvulsant effect as compared to free drug	[80]

(continued)

Table 3.1 (continued)

Architecture	Targeting ligand	Therapeutic moiety	Preparation method	Particle size	Neuronal disease/ disorder	In vitro/In vivo model	Application	Outcome	Ref.
Tet-1-curcumin-PLGA NPs	Tet-1	Curcumin	Single emulsion solvent evaporation	150–200 nm	Alzheimer's disease	GI-1 glioma cells, LAG cell line	Study anti-amyloid activity and antioxidant activity of curcumin-PLGA NPs	• The Tet-1 neuropeptide-conjugated PLGA NPs demonstrated that in vitro targeting efficiency was enhanced greatly • The curcumin-loaded PLGA NPs can be used as a potential tool in treating AD with respect to its anti-amyloid and antioxidant property	[81]
g7-FITC-albumin-PLGA NPs	g7	FITC-albumin	Double emulsion technique	254–261 nm	Lysosomal storage disorders	In vivo: C57BL/6 Idua knockout (ko) mouse and wild-type mouse	Targeted delivery platform loaded with high molecular weight molecules in lysosomal storage disorders	• In vivo study demonstrated that the g7-FITC-albumin-PLGA NPs were able to cross BBB and were distributed in brain parenchyma both in ko and wild-type mice, with higher efficiency in ko mice • g7-FITC-albumin-PLGA NPs followed the clathrin pathway to enter the neuronal cells	[82]

RVG29-PEG-PLGA-docetaxel	RVG29	Docetaxel	Nanoprecipitation method	110 nm	Glioma	C6, bEnd3 and HeLa cells	Brain-targeted drug delivery platform in the treatment of glioma	• The in vitro cellular uptake study results demonstrated that RVG29-conjugated NPs showed better targeting property as compared to nontargeted NPs • RVG29-PEG-PLGA NPs showed better BBB penetration in an in vitro model and were selectively accumulated in transcranial glioma tissue	[83]
Tf-PLGA NPs, RVG (rabies virus glycoprotein)-PLGA NPs, Tf-BSA NPs RVG-BSA NPs	Tf RVG	Oxytocin	Multiple emulsion solvent evaporation, Coacervation /nanoprecipitation method	100–278 nm	Autism spectrum disorder	Dendritic cells	Nanoformulations for sustained release and brain targeting	• The study demonstrated that the prepared formulation was found to be safe as it did not produce immunogenic and cytotoxic response • The release study revealed that BSA NPs showed faster initial burst of release compared to PLGA NPs, in addition to later sustained release	[84]

(continued)

Table 3.1 (continued)

Architecture	Targeting ligand	Therapeutic moiety	Preparation method	Particle size	Neuronal disease/ disorder	In vitro/In vivo model	Application	Outcome	Ref.
PEG-PLGA-epigallocatechin-3-gallate NPs	PEG	Epigallocatechin-3-gallate	Double emulsion method	169 nm	Temporal lobe epilepsy	In vitro: astrocytes, bEnd.3, and PC12 cells In vivo: 1-month-old wild-type C57BL/6J mice	Development of nanoplatform for the treatment of epilepsy	• Cytotoxicity assays showed that formulation was safe for delivery • Behavior test demonstrated that NPs reduced most number of episodes of epilepsy and their intensity as compared to free drug • Neurotoxicity and immunohistochemistry studies confirmed a decrease in neuronal death and neuroinflammation	[85]

Pep-apoE-PLGA NPs Lipocalin-type prostaglandin-D-synthase (L-PGDS)-PLGA NPs	Pep-apoE, L-PGDS	–	Single emulsion solvent evaporation method followed by conjugation with carbodiimide chemistry	198 nm	Brain diseases	DCs	Novel functionalization strategies for brain targeting	• The study revealed that targeted PLGA NPs, but not nontargeted PLGA NPs, were found in cerebral cortex parenchyma after 2 h of IV in mice • It was also found that NPs were mostly internalized by neurons and microglia • The finding showed that functionalization strategies were found to be safe as they did not elicit adverse immune response	[86]

(continued)

Table 3.1 (continued)

Architecture	Targeting ligand	Therapeutic moiety	Preparation method	Particle size	Neuronal disease/ disorder	In vitro/In vivo model	Application	Outcome	Ref.
hGDNF-polyamidoamine (PAMAM)-PEG-Lf NPs	Lf	hGDNF	Synthesis using Michael addition reaction	196 nm	Parkinson's disease	In vivo: Male Sprague Dawley rats	Platform for neuroprotection in a 6-hydroxydopamine-lesioned Parkinson's model	• The neuroprotective evaluation demonstrated that increase in the number of injections of NPs ameliorated locomotor activity, reduced dopaminergic neuronal loss, and enhanced monoamine neurotransmitter levels • Study also revealed that five injections of Lf-modified NPs loaded with hGDNF exhibited much more powerful neuroprotection than a single injection	[87]
83–14 mAb-polyacrylamide (PAA)-cardiolipin (CL)-PLGA-rosmarinic acid (RA) and curcumin (CUR)	83–14 mAb	RA and CUR	Emulsification followed by solvent displacement method	100–500 nm	Alzheimer's disease	In vitro: SK-N-MC cells	Development of promising platform for pharmacotherapy to permeate the BBB and reduce the fibrillar Aβ-induced neurotoxicity	• Study revealed that the increase in concentration of 83–14 mAb enhanced the permeability coefficient of RA and CUR using NPs •	[88]

mAb-CUR-PLGA NPs	mAb	CUR	Nanoprecipitation method	250 nm	Glioblastoma	In vitro: DKMG/ $EGFR_vIII$, DK-MG^{low}	In vitro photodynamic therapy on human glioblastoma cell line	• The experimental study demonstrated that mAb-CUR-PLGA NPs were able to show more effective photodynamic toxicity (56% vs. 24%) on the DKMG/ $EGFR_vIII$ cells as compared to CUR-PLGA NPs	[89]
GSH-PEG-CUR-PLGA NPs	GSH	CUR	Nanoprecipitation method followed by functionalization using click chemistry	149–180 nm	AD	In vitro: SK-N-SH cells	Investigation of the internalization pathway in neuronal cells	• Presence of GSH on the surface of NPs exhibited neuroprotective property against acrolein • GSH-PEG-CUR-PLGA NPs showed higher neuronal internalization as compared to free CUR	[90]

(continued)

Table 3.1 (continued)

Architecture	Targeting ligand	Therapeutic moiety	Preparation method	Particle size	Neuronal disease/ disorder	In vitro/In vivo model	Application	Outcome	Ref.
MNPs-T7-PTX/ CUR-PLGA NPs	MNPs, T7	PTX/CUR	Single emulsion solvent evaporation method followed by Michael addition	130 nm	Brain tumor therapy	In vitro: U87, bEnd.3 cells In vivo: Balb/c nude mice	Dual-targeting platform for brain tumor targeting	• The combination of drug yielded synergistic effects on inhibition of tumor growth via the mechanisms of apoptosis induction and cell cycle arrest, showing significantly increased efficacy than single use of each drug • Dual-targeting effect yielded over tenfold increase in cellular uptake studies and over fivefold enhancement in brain delivery compared to the nontargeted NPs	[91]
Alexa fluor-labeled VEGF-loaded liposomes	Alexa fluor	VEGF	Synthesized using conjugation of Alexa fluor with DSPE followed by rehydration process for liposomes	<200 nm	Ischemia	In vivo: C57BL/6J mice	Liposomes for ischemia tissue by controlling blood vessel permeability	• The study demonstrated that IV-administered liposomes target ischemic site via EPR effect in early stage of ischemia and maintain permeability of vessels for longer period of time (7 days) as compared to delivery of empty liposomes	[92]

Irinotecan hydrochloride/ metformin-PLGA NPs	–	Irinotecan hydrochloride/ metformin	Single emulsion solvent evaporation method	216 and 300 nm	Glioblastoma	U-87 MG cells	Effect of irinotecan hydrochloride/ metformin-PLGA NPs on in vitro/in vivo studies of glioblastoma	• The study demonstrated that 1 mM and 2 mM doses of metformin and metformin-loaded PLGA NPs, respectively, significantly reduce the volume of extracted cancer	[93]
p38 SiRNA-PLGA NPs	–	P38 SiRNA	Double emulsion method	153.1 nm	Neuropathic pain	In vitro: BV2, HT22, and U87MG cells	Effect of p38 SiRNA-PLGA NPs as a drug delivery platform in neuropathic pain in rats by inhibiting microglia activation	• The study revealed that p38 SiRNA-PLGA NPs significantly reduced mechanical allodynia as well as microgliosis in the spinal dorsal horns of SNL rats, consistent with a downregulation of p38-related proinflammatory mediators	[94]
Chitosan-Eugenol-PCL NPs	–	Eugenol	Double emulsification-solvent evaporation method	224 nm	Cerebral ischemia		Quantification and targeted delivery of eugenol-loaded NPs via intranasal route in the treatment of cerebral ischemia	• The toxicity studies revealed that the nanoformulation was found to be safe • Chitosan-eugenol-PCL NPs was found to effectively enhance the bioavailability drug in the brain when administered in rat	[95]

(continued)

Table 3.1 (continued)

Architecture	Targeting ligand	Therapeutic moiety	Preparation method	Particle size	Neuronal disease/ disorder	In vitro/In vivo model	Application	Outcome	Ref.
Lamotrigine-pluronic F127/ Carbopol 974P gel	–	Lamotrigine	Cold method	–	Epilepsy	In vivo: Male CD-1 mice	Development of nose to brain delivery platform for the treatment of epilepsy	• Biodistribution studies demonstrated that there is higher concentration of lamotrigine in olfactory bulb as compared to frontal cortex • It was also revealed that directly transfer of lamotrigine to the brain was done via olfactory neuronal pathway	[96]
CPP-chitosan-PLGA/NLC NPs	CPP	–	Melt emulsification technique Double emulsion solvent evaporation method	200 nm	CNS diseases	In vitro: Olfactory cell monolayer	Study transport of NPs across olfactory cell monolayer	• It was found that 0.7% of PLGA NPs was able to cross olfactory cell monolayer, whereas 8% and 22% of NLC and chitosan-coated NLC were transported across olfactory cell monolayer respectively • Additionally addition of CPP significantly enhanced their transport i.e. 46%	[97]

Chitosan-PLGA NPs; PLGA NPs	–	–	Solvent displacement method	250 nm	Nose to brain delivery in the treatment of CNS disease	In vivo: male Wistar rats	Study of effect of surface charge of the NPs on brain subregion localization	• Study demonstrated that both surface-charged NPs had reached the brain and persist in the brain for 48 h after intranasal administration • Surface charge on the NPs affects the pathways involved in their translocation from the nasal cavity to CNS • Furthermore, in brief, positively charged NPs followed the trigeminal pathway, whereas negatively charged NPs followed olfactory pathway	[98]
Chitosan-L-pGlu-(1-benzyl)-L-His-L-ProNH2 (NP-355)/L-pGlu-(2-propyl)-L-His-L-ProNH2 (NP-647)-PLGA NPs	–	NP-355 and NP-647	Emulsion solvent evaporation	110 nm and 163.6 nm	Epilepsy		Delivery platform for antiepileptic TRH analogs in the treatment of epilepsy via nose to brain delivery	• The toxicity studies revealed that NP-355- and NP-647-loaded PLGA NPs were found to be safe • The ability of these NPs to reach the brain was evaluated by utilizing quantum dots as fluorescent probes	[99]

(continued)

Table 3.1 (continued)

Architecture	Targeting ligand	Therapeutic moiety	Preparation method	Particle size	Neuronal disease/ disorder	In vitro/In vivo model	Application	Outcome	Ref.
Rivastigmine-mucoadhesive thermosensitive in situ gel	–	Rivastigmine	Cold method	–	AD	Ex vivo transnasal study	Rivastigmine-mucoadhesive thermosensitive in situ gel platform for targeting to brain via nasal route in the treatment of AD	• The optimal RV in situ gel (PF127 and 1% Carbopol 934) showed significant transnasal permeation (84%) which was reflected in better distribution to the brain (0.54 %ID/g), when compared to RV IN solution (0.16 %ID/g) and RV IV intravenous solution (0.15 %ID/g)	[100]
Lf-Rotigotine-NPs	Lf	Rotigotine	Nanoprecipitation method	200 nm	Parkinson's disease	In vivo: Sprague Dawley male rats	Study of neuroprotective effect of Lf-Rotigotine-NPs and its biodistribution and pharmacodynamics using nose to brain route	• Lf-R-NPs more efficiently supplied rotigotine to the brain (with a greater sustained amount of the drug delivered to this organ and with more effective targeting to the striatum) than R-NPs • Furthermore, Lf-R-NPs significantly alleviated nigrostriatal dopaminergic neurodegeneration in the rat model of 6-hydroxydopamine-induced PD	[101]

Lf-N-trimethylated chitosan-Huperzine A-PLGA NPs	Lf	Huperzine A	Emulsion solvent evaporation method	153.2 nm	AD	In vitro: 16HBE, SH-SY-5Y cells; In vivo: KM mice	Nanoformulation as a platform in the treatment of AD	• Lf-N-trimethylated chitosan-Huperzine A-PLGA NPs showed lower toxicity on 16HBE cells as compared to Huperzine A solution • Lf-N-trimethylated chitosan-Huperzine A-PLGA NPs showed higher accumulation as compared to nontargeted NPs, i.e., N-trimethylated chitosan-Huperzine A-PLGA NPs in 16HBE and SH-SY-5Y cells • In vivo imaging results showed that Lf-TMC NPs exhibited a higher fluorescence intensity in the brain and a longer residence time than nontargeted NPs	[102]

Table 3.2 Patents on nanoparticles for neuronal cell targeting

Application number	Title	Applicant	Status
JP2018065862	Drug-loaded polymeric nanoparticles and methods of making and using the same	Pfizer	Pending
WO2018146599 (A1)	Polymeric nanoparticles encapsulating a combination of natural bioactive trans-resveratrol (rsv) and celastrol (cl), a process for the preparation and use thereof in the treatment of prostate cancer	Univ DEGLI STUDI DI SASSARI	Pending
WO2018073740 (A1)	Therapeutic polymeric nanoparticles comprising lipids and methods of making and using same	Pfizer	Pending
US2017326085 (A1)	Glycolic acid and/or D-lactic acid for the treatment of neurodegenerative diseases	De max-planck-gesellschaft zur foerderung der wss e v	Pending
RU2009148718 (A)	Pharmaceutical composition for neurodegenerative diseases of hydrogenated pyrido(4,3-b)indole, method for preparing and based drug		Pending
US2014234402 (A1)	Intravenous infusion of curcumin and a calcium channel blocker	Signpath PHARMA INC.	Pending
5,955,506	Benzamides for neurodegenerative disorder treatment	Centaur PHARMACEUTICALS, INC.	September 21, 1999
7,081,345	Use of a polypeptide for detecting, preventing, or treating a pathological condition associated with a degenerative, neurological, or autoimmune disease	Biomerieux STEHLYS	July 25, 2006
7,255,874	Biocompatible polymers and adhesives: compositions, methods of making, and uses related thereto	Closure MEDICAL CORPORATION	August 14, 2007
7,262,189	Benzothiazine and benzothiadiazine derivatives, method for preparing same and *pharmaceutical compositions* containing same	Les LABORATOIRES SERVIER	August 28, 2007

(continued)

Table 3.2 (continued)

Application number	Title	Applicant	Status
7,273,618	Method for administering agents to the central nervous system	Chiron CORPORATION	September 25, 2007
7,445,931	Compositions and methods for enrichment of neural stem cells using ceramide analogs	Medical COLLEGE OF GEORGIA RESEARCH INSTITUTE	November 4, 2008
7,888,066	Methods for identifying substances for the treatment of Alzheimer's disease	Mount SINAI SCHOOL OF MEDICINE	February 15, 2011
8,288,444	Iontophoretic delivery of curcumin and curcumin analogs for the treatment of Alzheimer's disease	Codman & SHURTLEFF, INC.	October 16, 2012
8,329,719	Neuroprotective agents for the prevention and treatment of *neurodegenerative diseases*	Lixte BIOTECHNOLOGY, INC.	December 11, 2012
8,609,652	Method of administering a methylene blue-curcumin analog for the treatment of Alzheimer's disease	DePuy SYNTHES PRODUCTS, LLC	December 17, 2013
8,758,778	Polymeric nanocarriers with a linear dual-response mechanism and uses thereof	The REGENTS OF THE UNIVERSITY OF CALIFORNIA	June 24, 2014
8,778,904	Methods and compositions for treating diseases, disorders, or injuries of the CNS	Quark PHARMACEUTICALS, INC	July 15, 2014
8,835,387	Histidyl-tRNA synthetases for treating autoimmune and inflammatory diseases	Pangu BioPharma LIMITED	September 16, 2014
8,871,212	Amyloid-beta polypeptide vaccine	H. Lundbeck a/s	October 28, 2014
8,999,927	Glial cell line-derived neurotrophic factor (GDNF) compositions and use thereof	The UNITED STATES OF AMERICA, AS REPRESENTED BY THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES (WASHINGTON, DC)	April 7, 2015
9,295,689	Formulation and delivery of *plga* microspheres	Moderna THERAPEUTICS, INC.	March 29, 2016
9,486,559	Methods of treatment with a bioresorbable scaffold for neurologic drug delivery	Abbott CARDIOVASCULAR SYSTEMS INC	November 8, 2016

(continued)

Table 3.2 (continued)

Application number	Title	Applicant		Status
9,555,071	Methods and compositions for the treatment of axonal and neuronal degeneration	Nguyen; THIEN	Potomac	January 31, 2017
9,579,300	Nanoparticle and polymer formulations for thyroid hormone analogs, antagonists, and formulations thereof	NanoPharmaceuticals LLC		February 28, 2017
9,687,553	Polymeric nanocarriers with linear dual response mechanism	The REGENTS OF THE UNIVERSITY OF CALIFORNIA		June 27, 2017
9,827,353	Cross-linked fatty acid-based biomaterials	Atrium MEDICAL CORPORATION		November 28, 2017
9,913,877	Methods and compositions for the treatment of axonal and neuronal degeneration	Ewaleifoh; OSEFAME NGUYEN; THIEN		March 13, 2018
10,028,971	Compositions and methods for treating psychiatric disorders	Gosforth CENTRE (HOLDINGS) PTY LTD		July 24, 2018
10,034,918	Therapeutic use of a growth factor, metrnl	Hoba THERAPEUTICS APS		July 31, 2018
10,040,783	Prostaglandin receptor ep2 antagonists, derivatives, compositions, and uses related thereto	Emory UNIVERSITY		August 7, 2018

7 Concluding Remarks and Future Perspectives

In summary, therapeutic formulations fabricated owing to nanotechnology development have been extensively used in the treatment of CNS diseases. Although various formulations such as liposomes, micelles, nanostructured lipid carrier, dendrimers, nanotubes, and others are used in the treatment of various CNS diseases, PLGA-based polymeric-based nanostructures have been extensively studied. The literature of various studies demonstrated that despite BBB, intranasal route can be an effective route for brain targeting. PLGA NPs exhibits various advantages like better drug entrapment, ease in surface functionalization of ligands. PLGA NPs also has application in simultaneous diagnosis and therapy (theranostic). Surface functionalization of PLGA NPs and the attachment of the targeting moiety incorporate useful information and progress in this field. Additionally, the type of chemistry/conjugation of the nanocarrier with the ligands depends on the type of functional group present on both, i.e., ligand and nanocarrier. Dual-/multi-targeting strategy was employed to overcome two or more barriers present in the brain which may retard the delivery system to reach specific diseased area. It is generalized that dual-targeting delivery systems should function to penetration through nasal mucosa and/

or BBB and then specifically binds to cells of diseased brain. Until now, none of the ligand-targeted nanomedicines have been approved probably due to two major aspects: ambiguous active-targeting effect in CNS diseases and the difficulties encountered in large-scale and reproducible production while maintaining the activity of targeting ligands. Therefore, future studies should focus more on human CNS biology (e.g., by establishing models that are more close to CNS-specific disease). Besides, more attention should be paid to the design, production, and control aspects of dual-targeted nanomedicines to facilitate possible clinical translation.

Acknowledgment The authors are thankful for the support from the Institute of Pharmacy, Nirma University; the CSIR, Government of India, in the form of CSIR-SRF (Grant no. 09/1048 (007)/2018 EMR-I); the Prime Minister Fellowship (SERB/PM Fellow/CII-FICCI/Meeting/2018 dated 27.02.2018); and DST-INSPIRE for providing fellowship (IF131007) as a financial assistance.

Conflict of Interest The authors declared no conflict of interest.

References

1. *Neurologic diseases* [Internet]. Retrieved August 24, 2018, from https://medlineplus.gov/neurologicdiseases.html
2. WHO *What are neurological disorders?* [Internet]. WHO. Retrieved August 24, 2018, from http://www.who.int/features/qa/55/en/
3. Ferri, C. P., Prince, M., Brayne, C., Brodaty, H., Fratiglioni, L., Ganguli, M., et al. (2005). Global prevalence of dementia: A Delphi consensus study. *Lancet (London, England), 366*(9503), 2112–2117.
4. Singh, S. K., Srivastav, S., Yadav, A. K., Srikrishna, S., & Perry, G. (2016). Overview of Alzheimer's disease and some therapeutic approaches targeting Aβ by using several synthetic and herbal compounds. *Oxidative Medicine and Cellular Longevity* [Internet]*, 2016*. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4807045/
5. Cummings, J. L., Vinters, H. V., Cole, G. M., & Khachaturian, Z. S. (1998). Alzheimer's disease: Etiologies, pathophysiology, cognitive reserve, and treatment opportunities. *Neurology, 51*(1 Suppl 1), S2–17; discussion S65–67.
6. Wolters E. C. (2009). Non-motor extranigral signs and symptoms in Parkinson's disease. Parkinsonism & Related Disorders, 15(Suppl 3):S6–S12.
7. Obeso, J. A., Rodríguez-Oroz, M. C., Benitez-Temino, B., Blesa, F. J., Guridi, J., Marin, C., et al. (2008). Functional organization of the basal ganglia: Therapeutic implications for Parkinson's disease. *Movement Disorders: Official Journal of the Movement Disorder Society, 23*(Suppl 3), S548–S559.
8. Obeso, J. A., Rodriguez-Oroz, M. C., Goetz, C. G., Marin, C., Kordower, J. H., Rodriguez, M., et al. (2010). Missing pieces in the Parkinson's disease puzzle. *Nature Medicine, 16*(6), 653–661.
9. Ho, S. C., Woo, J., & Lee, C. M. (1989). Epidemiologic study of Parkinson's disease in Hong Kong. *Neurology, 39*(10), 1314–1318.
10. Braak, H., Rüb, U., Gai, W. P., & Del Tredici, K. (2003). Idiopathic Parkinson's disease: Possible routes by which vulnerable neuronal types may be subject to neuroinvasion by an unknown pathogen. *Journal of Neural Transmission (Vienna, Austria: 1996), 110*(5), 517–536.

11. Schapira, A. H. V. (2005). Present and future drug treatment for Parkinson's disease. *Journal of Neurology, Neurosurgery, and Psychiatry, 76*(11), 1472–1478.
12. Factor, S. A. (2008). Current status of symptomatic medical therapy in Parkinson's disease. *Neurotherapeutics, 5*(2), 164–180.
13. Ricard, D., Idbaih, A., Ducray, F., Lahutte, M., Hoang-Xuan, K., & Delattre, J.-Y. (2012). Primary brain tumours in adults. *Lancet (London, England), 379*(9830), 1984–1996.
14. Koo, Y.-E. L., Reddy, G. R., Bhojani, M., Schneider, R., Philbert, M. A., Rehemtulla, A., et al. (2006). Brain cancer diagnosis and therapy with nanoplatforms. *Advanced Drug Delivery Reviews, 58*(14), 1556–1577.
15. Lewandowsky, M. (1909). Zur Lehre der Cerebrospinalflussigkeit. *Zeitschrift für Klinische Medizin, 40*, 480–494.
16. Cardoso, F. L., Brites, D., & Brito, M. A. (2010). Looking at the blood-brain barrier: Molecular anatomy and possible investigation approaches. *Brain Research Reviews, 64*(2), 328–363.
17. Tapeinos, C., Battaglini, M., & Ciofani, G. (2017). Advances in the design of solid lipid nanoparticles and nanostructured lipid carriers for targeting brain diseases. *Journal of Controlled Release, 264*, 306–332.
18. Abbott, N. J., Bundgaard, M., & Cserr, H. F. (1985). Tightness of the blood-brain barrier and evidence for brain interstitial fluid flow in the cuttlefish, *Sepia officinalis*. *The Journal of Physiology, 368*, 213–226.
19. Abbott, N. J., Patabendige, A. A. K., Dolman, D. E. M., Yusof, S. R., & Begley, D. J. (2010). Structure and function of the blood-brain barrier. *Neurobiology of Disease, 37*(1), 13–25.
20. Cornford, E. M., & Hyman, S. (2005). Localization of brain endothelial luminal and abluminal transporters with immunogold electron microscopy. *NeuroRx: The Journal of the American Society for Experimental NeuroTherapeutics, 2*(1), 27–43.
21. Dinda, S. C., & Pattnaik, G. (2013). Nanobiotechnology-based drug delivery in brain targeting. *Current Pharmaceutical Biotechnology, 14*(15), 1264–1274.
22. Thorne, R. G., Emory, C. R., Ala, T. A., & Frey, W. H. (1995). Quantitative analysis of the olfactory pathway for drug delivery to the brain. *Brain Research, 692*(1–2), 278–282.
23. Shipley, M. T. (1985). Transport of molecules from nose to brain: Transneuronal anterograde and retrograde labeling in the rat olfactory system by wheat germ agglutinin-horseradish peroxidase applied to the nasal epithelium. *Brain Research Bulletin, 15*(2), 129–142.
24. Chapman, C. D., Frey, W. H., Craft, S., Danielyan, L., Hallschmid, M., Schiöth, H. B., et al. (2013). Intranasal treatment of central nervous system dysfunction in humans. *Pharmaceutical Research, 30*(10), 2475–2484.
25. Linazasoro, G., & Nanotechnologies for Neurodegenerative Diseases Study Group of the Basque Country (NANEDIS). (2008). Potential applications of nanotechnologies to Parkinson's disease therapy. *Parkinsonism & Related Disorders, 14*(5), 383–392.
26. Ravi Kumar, M. N. (2000). Nano and microparticles as controlled drug delivery devices. *Journal of Pharmacy & Pharmaceutical Sciences: A Publication of the Canadian Society for Pharmaceutical Sciences, Société canadienne des sciences pharmaceutiques, 3*(2), 234–258.
27. Jores, K., Mehnert, W., Drechsler, M., Bunjes, H., Johann, C., & Mäder, K. (2004). Investigations on the structure of solid lipid nanoparticles (SLN) and oil-loaded solid lipid nanoparticles by photon correlation spectroscopy, field-flow fractionation and transmission electron microscopy. *Journal of Controlled Release: Official Journal of the Controlled Release Society, 95*(2), 217–227.
28. Kreuter, J. (2001). Nanoparticulate systems for brain delivery of drugs. *Advanced Drug Delivery Reviews, 47*(1), 65–81.
29. Schroeder, U., Sommerfeld, P., Ulrich, S., & Sabel, B. A. (1998). Nanoparticle technology for delivery of drugs across the blood-brain barrier. *Journal of Pharmaceutical Sciences, 87*(11), 1305–1307.
30. Astete, C. E., & Sabliov, C. M. (2006). Synthesis and characterization of PLGA nanoparticles. *Journal of Biomaterials Science. Polymer Edition, 17*(3), 247–289.

31. Mohamed, F., & van der Walle, C. F. (2008). Engineering biodegradable polyester particles with specific drug targeting and drug release properties. *Journal of Pharmaceutical Sciences, 97*(1), 71–87.
32. Pillay, S., Pillay, V., Choonara, Y. E., Naidoo, D., Khan, R. A., du Toit, L. C., et al. (2009). Design, biometric simulation and optimization of a nano-enabled scaffold device for enhanced delivery of dopamine to the brain. *International Journal of Pharmaceutics, 382*(1–2), 277–290.
33. Gao, H., Yang, Z., Zhang, S., Cao, S., Shen, S., Pang, Z., et al. (2013). Ligand modified nanoparticles increases cell uptake, alters endocytosis and elevates glioma distribution and internalization. *Scientific Reports, 3*, 2534.
34. Gao, H., Pang, Z., & Jiang, X. (2013). Targeted delivery of nano-therapeutics for major disorders of the central nervous system. *Pharmaceutical Research, 30*(10), 2485–2498.
35. Gao, H. (2017). Perspectives on dual targeting delivery systems for brain tumors. *Journal of Neuroimmune Pharmacology, 12*(1), 6–16.
36. Du, J., Lu, W.-L., Ying, X., Liu, Y., Du, P., Tian, W., et al. (2009). Dual-targeting topotecan liposomes modified with tamoxifen and wheat germ agglutinin significantly improve drug transport across the blood-brain barrier and survival of brain tumor-bearing animals. *Molecular Pharmaceutics, 6*(3), 905–917.
37. Yin, T., Yang, L., Liu, Y., Zhou, X., Sun, J., & Liu, J. (2015). Sialic acid (SA)-modified selenium nanoparticles coated with a high blood-brain barrier permeability peptide-B6 peptide for potential use in Alzheimer's disease. *Acta Biomaterialia, 25*, 172–183.
38. Zhang, C., Zheng, X., Wan, X., Shao, X., Liu, Q., Zhang, Z., et al. (2014). The potential use of H102 peptide-loaded dual-functional nanoparticles in the treatment of Alzheimer's disease. *Journal of Controlled Release: Official Journal of the Controlled Release Society, 192*, 317–324.
39. Sengupta, S., Eavarone, D., Capila, I., Zhao, G., Watson, N., Kiziltepe, T., et al. (2005). Temporal targeting of tumour cells and neovasculature with a nanoscale delivery system. *Nature, 436*(7050), 568–572.
40. Gao, H., Xiong, Y., Zhang, S., Yang, Z., Cao, S., & Jiang, X. (2014). RGD and interleukin-13 peptide functionalized nanoparticles for enhanced glioblastoma cells and neovasculature dual targeting delivery and elevated tumor penetration. *Molecular Pharmaceutics, 11*(3), 1042–1052.
41. Ruan, S., Cao, X., Cun, X., Hu, G., Zhou, Y., Zhang, Y., et al. (2015). Matrix metalloproteinase-sensitive size-shrinkable nanoparticles for deep tumor penetration and pH triggered doxorubicin release. *Biomaterials, 60*, 100–110.
42. Sharon, N., & Lis, H. (2004). History of lectins: From hemagglutinins to biological recognition molecules. *Glycobiology, 14*(11), 53R–62R.
43. Bies, C., Lehr, C.-M., & Woodley, J. F. (2004). Lectin-mediated drug targeting: History and applications. *Advanced Drug Delivery Reviews, 56*(4), 425–435.
44. Kennedy, J. F., Palva, P. M. G., Corella, M. T. S., Cavalcanti, M. S. M., & Coelho, L. C. B. B. (1995). Lectins, versatile proteins of recognition: A review. *Carbohydrate Polymers, 26*(3), 219–230.
45. Ni, Y., & Tizard, I. (1996). Lectin-carbohydrate interaction in the immune system. *Veterinary Immunology and Immunopathology, 55*(1–3), 205–223.
46. Wen, Z., Yan, Z., Hu, K., Pang, Z., Cheng, X., Guo, L., et al. (2011). Odorranalectin-conjugated nanoparticles: Preparation, brain delivery and pharmacodynamic study on Parkinson's disease following intranasal administration. *Journal of Controlled Release: Official Journal of the Controlled Release Society, 151*(2), 131–138.
47. Bechara, C., & Sagan, S. (2013). Cell-penetrating peptides: 20 years later, where do we stand? *FEBS Letters, 587*(12), 1693–1702.
48. Trabulo, S., Cardoso, A. L., Mano, M., & de Lima, M. C. P. (2010). Cell-penetrating peptides—Mechanisms of cellular uptake and generation of delivery systems. *Pharmaceuticals, 3*(4), 961–993.

49. Bolhassani, A., Jafarzade, B. S., & Mardani, G. (2017). *In vitro* and *in vivo* delivery of therapeutic proteins using cell penetrating peptides. *Peptides, 87*, 50–63.
50. Rao, K. S., Reddy, M. K., Horning, J. L., & Labhasetwar, V. (2008). TAT-conjugated nanoparticles for the CNS delivery of anti-HIV drugs. *Biomaterials, 29*(33), 4429–4438.
51. Liu, L., Guo, K., Lu, J., Venkatraman, S. S., Luo, D., Ng, K. C., et al. (2008). Biologically active core/shell nanoparticles self-assembled from cholesterol-terminated PEG-TAT for drug delivery across the blood-brain barrier. *Biomaterials, 29*(10), 1509–1517.
52. Suk, J. S., Suh, J., Choy, K., Lai, S. K., Fu, J., & Hanes, J. (2006). Gene delivery to differentiated neurotypic cells with RGD and HIV Tat peptide functionalized polymeric nanoparticles. *Biomaterials, 27*(29), 5143–5150.
53. Tsui, B., Singh, V. K., Liang, J. F., & Yang, V. C. (2001). Reduced reactivity towards antiprotamine antibodies of a low molecular weight protamine analogue. *Thrombosis Research, 101*(5), 417–420.
54. Liang, J. F., Zhen, L., Chang, L.-C., & Yang, V. C. (2003). A less toxic heparin antagonist—Low molecular weight protamine. *Biochemistry. Biokhimiia, 68*(1), 116–120.
55. Xia, H., Gao, X., Gu, G., Liu, Z., Zeng, N., Hu, Q., et al. (2011). Low molecular weight protamine-functionalized nanoparticles for drug delivery to the brain after intranasal administration. *Biomaterials, 32*(36), 9888–9898.
56. Yan, L., Wang, H., Jiang, Y., Liu, J., Wang, Z., Yang, Y., et al. (2013). Cell-penetrating peptide-modified PLGA nanoparticles for enhanced nose-to-brain macromolecular delivery. *Macromolecular Research, 21*(4), 435–441.
57. Yang, Z.-Z., Zhang, Y.-Q., Wang, Z.-Z., Wu, K., Lou, J.-N., & Qi, X.-R. (2013). Enhanced brain distribution and pharmacodynamics of rivastigmine by liposomes following intranasal administration. *International Journal of Pharmaceutics, 452*(1–2), 344–354.
58. Kamei, N., & Takeda-Morishita, M. (2015). Brain delivery of insulin boosted by intranasal coadministration with cell-penetrating peptides. *Journal of Controlled Release: Official Journal of the Controlled Release Society, 197*, 105–110.
59. Lu, W., Tan, Y.-Z., Hu, K.-L., & Jiang, X.-G. (2005). Cationic albumin conjugated pegylated nanoparticle with its transcytosis ability and little toxicity against blood-brain barrier. *International Journal of Pharmaceutics, 295*(1–2), 247–260.
60. Qin, Y., Chen, H., Zhang, Q., Wang, X., Yuan, W., Kuai, R., et al. (2011). Liposome formulated with TAT-modified cholesterol for improving brain delivery and therapeutic efficacy on brain glioma in animals. *International Journal of Pharmaceutics, 420*(2), 304–312.
61. Van Weperen, W., & Gaillard, P. (2010). Enhanced blood to brain drug delivery. Innovations in Pharmaceutical Technology, 55–57.
62. Patel, P. J., Acharya, N. S., & Acharya, S. R. (2013). Development and characterization of glutathione-conjugated albumin nanoparticles for improved brain delivery of hydrophilic fluorescent marker. *Drug Delivery, 20*(3–4), 143–155.
63. Smeyne, M., & Smeyne, R. J. (2013). Glutathione metabolism and Parkinson's disease. *Free Radical Biology & Medicine, 62*, 13–25.
64. Martin, H. L., & Teismann, P. (2009). Glutathione—A review on its role and significance in Parkinson's disease. *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology, 23*(10), 3263–3272.
65. Reijerkerk, A., Kooij, G., van der Pol, S. M. A., Leyen, T., Lakeman, K., van Het Hof, B., et al. (2010). The NR1 subunit of NMDA receptor regulates monocyte transmigration through the brain endothelial cell barrier. *Journal of Neurochemistry, 113*(2), 447–453.
66. Sutariya, V. (2013). Blood-brain barrier permeation of glutathione-coated nanoparticle. *SOJ Pharmacy & Pharmaceutical Sciences* [Internet]*, 1*(1). Retrieved September 10, 2018, from http://symbiosisonlinepublishing.com/pharmacy-pharmaceuticalsciences/pharmacy-pharmaceuticalsciences03.php
67. Gaillard, P. J., Appeldoorn, C. C. M., Rip, J., Dorland, R., van der Pol, S. M. A., Kooij, G., et al. (2012). Enhanced brain delivery of liposomal methylprednisolone improved therapeutic efficacy in a model of neuroinflammation. *Journal of Controlled Release: Official Journal of the Controlled Release Society, 164*(3), 364–369.

68. Nobs, L., Buchegger, F., Gurny, R., & Allémann, E. (2004). Current methods for attaching targeting ligands to liposomes and nanoparticles. *Journal of Pharmaceutical Sciences, 93*(8), 1980–1992.
69. Nicolas, J., Mura, S., Brambilla, D., Mackiewicz, N., & Couvreur, P. (2013). Design, functionalization strategies and biomedical applications of targeted biodegradable/biocompatible polymer-based nanocarriers for drug delivery. *Chemical Society Reviews, 42*(3), 1147–1235.
70. Haugland, R. P., Spence, M. T. Z., Johnson, I. D., & Basey, A. (2005). *The handbook: A guide to fluorescent probes and labeling technologies* (10th ed.) [Internet]. Eugene, OR: Molecular Probes. Retrieved from http://lib.ugent.be/catalog/rug01:000926166
71. Greg, H. *Bioconjugate techniques* (2nd ed.) [Internet]. Retrieved September 10, 2018, from https://www.elsevier.com/books/bioconjugate-techniques/hermanson/978-0-12-370501-3
72. Chiu, S.-J., Ueno, N. T., & Lee, R. J. (2004). Tumor-targeted gene delivery via anti-HER2 antibody (trastuzumab, Herceptin) conjugated polyethylenimine. *Journal of Controlled Release: Official Journal of the Controlled Release Society, 97*(2), 357–369.
73. Werengowska-Ciećwierz, K., Wiśniewski, M., Terzyk, A. P., & Furmaniak, S. (2015). The chemistry of bioconjugation in nanoparticles-based drug delivery system [Internet]. *Advances in Condensed Matter Physics.* Retrieved September 10, 2018, from https://www.hindawi.com/journals/acmp/2015/198175/
74. Algar, W. R., Prasuhn, D. E., Stewart, M. H., Jennings, T. L., Blanco-Canosa, J. B., Dawson, P. E., et al. (2011). The controlled display of biomolecules on nanoparticles: A challenge suited to bioorthogonal chemistry. *Bioconjugate Chemistry, 22*(5), 825–858.
75. Kolb, H. C., Finn, M. G., & Sharpless, K. B. (2001). Click chemistry: Diverse chemical function from a few good reactions. *Angewandte Chemie (International Ed. in English), 40*(11), 2004–2021.
76. Hein, C. D., Liu, X.-M., & Wang, D. (2008). Click chemistry, a powerful tool for pharmaceutical sciences. *Pharmaceutical Research, 25*(10), 2216–2230.
77. Jeong, B., Bae, Y. H., Lee, D. S., & Kim, S. W. (1997). Biodegradable block copolymers as injectable drug-delivery systems. *Nature, 388*(6645), 860–862.
78. Liu, Y., Miyoshi, H., & Nakamura, M. (2007). Nanomedicine for drug delivery and imaging: A promising avenue for cancer therapy and diagnosis using targeted functional nanoparticles. *International Journal of Cancer, 120*(12), 2527–2537.
79. Mo, L., Hou, L., Guo, D., Xiao, X., Mao, P., & Yang, X. (2012). Preparation and characterization of teniposide PLGA nanoparticles and their uptake in human glioblastoma U87MG cells. *International Journal of Pharmaceutics, 436*(1–2), 815–824.
80. Zybina, A., Anshakova, A., Malinovskaya, J., Melnikov, P., Baklaushev, V., Chekhonin, V., et al. (2018). Nanoparticle-based delivery of carbamazepine: A promising approach for the treatment of refractory epilepsy. *International Journal of Pharmaceutics, 547*(1–2), 10–23.
81. Mathew, A., Fukuda, T., Nagaoka, Y., Hasumura, T., Morimoto, H., Yoshida, Y., et al. (2012). Curcumin loaded-PLGA nanoparticles conjugated with Tet-1 peptide for potential use in Alzheimer's disease. *PLoS One, 7*(3), e32616.
82. Salvalaio, M., Rigon, L., Belletti, D., D'Avanzo, F., Pederzoli, F., Ruozi, B., et al. (2016). Targeted polymeric nanoparticles for brain delivery of high molecular weight molecules in lysosomal storage disorders. *PLoS One, 11*(5), e0156452.
83. Hua, H., Zhang, X., Mu, H., Meng, Q., Jiang, Y., Wang, Y., et al. (2018). RVG29-modified docetaxel-loaded nanoparticles for brain-targeted glioma therapy. *International Journal of Pharmaceutics, 543*(1–2), 179–189.
84. Zaman, R. U., Mulla, N. S., Braz Gomes, K., D'Souza, C., Murnane, K. S., & D'Souza, M. J. (2018). Nanoparticle formulations that allow for sustained delivery and brain targeting of the neuropeptide oxytocin. *International Journal of Pharmaceutics, 548*(1), 698–706.
85. Cano, A., Ettcheto, M., Espina, M., Auladell, C., Calpena, A. C., Folch, J., et al. (2018). Epigallocatechin-3-gallate loaded PEGylated-PLGA nanoparticles: A new anti-seizure strategy for temporal lobe epilepsy. *Nanomedicine: Nanotechnology, Biology, and Medicine, 14*(4), 1073–1085.

86. Portioli, C., Bovi, M., Benati, D., Donini, M., Perduca, M., Romeo, A., et al. (2017). Novel functionalization strategies of polymeric nanoparticles as carriers for brain medications: PEPTIDIC MOIETIES ENABLE BBB TRAVERSAL OF THE NPs. *Journal of Biomedical Materials Research. Part A, 105*(3), 847–858.
87. Huang, R., Han, L., Li, J., Ren, F., Ke, W., Jiang, C., et al. (2009). Neuroprotection in a 6-hydroxydopamine-lesioned Parkinson model using lactoferrin-modified nanoparticles. *The Journal of Gene Medicine, 11*(9), 754–763.
88. Kuo, Y.-C., & Tsai, H.-C. (2018). Rosmarinic acid- and curcumin-loaded polyacrylamide-cardiolipin-poly(lactide- co -glycolide) nanoparticles with conjugated 83-14 monoclonal antibody to protect β-amyloid-insulted neurons. *Materials Science and Engineering: C, 91*, 445–457.
89. Jamali, Z., Khoobi, M., Hejazi, S. M., Eivazi, N., Abdolahpour, S., Imanparast, F., et al. (2018). Evaluation of targeted curcumin (CUR) loaded PLGA nanoparticles for *in vitro* photodynamic therapy on human glioblastoma cell line. *Photodiagnosis and Photodynamic Therapy, 23*, 190–201.
90. Paka, G. D., & Ramassamy, C. (2017). Optimization of curcumin-loaded PEG-PLGA nanoparticles by GSH functionalization: Investigation of the internalization pathway in neuronal cells. *Molecular Pharmaceutics, 14*(1), 93–106.
91. Cui, Y., Zhang, M., Zeng, F., Jin, H., Xu, Q., & Huang, Y. (2016). Dual-targeting magnetic PLGA nanoparticles for codelivery of paclitaxel and curcumin for brain tumor therapy. *ACS Applied Materials & Interfaces, 8*(47), 32159–32169.
92. Nam, M., Lee, J., Lee, K. Y., & Kim, J. (2018). Sequential targeted delivery of liposomes to ischemic tissues by controlling blood vessel permeability. *ACS Biomaterials Science & Engineering, 4*(2), 532–538.
93. Taghizadehghalehjoughi, A., Hacimuftuoglu, A., Cetin, M., Ugur, A. B., Galateanu, B., Mezhuev, Y., et al. (2018). Effect of metformin/irinotecan-loaded poly-lactic-co-glycolic acid nanoparticles on glioblastoma: *In vitro* and *in vivo* studies. *Nanomedicine, 13*(13), 1595–1606.
94. Shin, J., Yin, Y., Park, H., Park, S., Triantafillu, U. L., Kim, Y., et al. (2018). p38 siRNA-encapsulated PLGA nanoparticles alleviate neuropathic pain behavior in rats by inhibiting microglia activation. *Nanomedicine, 13*(13), 1607–1621.
95. Ahmad, N., Ahmad, R., Alam, M., & Ahmad, F. (2018). Quantification and brain targeting of eugenol-loaded surface modified nanoparticles through intranasal route in the treatment of cerebral ischemia. *Drug Research* [Internet]*, 18*. Retrieved September 10, 2018, from http://www.thieme-connect.de/DOI/DOI?10.1055/a-0596-7288
96. Serralheiro, A., Alves, G., Fortuna, A., & Falcão, A. (2015). Direct nose-to-brain delivery of lamotrigine following intranasal administration to mice. *International Journal of Pharmaceutics, 490*(1–2), 39–46.
97. Gartziandia, O., Egusquiaguirre, S. P., Bianco, J., Pedraz, J. L., Igartua, M., Hernandez, R. M., et al. (2016). Nanoparticle transport across *in vitro* olfactory cell monolayers. *International Journal of Pharmaceutics, 499*(1–2), 81–89.
98. Bonaccorso, A., Musumeci, T., Serapide, M. F., Pellitteri, R., Uchegbu, I. F., & Puglisi, G. (2017). Nose to brain delivery in rats: Effect of surface charge of rhodamine B labeled nanocarriers on brain subregion localization. *Colloids and Surfaces. B, Biointerfaces, 154*, 297–306.
99. Kaur, S., Manhas, P., Swami, A., Bhandari, R., Sharma, K. K., Jain, R., et al. (2018). Bioengineered PLGA-chitosan nanoparticles for brain targeted intranasal delivery of antiepileptic TRH analogues. *Chemical Engineering Journal, 346*, 630–639.
100. Abouhussein, D. M. N., Khattab, A., Bayoumi, N. A., Mahmoud, A. F., & Sakr, T. M. (2018). Brain targeted rivastigmine mucoadhesive thermosensitive in situ gel: Optimization, *in vitro* evaluation, radiolabeling, *in vivo* pharmacokinetics and biodistribution. *Journal of Drug Delivery Science and Technology, 43*, 129–140.

101. Yan, X., Xu, L., Bi, C., Duan, D., Chu, L., Yu, X., et al. (2018). Lactoferrin-modified rotigotine nanoparticles for enhanced nose-to-brain delivery: LESA-MS/MS-based drug biodistribution, pharmacodynamics, and neuroprotective effects. *International Journal of Nanomedicine, 13*, 273–281.
102. Meng, Q., Wang, A., Hua, H., Jiang, Y., Wang, Y., Mu, H., et al. (2018). Intranasal delivery of Huperzine A to the brain using lactoferrin-conjugated N-trimethylated chitosan surface-modified PLGA nanoparticles for treatment of Alzheimer's disease. *International Journal of Nanomedicine, 13*, 705–718.

Chapter 4
Surface-Functionalized Lipid Nanoparticles for Site-Specific Drug Delivery

Diana P. Gaspar and António J. Almeida

Abstract Nanoparticles have been sought as drug carriers to increase bioavailability at the target site of action, improving drug therapeutic index. Among the several nanoparticulate systems proposed in the field of pharmaceutical technology, lipid-based nanoparticles (NPs) have attracted increasing attention due to their unique size-dependent properties, use of common well-tolerated, pharmaceutically accepted excipients, thus allowing for developing new delivery systems that could hold great promise for attaining the bioavailability enhancement along with controlled and site specific drug delivery. In the last years, passive and active modification approaches have been proposed to surface functionalization of lipid NPs intended for specific targeting. Internalizable ligands, specific targeted peptides, saccharide ligands, or even therapeutic molecules (e.g. antibodies or enzymes) are used for this purpose. Physicochemical properties of NPs can also be adjusted to improve drug targeting. The present chapter describes the recent advances in surface functionalization of lipid NPs, either solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC), lipid–drug conjugate nanoparticles (LDC), or lipid nanocapsules (LNC) with specific ligands to enhance drug targeting performance as well as its therapeutic effect.

Keywords Lipid nanoparticles · Active targeting · Passive targeting · Surface functionalization · Cell/receptor-specific ligands · Target site

1 Introduction

For the last 30 years particulate systems have been extensively investigated as drug delivery carriers to prevent or treat diseases. After administration, many active substances might suffer premature degradation, or elimination resulting in rapid clearance from the target site, reducing the therapeutic effect [1]. In this context,

D. P. Gaspar · A. J. Almeida (✉)
Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal
e-mail: aalmeida@ff.ulisboa.pt

Y. V Pathak (ed.), *Surface Modification of Nanoparticles for Targeted Drug Delivery*, https://doi.org/10.1007/978-3-030-06115-9_4

nanoformulations are extremely useful to overcome the challenges created not only by many active substances, such as poor drug solubility, physicochemical stability, permeability, and good access to the site of action, but also by extending their therapeutic and commercial value of existing drugs [2, 3]. Therefore, microencapsulation or nanoencapsulation into particulate systems emerged as an alternative to overcome those physicochemical and biological barriers [4–6].

Development of nanoparticles (NPs, most commonly referred as solid colloidal particles ranging in size from 1 to 1000 nm) for drug delivery began in the 1960s with the works of Peter Paul Speiser [7]. Due to their small size, they exhibit unique physicochemical and biological characteristics (e.g., an enhanced reactive area as well as an ability to overcome cell and tissue barriers) that make them a favorable material for biomedical applications, while promoting uptake by the cells [8]. They are able to carrying the therapeutic molecule to the site of action, thus minimizing unwanted side effects. In addition, they also protect the drug from rapid degradation or clearance and enhance drug concentration in target tissues, therefore reducing therapeutic doses [8]. In theory, a perfect nanoparticulate drug delivery system should (1) target the molecule to the site of action sites and keep it there for the desired time; (2) guarantee minimum drug degradation during transportation to target; (3) enable drug transport into the cell (if the target site is intracellular); (4) be biocompatible, biodegradable, and nonantigenic; and (5) be easily formulated at an industrial scale [6].

Many nanoparticulate systems have been reported to carry hydrophilic and hydrophobic drugs, proteins, biological macromolecules, and nucleic acids for targeted delivery, or made for long-term systemic circulation [9]. Several strategies have also been proposed to associate and release the drug, manipulating the materials used to formulate NPs, as well as with their structural design. Drugs can be entrapped in the carrier matrix, encapsulated in a core or surrounded by a shell, chemically conjugated to the NP matrix (covalent bonds, electrostatic interactions, etc.) or bound to the particle's surface by adsorption [8–10]. Thus, numerous methods exist for synthesizing NPs based on the type of drug used and the desired delivery route. Once a protocol is chosen, the parameters must be tailored to create the best possible characteristics for the NPs. The most important characteristics of NPs include particle size and distribution, encapsulation efficiency, surface charge, hydrophobicity, and release characteristics [9].

NPs exist in a wide variety of nature, size, shape, and composition, including metals and inorganic particles, such as gold, silver, metal oxides, polysaccharides (alginate and chitosan), proteins (gelatin and albumin), synthetic polymers (polylactide-co-glycolide and poly-ε-caprolactone), lipid-based particles (liposomes, solid lipid nanoparticles, and nanoemulsions). Each material presents its own inherent physicochemical characteristics that can be engineered to trigger different biological responses [11].

As stated above, the ability to provide specific drug delivery to target sites is among the characteristics of an ideal drug delivery system. In this context, NPs functionalization, resulting in a stealth surface avoiding opsonization, is necessary to increase circulation times by escaping the mononuclear phagocyte system (MPS).

For that purpose, NPs may be conjugated, grafted, or coated with hydrophilic polymers (e.g. polyethylene glycol (PEG), affording steric stabilization, conferring stealth properties, and being the most frequent and successful approach to delay the circulation time of nanoparticulate systems and protein drugs in the blood flow) [12, 13]. Moreover, with the aim to improve targeting, the NPs surface can also be functionalized using specific ligands (aptamers, peptides, antibodies/antibody fragments, and small molecules, among others) [6]. Functionalization can also be performed to avoid some bioactivity problems that, according to several authors [14], may be caused by (1) poor drug loading (DL) capacity, which causes a pharmacologically deficient drug concentration at the target site; (2) premature or burst release of the drug after administration before reaching the action site; and (3) cytotoxicity after internalization into cells. Hence, improved, multifunctional particulate carriers with targeting properties are urgently needed [14]. In this context, functionalization of NPs has been investigated for passive or active drug targeting, using a variety of ligands that may be functionally attached to nanoparticulate carriers, offering binding and catalytic capacity, including various ligands, specific targeted peptides, saccharide ligands, and therapeutic molecules (e.g., antibodies or enzymes).

The present chapter focuses on the surface modification of lipid nanoparticles, either solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC), lipid–drug conjugate nanoparticles (LDC), or lipid nanocapsules (LNC) with specific molecules with the aim of improving its therapeutic performance.

2 Lipid-Based NPs

Among the pioneer works of Peter Paul Speiser is the first description of NPs based on solid lipids, proposed for oral administration and called lipid nanopellets [15]. Using a similar approach Abraham Domb reported in 1993 the so-called lipospheres [16]. In the 1990s, Rainer Müller described the solid lipid nanoparticles (SLN) made of solid physiological lipids, stabilized by surfactants [17] and produced by high-pressure homogenization [18], while Maria Rosa Gasco proposed the microemulsion-based technique [19]. At the same time, the group of Kristen Westesen was also working in the field [20]. Both preparation methods are easily scalable, which is an obvious advantage [21–25]. Another interesting advantage is the fact that of SLN preparation does not require organic solvents, thus minimizing the toxicity. Produced using different solid monoacylglycerols, diacylglycerols, and triacylglycerols (e.g., glyceryl distearate and stearate, tripalmitin and trimyristin, cetyl palmitate and stearic acid), SLN have received increasing attention over the last years because, depending on the incorporation model, they provide drug protection, suitable physicochemical stability, and biocompatibility, allowing for modified and targeted drug release [21, 23, 25–28]. In fact, three drug incorporation models have been established: (1) homogenous matrix, (2) drug-enriched shell, and (3) drug-enriched core [29], as represented in Fig. 4.1. The type of model and, consequently, the nanoparticle inner structure, will depend basically on the formulation

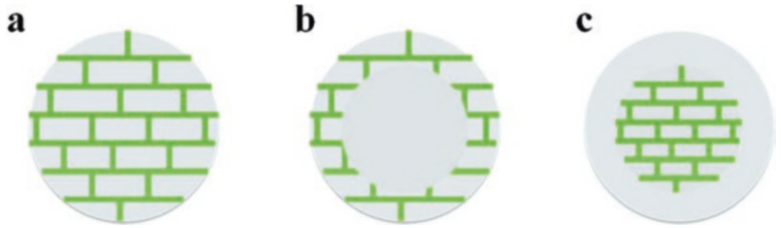


Fig. 4.1 Incorporation models of drugs (green) into SLN: (**a**) Type I—homogeneous matrix; (**b**) Type II—drug-enriched outer shell; (**c**) Type III—drug-enriched core with lipid shell

components and production conditions [23, 30, 31]. In the Type I model the drug is molecularly and homogeneously dispersed in the lipid matrix of the particles. Hence, drug release occurs via diffusion from the solid lipid matrix and/or by degradation of lipid matrix. This type of structure can provide controlled release properties. In the Type II model the drug is concentrated on the outer shell of the nanoparticles [23, 26, 32], since during preparation the lipid precipitates first forming a practically compound-free lipid core [23, 32]. This model is not suitable for prolonged drug release; nonetheless, it may be used to obtain a burst release of the drug [23, 26]. In contrast to drug-enriched shell model, the drug-enriched core model (Type III) is formed when precipitation of the drug is faster than lipid. Generally, prolonged drug release is observed from these SLN [23, 26, 32, 33].

However, some disadvantages of SLN have been pointed out, such as low DL capacity, drug expulsion after lipid polymorphic transition during storage and relatively high water content of the dispersions [17, 34–37]. To overcome some of these limitations, particularly to increase DL, modifications of the lipid structure have been introduced resulting in the so-called nanostructured lipid carriers (NLC) and lipid–drug conjugate (LDC) [38, 39] (Fig. 4.2). The NLC are composed of blends of solid and liquid lipids, improving DL and drug retention during storage [23]. In this case, the DL increased, while the drug loss during storage reduced by preventing the formation of perfect crystals [31].

In addition, SLN also present a reduced ability to encapsulate hydrophilic drugs, which will favorably partition to the outer aqueous phase during the formulation process [17, 29, 40]. Some authors have tried to increase the DL of hydrophilic drugs through solid lipid nanocapsules produced using a double emulsion (w/o/w) solvent evaporation method [41], which involves the use of organic solvents, bringing back the toxicological risk. For this reason, LDC were developed aiming at increasing the loading capacity for hydrophilic drugs [29, 39, 42]. LDC are based on a drug–lipid conjugation, which is then handled with an aqueous surfactant through a suitable preparation method [29, 42]. Overall, these three lipid-based nanosystems (SLN, NLC, and LDC) have been described for drug encapsulation with applications in diagnosis, prevention and treatment of diseases, as well as drug delivery by different administration routes (oral, rectal, topical, pulmonary, ocular, nasal, nose-to-brain, and parenteral) [43, 44].

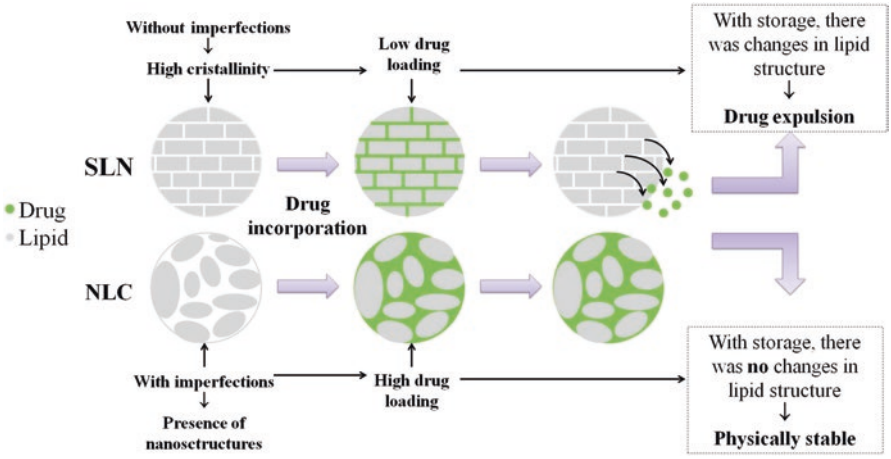


Fig. 4.2 SLN *versus* NLC. The main difference between SLN and NLC is the fact that the concept of the latter is performed by nanostructuring the lipid matrix, in order to increase DL and to prevent leakage, conferring more flexibility for drug release modulation. This goal is achieved by mixing solid lipids with liquid lipids (in NLC) instead of highly purified lipids with relatively similar molecules (as in SLN). The result is a less-ordered lipid matrix, with many imperfections, which can accommodate higher amounts of drug

3 Parameters Affecting the In Vivo Fate of Lipid NPs

Drug targeting is still one of the most complex tasks in pharmaceutical development. The involved phenomena depend on the nature of the drug and the carrier, the mode and duration desired for the delivery, and the methods used to prepare the NPs. In particular, the body recognizes hydrophobic particles as foreign and thus they are rapidly taken up by the MPS [9]. If the goal were to treat a condition in a MPS organ, the proper choice would be a hydrophobic NP system, which will be rapidly taken up by macrophages, ending in the liver or the spleen. However, if prolonged systemic circulation is required, the hydrophobic surface of NPs must be modified to prevent phagocytosis. Surface modification protects NPs from clearance from the blood, increasing both circulation time and drug uptake by target cells [45]. In addition, NPs surface may be functionalized with multivalent targeting moieties allowing not only to increase drug efficacy but also to decrease the dose [45]. The last 20 years have witnessed relevant advances in the strategies to enhancing NPs circulation time, reaching specific therapeutic targets through surface functionalization [6, 9] (Fig. 4.3). In general, active targeting means the directed homing of NPs to diseased sites by modifying particle surface with specific ligands, e.g. to biomarkers overexpressed in target cells. On the other hand, the passive targeting is related with the enhancement of permeability and retention (EPR) effect and refers the NPs ability to reach the target site only influenced by their physicochemical characteristics [45].

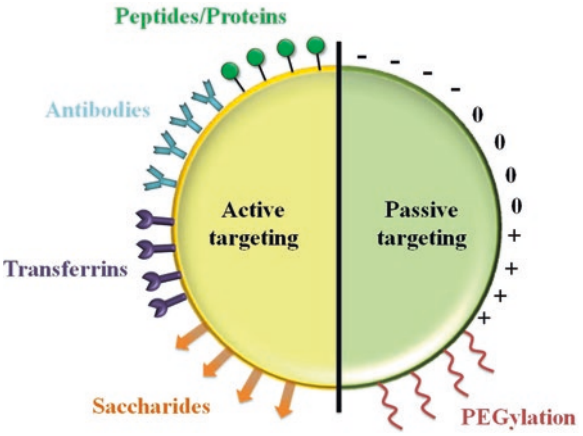


Fig. 4.3 Surface functionalization of NPs aiming at increasing circulation time, reducing nonspecific distribution and targeting specific tissues or cells. The chemistry at surface and bulk levels, as well as physical features such as size, porosity, surface topography, shape, and compartmentalization, should be carefully considered during the design/optimization and targeting of NPs for biomedical applications

Almost any physical modification made in NPs may have important repercussions in cellular uptake, biodistribution, and pharmacokinetics [12, 45–47]. These may be improved using passive targeting, providing a longer circulation by evading scavenging by the MPS. Passive targeting is closely related with the EPR which refers to the fact that some cells or organs (e.g., tumor cells) have the ability to concentrate more nanocarriers than other tissues [45]. This type of modification is also associated on both the nano-size of nanocarriers and the leaky tissue vasculature. Passively targeted NPs have been demonstrated to non-specifically enter the organ, tissue or cells as a mere consequence of their size and/or shape [48]. These are also influenced by the lymphatic system, which retains some particles, delaying their biodistribution and consequently their clearance. Overall, drug targeting occurs as a natural response to physicochemical characteristics of the molecules or drug carrier system and, in this aspect, lipid-based NPs are not different from other nanoparticulate carriers.

3.1 Influence of Particle Size

One of the major benefit of the NPs applicability is related with their submicron size, allowing for deep penetration into tissues through fine capillaries, crossing the fenestrations present in the epithelial lining (e.g., liver) [49]. Despite their reduced dimensions NPs size must be large enough to remain into blood capillaries [6]. If bloodstream accumulation is the goal, NPs must have a size between 20 and 200 nm

[50]. Nevertheless, particles larger than 2 nm cannot cross the tight junctions in normal vasculature. However, at a tumor condition, the tight junctions are dysfunctional, allowing particles from 10 to 500 nm to accumulate within the tumor interstitium [45]. This also occurs in inflammatory sites [51]. Other size-related advantages of NPs, over larger microparticles, include their better ability to cross the blood–brain barrier (BBB) [6, 12].

After intravenous injection actarit-loaded SLN of 241 ± 23 nm presented plasma concentrations 1.88-fold greater than those of actarit dissolved in 50% propylene glycol solution [52]. It was recently shown that NLC improved saquinavir permeability across the intestinal barrier up to 3.5-fold, through a particle size-dependent mechanism [53].

3.2 Influence of Surface Charge

The surface charge is one of the crucial physical properties of NPs. In general, surface charge is mostly affected by particle composition, particularly the surfactants, with positively charged NPs being preferentially taken up by cells when compared to neutral or negatively charged ones [22, 54, 55]. So coating lipid nanoparticles with chitosan (CS) has become a common strategy to create a positively charged NPs. Chitosan is a natural biocompatible, biodegradable, and positively charged polysaccharide, presenting mucoadhesion and permeation-enhancing characteristics, thus becoming one of the most studied ligands for mucosal drug delivery [9, 56]. It also regulates the opening of tight junctions between adjacent epithelial cells, enabling drug paracellular transport, thus improving bioavailability at the target site [57]. In fact, after coating with CS, SLN containing ferulic acid and acetylsalicylic acid, presented a positive surface charge that improved mucoadhesion and NPs residence time in the negatively charged intestinal epithelium, thus increasing the drug concentration at the site of absorption [57]. Likewise, CS-coated Witepsol 85E SLN developed for intestinal delivery of insulin showed increased insulin retention time and permeation [58].

In a different study using the intravenous route, the biodistribution and hepatic accumulation of SLN with different surface charges was compared, revealing that anionic SLN achieved higher uptake and longer retention in the liver than cationic SLN, although these results may have been influenced by particle size (260 nm for anionic particles and 170 nm for cationic particles) [59]. The same type of comparison had already been performed to improve the oral absorption of salmon calcitonin [60]. Briefly, after oral administration in a murine model CS-coated lipid NPs produced a substantial decrease in serum calcium levels, whereas the PEG-coated NPs induced a response similar to that of the calcitonin solution. CS- and PEG-coated SLN intended for oral delivery of lipophilic drugs were also compared in vitro with similarly coated PLGA nanoparticles. The two coating materials were used to modify NPs surface in order to increase adhesion to the oral mucosa and, consequently,

improve drug absorption. Coating with the polycationic CS led to a higher interaction with Caco-2 cells and a limited uptake in THP1 cells, while coating with PEG led to a reduced uptake in Caco-2 cells and avoided uptake by THP1 cells [61]. Interestingly, both formulations contained retinol, which may have acted as a targeting ligand to the liver through the retinol binding protein.

3.3 Influence of Hydrophobicity

Biodistribution of intravenously injected lipid NPs is intimately related with their interaction with blood components. When in circulation, lipid NPs are quickly opsonized and cleared from the blood circulation by the MPS, ending mostly in the liver and spleen [6, 12, 62]. This phenomenon is related to surface hydrophobicity, which plays a role on the amount and type of opsonins adsorbed at the NPs surface, thus enhancing uptake by macrophages [6, 12, 51, 63]. If drug targeting for a specific site is required, opsonization must be reduced, and several strategies have been described to diminish capture by macrophages and increase the bloodstream circulation, such as formulating or functionalizing NPs with hydrophilic polymers and/or surfactants or even coating with biodegradable hydrophilic polymers, such as PEG (discussed in the next section), polysorbate 80, polyethylene oxide (PEO), and poloxamers [6, 9]. Among these excipients, polysorbate 80 and poloxamer 188 have also been studied as coating materials for SLN formulations intended for brain targeting, to promote particle translocation across the blood–brain barrier (BBB) through the adsorption of apolipoproteins [62]. Apolipoprotein E (ApoE) simulates low density lipoprotein promoting NPs translocation into the brain [9]. Polysorbate 80-coated SLN had a higher ability to cross the BBB due to specific binding to ApoE, while poloxamer 188-NPs interacted with many apolipoproteins as well, but not with ApoE [62, 64–67]. Subsequently, the same authors correlated the ApoE amount bound to SLN with the PEO monomer chain length on the poloxamer coated-lipid NPs, concluding that there was a higher ApoE adsorption when small PEO chains were attached to the SLN [68]. The effect of coating lipid nanocapsules with poloxamer, CS and PEG on the immune responses was also reported [69]. Poloxamer-coated lipid nanocapsules modulated the innate immunity by inducing monocyte activation, while CS-coated lipid nanocapsules stimulated monocytes and T-lymphocytes, and PEG-coated particles seemed to be inert for the immune system. In the same way, the encapsulation of a neuroactive drug (URB597) polysorbate 80-coated SLN improved blood circulation time and brain targeting [70]. Recently, polymer–lipid hybrid NPs were developed to enhance intestinal absorption of cabazitaxel. The particles' surface was modified (1) with octadecylamine and poloxamer 188 to produce positively charged particles and (2) with PEO to increase hydrophilicity. This construct improved drug oral adsorption by rapidly linking to and accumulating at the intestinal mucus surface, facilitating enterocyte uptake [71].

4 Passive Targeting Based on Pegylation

PEGylation is a physicochemical approach where PEG chains are conjugated at the NPs surface with the aim to prolong residence time in the bloodstream [51, 72]. This is achieved due to the fact that PEG chains have the ability to decrease proteins adsorption on the NPs surface providing opsonization resistance. Moreover, attaching PEG molecules to NPs surface allows for circumventing capture by the MPS, making PEGylation the most common strategy for NPs surface functionalization [50, 73, 74]. PEG is a biocompatible hydrophilic nonionic polymer that can be associated to the NPs using a several approaches, such as covalent bonding during NPs formulation or by surface adsorption/coating in a post-production step [9, 62].

At the NPs surface PEG chains may acquire a low-density configuration (mushroom configuration) and a high-density configuration (brush-type configuration), which highly influence the efficacy of PEGylation. According to some authors, mushroom-like structures elicit complement activation and phagocytosis by MPS while brush-like configurations decreased those processes [12, 51, 75].

PEGylation is also a useful specific-targeting tool for cancer treatment using lipid nanoparticles, since it enhances EPR effect minimizing the non-specific uptake by normal cells [76, 77]. Thus, it is frequently used in treatment of tumors, since PEG chains are naturally cleaved by matrix metalloproteinases (MMP), whose concentration is increased at tumor's surrounding areas [78]. In fact, docetaxel-loaded LNC coated with PEG-distearoylphosphatidylethanolamine (DSPE) enhanced NPs accumulation at tumors in a murine model of colon adenocarcinoma, when compared to the free drug [79]. In addition, the longer the PEG chains, the greater was NPs accumulation at the target sites, due to increased particles residence time in the bloodstream. Different research groups have corroborated these observations [80–83]. Remarkably, Zheng et al. developed paclitaxel (PTX)-loaded SLN for tumor targeting, with reversibly associated PEG chains, confirming the strategy increased the circulation time and, after reaching the tumor site, PEG chains were cleaved by tumor-secreted MMPs, promoting drug uptake [84] (Fig. 4.4).

PEGylation is also an important approach for oral administration, since it improves the traffic of biomolecules across gastrointestinal mucus [85]. In a comparative study between PEG_{2000}-stearic-modified SLN and unmodified ones, the functionalized NPs showed a reduced permeation through Caco-2 cell monolayers while an increment was observed in a mucus-secreting Caco-2/HT29 coculture cell monolayer. Furthermore, PEGylated SLN could easily cross the mucus layer, while the unmodified NPs were entrapped in the intestinal mucus [85]. Recently, PEG monostearate (PEG-SA) surface-modified SLN provided improved permeability of poorly absorbed drugs across epithelial cells, with a 70-fold increase in bioavailability when compared to drug solution [86].

Despite being a most useful targeting tool due to inhibition of nonspecific connections with serum components, PEGylation may hinder the interaction of siRNA-loaded nanocarriers with endosomal membranes, leading to a poor cytosolic delivery. Hashiba et al. optimized a nanosystem composed by PEGylated siRNA-loaded SLN,

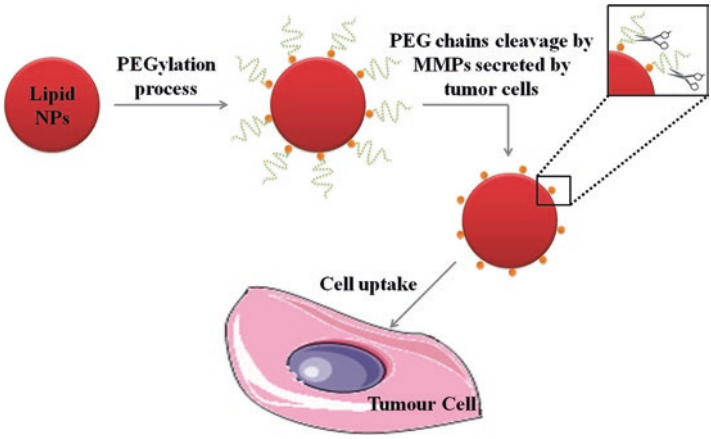


Fig. 4.4 Diagram representing the drug targeting process occurring when PEG-modified lipid NPs reach the tumor target site, as described in [84]. PEG chains suffered specific-cleavage by MMPs produced in situ, favoring drug release and then NPs uptake by tumor cells

aiming at overpassing this limitation. The functionalization was made with PEG chains through maleic anhydride, a pH-sensitive linker. In vivo studies demonstrated a pH-labile detachment of PEG chains from SLN that increased the efficiency of siRNA delivery to hepatocytes [87].

In order to improve tumor permeability and reduce nonspecific uptake by the MPS, hybrid nanosystems composed of pH-sensitive cationic PEG-modified SLN have been proposed [88]. In vivo experiments carried out with these nanoparticles demonstrated high nanocarrier concentration in various types of tumors, as well as a clear improvement in therapeutic efficiency of the encapsulated drug [88].

5 Active Targeting

However, passive targeting has some associated problems, such as deficient biodistribution since lipid NPs are mostly retained in the main MPS organs (i.e., liver and spleen), reducing site specific drug delivery [45, 89]. Therefore, novel targeting strategies are needed, mainly for extending blood circulation, while allowing intracellular drug delivery [45]. Several approaches are currently used to take advantage of the stealth effect of PEGylation, by adding other targeting moieties through covalent linking of PEG chains to reactive carboxylic acids, amines, or sulfhydryl groups present in different targeting ligands [6]. This type of functionalization increases selectivity, facilitates site-specific delivery, and results in less adverse effects with enhanced therapeutic efficacy [6, 45].

Active targeting emerged as a strategy to deliver active substances specifically to a certain organ, tissue, cell or intracellular compartment by binding these specific

moieties to particular ligands expressed on, or around, the target local due to highly specific interactions between the targeting ligand and biomolecules [90, 91]. These targeting molecules can also facilitate the translocation of NPs across the cellular membranes of the diseased tissue [50]. The potential of a lectin-functionalized SLN formulation containing insulin was evaluated in vivo, following oral administration to rats, and showed to promote the absorption of insulin, thus providing higher pharmacological bioavailability than the unmodified formulation [92, 93].

Many targeting moieties have been described, such as vitamins, glycoproteins, antibodies and fragments, peptides, and aptamers [6, 50]. A variety of ligands may be functionally linked to lipid NPs, including various therapeutic molecules (antibodies or enzymes), specific targeted peptides, saccharide ligands, and internalizing ligands (biotin, folic acid, transferrin) (Table 4.1).

5.1 Functionalization with Antibody Ligands

Due to their small size and low immunogenicity, decorating lipid NPs with antibody ligands has been proposed to improve site-specific delivery in cancer therapy [13]. Modification can be directly made at NPs surface or through linker ligands, such as PEG, using either a random or a site-specific ligation, using simple formulation processes that are easy to scale up [13, 104]. As antibodies are able to identify specific sites of a precise target, they provide the opportunity to distinguish a diversity of antigens, or binding sites [105].

For example, several attempts have been made to use this strategy to overcome the BBB. Surface-functionalized SLN with 83-14 monoclonal antibody presented reduced phagocytosis by the MPS and lymphatic NPs uptake, with enhanced drug bioavailability through brain endothelia [106]. Remarkably, PEG-SLN functionalized with antibody Fas ligand that is specifically expressed on ischemic brain efficiently delivered the neuroprotective drug butylphthalide to ischemic brain regions in a murine model [107]. Also with the purpose of overcoming the BBB, PEGylated SLN were functionalized with anti-contactin-2 or anti-neurofascin, using the carbodiimide reaction (Fig. 4.5). Contactin-2 and anti-neurofascin are two axo-glial glycoprotein antigens considered as the main targets of autoimmune reaction in multiple sclerosis. Functionalization anti-contactin-2 or anti-neurofascin improved NPs uptake by U87MG cells by 4- and 8-fold, respectively, when compared to controls. Moreover, these modified nanocarriers are also able to cross the BBB, being a promising strategy for multiple sclerosis treatment [108].

Some studies demonstrate the versatility of antibody surface functionalization of lipid NPs. As T lymphocyte transfection is usually difficult to achieve, lipid NPs functionalized with anti-CD4 monoclonal antibody were developed to enable siRNA delivery to murine $CD4^+$ T cells, thus allowing manipulating T cell functionality and treat leukocyte-associated diseases. The so-produced nanoformulation specifically bound to and was taken up only by primary $CD4^+$ T lymphocytes in different organs without reaching the other cell types [109]. Delivery of siRNA was

Table 4.1 Some examples of molecules incorporated into surface-modified lipid nanoparticles

Encapsulated drug	Lipid matrix	Surface modification	Advantages	Nanosystem	Ref.
–	Glyceryl behenate and cetylpalmitate	Hydrophilic poloxamine 908 and poloxamer 407 block-copolymers coating	Reduction of the phagocytic uptake by the RES after i.v. injection	SLN	[63]
Salmon calcitonin	Tripalmitin or a mixture of a liquid and a solid triglyceride (Miglyol® 812 and tripalmitin)	CS coating	Slow release of the associated peptide	SLN	[94]
Insulin	Steric acid	Wheat germ agglutinin-*N*-glutaryl-phosphatidylethanolamine (PE) coating	Insulin oral absorption	SLN	[92]
Quinine dihydrochloride	Hydrogenated soya phosphatidyl choline, triolein, cholesterol and DSPE	Transferrin conjugation	Improved brain targeting	SLN	[95]
pEGFP	Glyceryl monostearate	Mannan-based PE-grafted ligand coating	Active targeting for gene delivery	SLN	[96]
Carvedilol	Glyceryl monostearate and soya lecithin	*N*-carboxymethyl CS coating	Improved drug bioavailability after oral administration	SLN	[97]
5-Fluorouracil	Glyceryl monostearate	Galactosylation	Increased uptake by hepatic cells	NLC	[98]
Docetaxel and ketoconazole	Glyceryl monostearate	Folic acid modification	Increased brain permeation	SLN	[99]
siRNA	Cholesterol, 1,2-dioleoyl-3-trimethylammoniumpropane and DOPE (1,2-dioleoyl-sn-glycero-3-phosphoethanolamine	PEGylation	In vivo melanoma- metastasis targeting	LNC	[100]
Salmon calcitonin	Soybean phosphatidyl choline, stearic acid and tripalmitin	Cell-penetrating peptides (CPP) conjugation	Enhanced SLN intestinal adsorption	SLN	[101]
Dexamethasone	Steric acid	Folate coating	Improved cell targeting ability	SLN	[102]
Curcumin	Palmitic acid	*N*-trimethyl CS conjugation	Prolonged stability with controlled release and brain targeting	SLN	[103]

SLN solid lipid nanoparticles, *NLC* nanostructured lipid carriers, *LDC* lipid nanocapsules

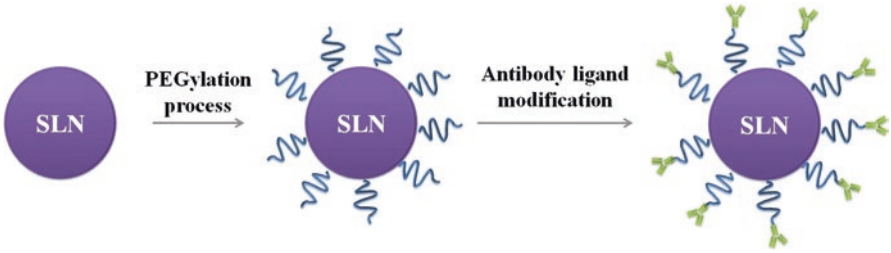


Fig. 4.5 Representation of PEGylated NPs with modified surface using antibodies. Based on the construct described in [108]

also achieved using a nanosystem based on ionisable cationic lipids, functionalized with coating NPs with a single-chain antibody, specific to murine endocytic receptor DEC205, which is expressed at high levels by dendritic cells, which are major antigen presenting cells. This resulted in a clear gene knockdown leading to a considerable inhibition of some immune responses [110].

Association with lipid NPs may avoid the rapid blood clearance of antitumor single chain antibody fragments as shown by Wong et al. [111], who modified lipid NPs with an anti-prostate membrane antigen single chain through the copolymer DSPE-PEG-maleimide that self assembles into nanoparticles. These showed a significant increase in tumor uptake and tumor targeting over the blank NPs. In another study, an anti-epidermal growth factor receptor variant III (anti-EGFRvIII) monoclonal antibody was linked to doxorubicin (Dox)-NLC improving targeting specificity to EGFRvIII-overexpressing tumor cells [112].

5.2 Functionalization with Saccharide ligands

Saccharide moieties have gained increased attention as surface-modification ligands since they are present in all type of cells mediating many biological processes, such as cell-cell communication, proliferation and differentiation, tumor metastasis, inflammatory or viral response [113].

Mannosylation is a most studied active functionalization strategy based on saccharide ligands, since mannose receptors are highly expressed on macrophages. Thus, it is suitable for specific delivery of antimycobacterial drugs to alveolar macrophages. In preliminary experiments, internalization studies revealed higher internalization rates of mannosylated SLN presented when compared to blank SLN [114]. Actually, while pulmonary applications of mannosylated SLN were also investigated for drug targeting in lung cancer treatment [115], their main purpose is still antimycobacterial drug delivery by promoting internalization by alveolar macrophages. Investigators have proposed pH dependent mannosylated NLC, which favored rifabutin release under the acidic environment of phagosomes and

phagolysosomes, where *Mycobacterium tuberculosis* usually persists [116]. Similar studies have been carried out using mannosylated NLC containing rifampicin, which were easily and specifically internalized by NR8383 cells and alveolar macrophages [117]. Recently, isoniazid-loaded mannosylated SLN, presenting a positive surface charge, were more efficiently internalized by macrophages than unmodified SLN. Previous incubation of macrophages with mannose reduced particle uptake, suggesting the saturation of receptors and that phagocytosis could be mediated by mannose receptors [118]. The same approach was adopted for targeted gene delivery to alveolar macrophages. These authors modified SLN surface with mannan-based PE-grafted ligands. The resulting functionalized NPs provided higher gene expression, making this nanosystem suitable for specific uptake as well as active targeting to precise cell lines, such as alveolar macrophages [96].

Surface NPs modification with hyaluronic acid (HA) has also become an interesting platform due to its selectivity for cell binding and uptake, since HA is a negative hydrophilic linear polysaccharide, preventing unspecific adsorption, thus reducing MPS internalization. HA is the major extracellular matrix component, binding specifically to CD44 cell membrane receptor, which are overexpressed in tumor diseases [105, 119, 120]. Therefore, HA-modified SLN have been engineered for tumor targeting, being easily internalized by CD44 overexpressing cells. Furthermore, the presence of HA in lipid NPs provided longer blood circulation and improved the bioavailability of vorinostat [120]. Similarly, HA-coated SLN containing PTX were efficiently internalized in $CD44^+$ lung tumor model, leading a high in vitro cell death [121].

5.3 Functionalization with Transferrins

Transferrins (Tf) are serum non-heme iron-binding glycoproteins that are internalized by cells via receptor-mediated endocytosis after binding to their specific cell surface receptors. As tumoral and drug-resistant cells have a high amount of Tf receptors at the surface compared to healthy cells, functionalization of NPs through surface adsorption with Tf has emerged as a valuable strategy for specific cell targeting [13, 45, 51, 105, 122]. Tf surface-modified-NLC for PTX delivery were efficiently and specifically taken up in relevant cell lines, presenting high antitumor activity both in vitro and in vivo [123]. Lipid NPs functionalized with Tf, containing siRNA LOR-1284 for tumor treatment not only protected the siRNA from serum nuclease degradation, but also had a longer circulation time, enhanced accumulation at the tumor, and specific internalization by MV4-11 cells, with high reduction levels of the R2 mRNA in a murine xenograft model [124].

Within the Tf family lactoferrin (Lf), also called lactotransferrin, is an interesting mammalian cationic iron-binding glycoprotein, whose receptors are mainly restricted to the BBB and the surface of gliobastoma cells [105, 125]. Docetaxel-loaded SLN targeted with Lf were developed with the goal to cross the BBB, enhancing brain uptake and improving drug efficacy [125]. Similar results were

obtained by other investigators who reported a tenfold increase in drug BBB permeability using Lf-modified SLN [126], and a 5.6-fold increase using catanionic SLN functionalized with Lf and a lectin (wheat germ agglutinin) [127].

5.4 Functionalization with Cell-Penetrating Peptides

Surface modification based on cell-penetrating peptides (CPP) is one of the most used platform to promote the uptake of lipid NPs by target cells since they have the ability to bind with high specificity to many targets. CPP are membrane permeable short sequences of 5–30 cationic and/or amphiphilic peptides, like as arginine, lysine, and leucine [50, 128–130]. Modification of lipid NPs with CPP promotes drug delivery by endocytosis or direct penetration associated to the positive charge of CPP [50, 130–133]. CPP derived from protamine were used to modify lipid NPs intended for siRNA delivery, confirming that nanoencapsulation led to high siRNA stability in vivo, and high uptake into B16F10 murine melanoma cells, followed by release in the cytoplasm, while nontargeted NPs were poorly internalized by the same cells. Functionalized NPs were mostly internalized via macropinocytosis and heparan sulfate-mediated endocytosis promoting an enhanced gene silencing [134]. Folic acid and a tumor microenvironment-sensitive polypeptide were used to modify NLC with the goal to target the folate receptor and the up regulation of matrix metalloproteinase-2 (MMP-2) in tumor microenvironment. The nanosystem reached the tumor via the EPR effect, allowing for specific binding to folate receptors, followed by CPP action triggered by MMP-2 protease oversecretion, leading to enhanced tumor cell recognition and internalization, as well as tumor cell apoptosis [133]. This interaction between CPP and MMP, is particularly important due to the MMP-2 overexpression in the tumor microenvironment [135]. Thus, functionalization of NPs with MMP-2 specific sequences is becoming an encouraging approach to cancer treatment. As PEGylation enables prolonged blood circulation, a combined platform involving PEGylated NPs modified with MMP-substrate peptide has been proposed [84]. Briefly, CPP was conjugated with PEG chains to take advantage of a stealth effect, allowing for increased uptake by tumor cells, without side effects. Based on this approach, LNC were loaded by adsorption with the neurofilament-derived peptide NFL-TBS.40-63 (NFL) for neural stem cell targeting. It was found that NFL-LNC were not taken up by spinal cord neural stem cells but were specifically internalized by neural stem cells from the brain [136]. Another interesting CPP used in drug delivery is the HIV-1 derived transactivator of transcription (TAT), which presents a high fraction of positively charged amino acids [50]. It enhances biomolecule internalization, decreasing the adverse effects while overpassing drug resistance. When conjugated with SLN containing PTX and α-tocopherol succinate-cisplatin prodrug, it resulted in increased antitumor activity against cervical cancer, showing a synergistic outcome in the reduction of cervical tumor cell development in comparison with nontailored [137]. In a different study, NLC were functionalized with chitosan-linked CPP and their ability to penetrate in vitro olfactory cell

monolayers was assessed as an alternative nose-to-brain drug delivery system [138]. Finally, the potential of SLN loaded with a CPP (octaarginine) for oral administration of insulin was evaluated in vivo with promising results [139].

5.5 *Functionalization Based on Avidin–Biotin Affinity*

The avidin–biotin affinity strategy is considered one of the most used strategies for NPs functionalization because it is a stable noncovalent interaction that is resistant to proteolytic enzymes, harsh temperatures and organic solvents, pH and other denaturing agents, being far more stable than antigen–antibody interactions. It is based on the specific molecular recognition of biotin (vitamin B_7) by the four subunits of its specific receptor, the globular protein avidin. As biotin is a low-molecular-weight molecule, with a free carboxylic group accessible for chemical modification (e.g., $–NH_2$ or –NHS), NPs modification with avidin–biotin system is a relatively easy and stable process (Fig. 4.6) [140, 141].

Based on this approach, a novel SLN formulation has been proposed for brain targeting, with the goal to enhance the permeability through the BBB. The SLN were modified with biotinylated ApoE, without compromising their activity, through the conjugation of active avidins at the surface NPs using DSPE-PEG-avidin or a palmitate-avidin conjugate, originating two different ApoE-functionalized SLN: SLN-DSPE-ApoE and SLN-palmitate-ApoE [142]. In a similar study, the same authors developed a novel system based on resveratrol-encapsulated SLN modified

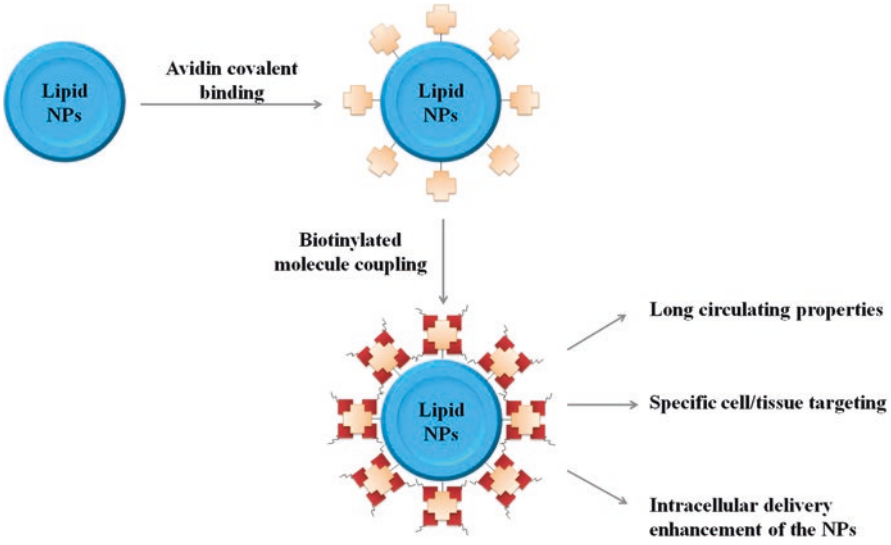


Fig. 4.6 Surface functionalization based on avidin conjugation with subsequent biotinylated molecule attachment

with ApoE. This nanocarrier had the ability to specifically identify the low-density lipoprotein receptors, which overexpressed on the BBB. The authors concluded that targeted SLN were protected from degradation in circulation and increased their permeability in relevant cell lines [143]. DOX-containing SLN were surface-functionalized with epidermal growth factor (EGF) for liver tumor treatment since its receptor (EGFR) is also overexpressed in many tumor cells. The strategy consisted of adsorbing avidin molecules at the NPs surface, followed by coupling with biotinylated EGF and resulted in a significant tumor reduction comparing to unmodified NPs [144].

5.6 The Antiangiogenic Strategy

Endothelial cells express a large number of integrins at their surface with ability to change cell proliferation, differentiation and migration at some point in angiogenesis step. This allows an antiangiogenic active drug targeting approach for the upregulated cell surface receptors mainly on tumor neovasculature [145]. The majority of tumors and tumor vascular endothelium present an overexpression of $\alpha_v\beta_3$, an integrin that is a receptor for extracellular matrix that contains the tripeptide Arg-Gly-Asp sequence [145]. A $\alpha_v\beta_3$-targeted SLN formulation through conjugation to the $\alpha_v\beta_3$ integrin-specific ligand cyclic Arg-Gly-Asp (cRGD) was suggested for overcoming the poor hepatic accumulation of nonfunctionalized SLN [146]. Targeted RGD-SLN presented high distribution in the liver, spleen, and kidneys, as well as higher tumor accumulation and tumor residence time. Another research group constructed Tyr-3-octreotide-modified PEGylated SLN loaded with PTX to promote recognition by the subtype 2 somatostatin receptors that are overexpressed in tumor neovasculature, mainly in endothelial and glioma cells [147]. The so modified SLN promoted, both in vitro and in vivo, the PTX-induced apoptosis and the PTX antiangiogenic capacity. Moreover, the modified SLN also had a greater specific retention at the glioma site with an improved anti-glioma performance over unmodified nanocarriers.

6 Conclusions

The physicochemical properties of lipid NPs play a major role in the in vitro and in vivo behavior of lipid NPs, influencing mainly their biodistribution. Passive targeting is highly affected by particle size, since NPs with size <7 nm are mainly retained by renal filtration and then excreted by urine, while NPs higher than 100 nm are usually withdrawn from the bloodstream by the MPS. Surface charge also affects NPs' performance. For example, positively charged NPs are favorably internalized by cells. Surface modification of lipid NPs with hydrophilic polymers allows higher circulation half-lives and reduced clearance by the MPS, thus enabling

NPs accumulation at the target site, as in the specific case of PEGylation. Many other hydrophilic polymers or surfactants, such as chitosan, hyaluronic acid, dextran, polyvinyl pyrrolidone (PVP), polyvinyl alcohol (PVA), poloxamines, and poloxamers, have been used for stealth coating of NPs. However, passive targeting can have a negative effect on NPs internalization into relevant cell lines. Thus, surface functionalization with specific moieties emerges as an alternative to overpass this limitation, resulting in active drug targeting. This strategy involves surface tailoring of NPs with cell/receptor-specific ligands, enhancing NP interactions with the cells through ligand recognition by receptors present on the cell's surface and leading to NPs uptake via receptor-mediated mechanisms. Such is the case of Tf, antibodies and their fragments, and biotin, which are frequently used as NP functionalization ligands because their receptors are overexpressed on many diseased cells, enhancing NP uptake and improving the therapeutic performance. Recently, dual-targeting NPs have been described as an alternative to single-targeting nanocarriers since they have a synergetic effect by binding two different target sites. This strategy, in addition to increasing NPs' affinity towards their target site, promotes the reduction the off-target binding to cells.

Acknowledgments The authors acknowledge FEDER and Fundação para a Ciência e Tecnologia, Portugal, for financial support (UID/DTP/04138/2013, SFRH/BD/89520/2012 and SFRH/BSAB/1210/2011).

References

1. Lundqvist, T., & Bredenberg, S. (2013). Pharmaceutical development. In R. G. R. H. Hill (Ed.), *Drug discovery and development – Technology in transition* (pp. 227–238). Edinburgh: Churchull Livingstone/Elsevier.
2. Peppas, N. A. (2013). Historical perspective on advanced drug delivery: How engineering design and mathematical modeling helped the field mature. *Advanced Drug Delivery Reviews, 65*(1), 5–9.
3. De Koker, S., De Cock, L. J., Rivera-Gil, P., Parak, W. J., Velty, R. A., Vervaet, C., et al. (2011). Polymeric multilayer capsules delivering biotherapeutics. *Advanced Drug Delivery Reviews, 63*(9), 748–761.
4. Bao, G., Mitragotri, S., & Tong, S. (2013). Multifunctional nanoparticles for drug delivery and molecular imaging. *Annual Review of Biomedical Engineering, 15*(1), 253–282.
5. Lima, A. C., Alvarez-Lorenzo, C., & Mano, J. F. (2016). Design advances in particulate systems for biomedical applications. *Advanced Healthcare Materials, 5*(14), 1687–1723.
6. Gaspar, D., Faria, V., Quintas, Q., & Almeida, A. J. (2017). Targeted delivery of lipid nanoparticles by means of surface chemical modification. *Current Organic Chemistry, 21*(1), 2360–2375.
7. Birrenbach, G., & Speiser, P. (1976). Polymerized micelles and their use as adjuvants in immunology. *Journal of Pharmaceutical Sciences, 65*(12), 1763–1766.
8. Wilczewska, A. Z., Niemirowicz, K., Markiewicz, K. H., & Car, H. (2012). Nanoparticles as drug delivery systems. *Pharmacological Reports, 64*(5), 1020–1037.
9. Hans, M., & Lowman, A. (2002). Biodegradable nanoparticles for drug delivery and targeting. *Current Opinion in Solid State and Materials Science, 6*(4), 319–327.
10. Sung, J. C., Pulliam, B. L., & Edwards, D. A. (2007). Nanoparticles for drug delivery to the lungs. *Trends in Biotechnology, 25*(12), 563–570.

11. Naahidi, S., Jafari, M., Edalat, F., Raymond, K., Khademhosseini, A., & Chen, P. (2013). Biocompatibility of engineered nanoparticles for drug delivery. *Journal of Controlled Release, 166*(2), 182–194.
12. Singh, R., & Lillard, J. W. (2009). Nanoparticle-based targeted drug delivery. *Experimental and Molecular Pathology, 86*(3), 215–223.
13. Byrne, J. D., Betancourt, T., & Brannon-Peppas, L. (2008). Active targeting schemes for nanoparticle systems in cancer therapeutics. *Advanced Drug Delivery Reviews, 60*(15), 1615–1626.
14. Couvreur, P. (2013). Nanoparticles in drug delivery: Past, present and future. *Advanced Drug Delivery Reviews, 65*(1), 21–23.
15. Speiser, P. (1990). Lipidnanopellets als Trägersystem für Arzneimittel zur peroralen Anwendung.
16. Domb, A. J., Bergelson, L., & Amselem, S. (1996). Lipospheres for controlled delivery of substances patent 0360-2583.
17. Schwarz, C., Mehnert, W., Lucks, J., & Müller, R. H. (1994). Solid lipid nanoparticles (SLN) for controlled drug delivery. I. Production, characterization and sterilization. *Journal of Controlled Release, 30*(1), 83–96.
18. Müller, R. H., & Lucks, J. (1996). Arzneistoffträger aus festen lipidteilchen, feste lipidnanosphären (SLN).
19. Gasco, M. R. (1993) Method for producing solid lipid microspheres having a narrow size distribution.
20. Siekmann, B., & Westesen, K. (1992). Submicron-sized parenteral carrier systems based on solid lipids. *Pharmaceutical and Pharmacological Letters, 1*(3), 123–126.
21. Lopes, R., Eleutério, C., Gonçalves, L. M. D., Cruz, M., & Almeida, A. J. (2012). Lipid nanoparticles containing oryzalin for the treatment of leishmaniasis. *European Journal of Pharmaceutical Sciences, 45*(4), 442–450.
22. Mancini, G., Lopes, R. M., Clemente, P., Raposo, S., Gonçalves, L., Bica, A., et al. (2015). Lecithin and parabens play a crucial role in tripalmitin-based lipid nanoparticle stabilization throughout moist heat sterilization and freeze-drying. *European Journal of Lipid Science and Technology, 117*(12), 1947–1959.
23. Souto, E., Almeida, A. J., & Müller, R. H. (2007). Lipid nanoparticles (SLN®, NLC®) for cutaneous drug delivery: Structure, protection and skin effects. *Journal of Biomedical Nanotechnology, 3*(4), 317–331.
24. Vitorino, C., Carvalho, F., Almeida, A. J., Sousa, J., & Pais, A. (2011). The size of solid lipid nanoparticles: An interpretation from experimental design. *Colloids and Surfaces B: Biointerfaces, 84*(1), 117–130.
25. Gaspar, D. P., Faria, V., Gonçalves, L. M. D., Taboada, P., Remuñán-López, C., & Almeida, A. J. (2016). Rifabutin-loaded solid lipid nanoparticles for inhaled antitubercular therapy: Physicochemical and *in vitro* studies. *International Journal of Pharmaceutics, 497*(1–2), 199–209.
26. Das, S., & Chaudhury, A. (2011). Recent advances in lipid nanoparticle formulations with solid matrix for oral drug delivery. *AAPS PharmSciTech, 12*(1), 62–76.
27. Silva, A., González-Mira, E., García, M., Egea, M., Fonseca, J., Silva, R., et al. (2011). Preparation, characterization and biocompatibility studies on risperidone-loaded solid lipid nanoparticles (SLN): High pressure homogenization *versus* ultrasound. *Colloids and Surfaces B: Biointerfaces, 86*(1), 158–165.
28. Gaspar, D. P., Gaspar, M. M., Eleutério, C. V., Grenha, A., Blanco, M., Gonçalves, L. M. D., et al. (2017). Microencapsulated solid lipid nanoparticles as a hybrid platform for pulmonary antibiotic delivery. *Molecular Pharmaceutics, 14*(9), 2977–2990.
29. Wissing, S., Kayser, O., & Müller, R. (2004). Solid lipid nanoparticles for parenteral drug delivery. *Advanced Drug Delivery Reviews, 56*(9), 1257–1272.
30. Jensen, L., Magnussson, E., Gunnarsson, L., Vermehren, C., Nielsen, H., & Petersson, K. (2010). Corticosteroid solubility and lipid polarity control release from solid lipid nanoparticles. *International Journal of Pharmaceutics, 390*(1), 53–60.

31. Fathi, M., Mozafari, M., & Mohebbi, M. (2012). Nanoencapsulation of food ingredients using lipid based delivery systems. *Trends in Food Science and Technology, 23*(1), 13–27.
32. Üner, M., & Yener, G. (2007). Importance of solid lipid nanoparticles (SLN) in various administration routes and future perspectives. *International Journal of Nanomedicine, 2*(3), 289–300.
33. Müller, R. H., Radtke, M., & Wissing, S. (2002). Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. *Advanced Drug Delivery Reviews, 54*(1), 131–155.
34. Westesen, K., Siekmann, B., & Koch, M. H. (1993). Investigations on the physical state of lipid nanoparticles by synchrotron radiation X-ray diffraction. *International Journal of Pharmaceutics, 93*(1), 189–199.
35. Mehnert, W., & Mäder, K. (2001). Solid lipid nanoparticles: Production, characterization and applications. *Advanced Drug Delivery Reviews, 47*(2), 165–196.
36. Bunjes, H., Westesen, K., & Koch, M. H. (1996). Crystallization tendency and polymorphic transitions in triglyceride nanoparticles. *International Journal of Pharmaceutics, 129*(1), 159–173.
37. Müller, R. H., Mäder, K., & Gohla, S. (2000). Solid lipid nanoparticles (SLN) for controlled drug delivery – A review of the state of the art. *European Journal of Pharmaceutics and Biopharmaceutics, 50*(1), 161–177.
38. Müller, R. H., Radtke, M., & Wissing, S. (2002). Nanostructured lipid matrices for improved microencapsulation of drugs. *International Journal of Pharmaceutics, 242*(1), 121–128.
39. Olbrich, C., Gessner, A., Kayser, O., & Müller, R. H. (2002). Lipid-drug-conjugate (LDC) nanoparticles as novel carrier system for the hydrophilic antitrypanosomal drug diminazene-diaceturate. *Journal of Drug Targeting, 10*(5), 387–396.
40. Almeida, A. J., Runge, S., & Müller, R. H. (1997). Peptide-loaded solid lipid nanoparticles (SLN): Influence of production parameters. *International Journal of Pharmaceutics, 149*(2), 255–265.
41. Garcìa-Fuentes, M., Torres, D., & Alonso, M. (2003). Design of lipid nanoparticles for the oral delivery of hydrophilic macromolecules. *Colloids and Surfaces B: Biointerfaces, 27*(2), 159–168.
42. Attama, A., Momoh, M., & Builders, P. (2012). Chapter 5 – Lipid nanoparticulate drug delivery systems: A revolution in dosage form design and development. In A. Demir (Ed.), *Recent advances in novel drug carrier systems* (pp. 107–140). Croatia: InTech.
43. Beloqui, A., Solinís, M. Á., Rodríguez-Gascón, A., Almeida, A. J., & Préat, V. (2016). Nanostructured Lipid Carriers: Promising drug delivery systems for future clinics. *Nanomedicine: Nanotechnology, Biology and Medicine, 12*(1), 143–161.
44. Patel, S., Chavhan, S., Soni, H., Babbar, A., Mathur, R., Mishra, A., et al. (2011). Brain targeting of risperidone-loaded solid lipid nanoparticles by intranasal route. *Journal of Drug Targeting, 19*(6), 468–474.
45. Xu, X., Ho, W., Zhang, X., Bertrand, N., & Farokhzad, O. (2015). Cancer nanomedicine: From targeted delivery to combination therapy. *Trends in Molecular Medicine, 21*(4), 223–232.
46. Chono, S., Tanino, T., Seki, T., & Morimoto, K. (2007). Uptake characteristics of liposomes by rat alveolar macrophages: Influence of particle size and surface mannose modification. *The Journal of Pharmacy and Pharmacology, 59*(1), 75–80.
47. Gao, H., Shi, W., & Freund, L. B. (2005). Mechanics of receptor-mediated endocytosis. *Proceedings of the National Academy of Sciences of the United States of America, 102*(27), 9469–9474.
48. Sykes, E. A., Chen, J., Zheng, G., & Chan, W. C. (2014). Investigating the impact of nanoparticle size on active and passive tumor targeting efficiency. *ACS Nano, 8*(6), 5696–5706.
49. Gan, Q., Wang, T., Cochrane, C., & McCarron, P. (2005). Modulation of surface charge, particle size and morphological properties of chitosan–TPP nanoparticles intended for gene delivery. *Colloids and Surfaces B: Biointerfaces, 44*(2-3), 65–73.

50. Pearce, T. R., Shroff, K., & Kokkoli, E. (2012). Peptide targeted lipid nanoparticles for anticancer drug delivery. *Advanced Materials, 24*(28), 3803–3822.
51. Cho, K., Wang, X., Nie, S., & Shin, D. M. (2008). Therapeutic nanoparticles for drug delivery in cancer. *Clinical Cancer Research, 14*(5), 1310–1316.
52. Ye, J., Wang, Q., Zhou, X., & Zhang, N. (2008). Injectable actarit-loaded solid lipid nanoparticles as passive targeting therapeutic agents for rheumatoid arthritis. *International Journal of Pharmaceutics, 352*(1–2), 273–279.
53. Beloqui, A., Solinís, M. Á., Gascón, A. R., del Pozo-Rodríguez, A., des Rieux, A., & Préat, V. (2013). Mechanism of transport of saquinavir-loaded nanostructured lipid carriers across the intestinal barrier. *Journal of Controlled Release, 166*(2), 115–123.
54. Lim, S.-J., & Kim, C.-K. (2002). Formulation parameters determining the physicochemical characteristics of solid lipid nanoparticles loaded with all-trans retinoic acid. *International Journal of Pharmaceutics, 243*(1), 135–146.
55. Alexis, F., Pridgen, E., Molnar, L. K., & Farokhzad, O. C. (2008). Factors affecting the clearance and biodistribution of polymeric nanoparticles. *Molecular Pharmaceutics, 5*(4), 505–515.
56. Cadete, A., Figueiredo, L., Lopes, R., Calado, C. C. R., Almeida, A. J., & Gonçalves, L. M. D. (2012). Development and characterization of a new plasmid delivery system based on chitosan – Sodium deoxycholate nanoparticles. *European Journal of Pharmaceutical Sciences, 45*(4), 451–458.
57. Thakkar, A., Chenreddy, S., Wang, J., & Prabhu, S. (2015). Ferulic acid combined with aspirin demonstrates chemopreventive potential towards pancreatic cancer when delivered using chitosan-coated solid-lipid nanoparticles. *Cell & Bioscience, 5*(1), 1–14.
58. Fonte, P., Nogueira, T., Gehm, C., Ferreira, D., & Sarmento, B. (2011). Chitosan-coated solid lipid nanoparticles enhance the oral absorption of insulin. *Drug Delivery and Translational Research, 1*(4), 299–308.
59. Pan, T.-L., Wang, P.-W., Hung, C.-F., Aljuffali, I. A., Dai, Y.-S., & Fang, J.-Y. (2016). The impact of retinol loading and surface charge on the hepatic delivery of lipid nanoparticles. *Colloids and Surfaces B: Biointerfaces, 141*(1), 584–594.
60. Garcia-Fuentes, M., Prego, C., Torres, D., & Alonso, M. (2005). A comparative study of the potential of solid triglyceride nanostructures coated with chitosan or poly (ethylene glycol) as carriers for oral calcitonin delivery. *European Journal of Pharmaceutical Sciences, 25*(1), 133–143.
61. Durán-Lobato, M., Martín-Banderas, L., Gonçalves, L. M. D., Fernández-Arévalo, M., & Almeida, A. J. (2015). Comparative study of chitosan-and PEG-coated lipid and PLGA nanoparticles as oral delivery systems for cannabinoids. *Journal of Nanoparticle Research, 17*(2), 1–17.
62. Aggarwal, P., Hall, J. B., McLeland, C. B., Dobrovolskaia, M. A., & McNeil, S. E. (2009). Nanoparticle interaction with plasma proteins as it relates to particle biodistribution, biocompatibility and therapeutic efficacy. *Advanced Drug Delivery Reviews, 61*(6), 428–437.
63. Müller, R. H., Maaben, S., Weyhers, H., & Mehnert, W. (1996). Phagocytic uptake and cytotoxicity of solid lipid nanoparticles (SLN) sterically stabilized with poloxamine 908 and poloxamer 407. *Journal of Drug Targeting, 4*(3), 161–170.
64. Blasi, P., Giovagnoli, S., Schoubben, A., Ricci, M., & Rossi, C. (2007). Solid lipid nanoparticles for targeted brain drug delivery. *Advanced Drug Delivery Reviews, 59*(6), 454–477.
65. Dhawan, S., Kapil, R., & Singh, B. (2011). Formulation development and systematic optimization of solid lipid nanoparticles of quercetin for improved brain delivery. *The Journal of Pharmacy and Pharmacology, 63*(3), 342–351.
66. Martins, S. M., Sarmento, B., Nunes, C., Lúcio, M., Reis, S., & Ferreira, D. C. (2013). Brain targeting effect of camptothecin-loaded solid lipid nanoparticles in rat after intravenous administration. *European Journal of Pharmaceutics and Biopharmaceutics, 85*(3), 488–502.
67. Blasi, P., Schoubben, A., Traina, G., Manfroni, G., Barberini, L., Alberti, P. F., et al. (2013). Lipid nanoparticles for brain targeting III. Long-term stability and *in vivo* toxicity. *International Journal of Pharmaceutics, 454*(1), 316–323.

68. Göppert, T. M., & Müller, R. H. (2005). Protein adsorption patterns on poloxamer-and poloxamine-stabilized solid lipid nanoparticles (SLN). *European Journal of Pharmaceutics and Biopharmaceutics, 60*(3), 361–372.
69. Farace, C., Sánchez-Moreno, P., Orecchioni, M., Manetti, R., Sgarrella, F., Asara, Y., et al. (2016). Immune cell impact of three differently coated lipid nanocapsules: Pluronic, chitosan and polyethylene glycol. *Scientific Reports*, *6*(18423), 1–14.
70. Esposito, E., Drechsler, M., Mariani, P., Carducci, F., Servadio, M., Melancia, F., et al. (2017). Lipid nanoparticles for administration of poorly water soluble neuroactive drugs. *Biomedical Microdevices, 19*(3), 44.
71. Ren, T., Wang, Q., Xu, Y., Cong, L., Gou, J., Tao, X., et al. (2018). Enhanced oral absorption and anticancer efficacy of cabazitaxel by overcoming intestinal mucus and epithelium barriers using surface polyethylene oxide (PEO) decorated positively charged polymer-lipid hybrid nanoparticles. *Journal of Controlled Release, 269*(1), 423–438.
72. Almeida, A. J., & Souto, E. (2007). Solid lipid nanoparticles as a drug delivery system for peptides and proteins. *Advanced Drug Delivery Reviews, 59*(6), 478–490.
73. Buse, J., & El-Aneed, A. (2010). Properties, engineering and applications of lipid-based nanoparticle drug-delivery systems: Current research and advances. *Nanomedicine, 5*(8), 1237–1260.
74. van Vlerken, L. E., Vyas, T. K., & Amiji, M. M. (2007). Poly (ethylene glycol)-modified nanocarriers for tumor-targeted and intracellular delivery. *Pharmaceutical Research, 24*(8), 1405–1414.
75. Olivier, J. C. (2005). Drug transport to brain with targeted nanoparticles. *NeuroRx, 2*(1), 108–119.
76. Madan, J., Pandey, R. S., Jain, V., Katare, O. P., Chandra, R., & Katyal, A. (2013). Poly (ethylene)-glycol conjugated solid lipid nanoparticles of noscapine improve biological half-life, brain delivery and efficacy in glioblastoma cells. *Nanomedicine: Nanotechnology, Biology, and Medicine, 9*(4), 492–503.
77. Bastiat, G., Hirsjärvi, S., & Benoît, J.-P. (2012). Drug delivery strategies: Lipid nanocapsules. In M. Alonso & N. Csaba (Eds.), *Nanostructured biomaterials for overcoming biological barriers* (pp. 483–497). Cambridge: RSC Publishing.
78. Xu, H., Deng, Y., Chen, D., Hong, W., Lu, Y., & Dong, X. (2008). Esterase-catalyzed dePEGylation of pH-sensitive vesicles modified with cleavable PEG-lipid derivatives. *Journal of Controlled Release, 130*(3), 238–245.
79. Khalid, M. N., Simard, P., Hoarau, D., Dragomir, A., & Leroux, J.-C. (2006). Long circulating poly (ethylene glycol)-decorated lipid nanocapsules deliver docetaxel to solid tumors. *Pharmaceutical Research, 23*(4), 752–758.
80. Hoarau, D., Delmas, P., Roux, E., & Leroux, J.-C. (2004). Novel long-circulating lipid nanocapsules. *Pharmaceutical Research, 21*(10), 1783–1789.
81. Morille, M., Montier, T., Legras, P., Carmoy, N., Brodin, P., Pitard, B., et al. (2010). Long-circulating DNA lipid nanocapsules as new vector for passive tumor targeting. *Biomaterials, 31*(2), 321–329.
82. Morille, M., Passirani, C., Dufort, S., Bastiat, G., Pitard, B., Coll, J.-L., et al. (2011). Tumor transfection after systemic injection of DNA lipid nanocapsules. *Biomaterials, 32*(9), 2327–2333.
83. Vonarbourg, A., Passirani, C., Desigaux, L., Allard, E., Saulnier, P., Lambert, O., et al. (2009). The encapsulation of DNA molecules within biomimetic lipid nanocapsules. *Biomaterials, 30*(18), 3197–3204.
84. Zheng, J., Wan, Y., Elhissi, A., Zhang, Z., & Sun, X. (2014). Targeted paclitaxel delivery to tumors using cleavable PEG-conjugated solid lipid nanoparticles. *Pharmaceutical Research, 31*(8), 2220–2233.
85. Yuan, H., Chen, C.-Y., Chai, G.-h., Du, Y.-Z., & Hu, F.-Q. (2013). Improved transport and absorption through gastrointestinal tract by PEGylated solid lipid nanoparticles. *Molecular Pharmaceutics, 10*(5), 1865–1873.

86. Kang, X., Chen, H., Li, S., Jie, L., Hu, J., Wang, X., et al. (2018). Magnesium lithospermate B loaded PEGylated solid lipid nanoparticles for improved oral bioavailability. *Colloids and Surfaces B: Biointerfaces, 161*(1), 597–605.
87. Hashiba, K., Sato, Y., & Harashima, H. (2017). pH-labile PEGylation of siRNA-loaded lipid nanoparticle improves active targeting and gene silencing activity in hepatocytes. *Journal of Controlled Release, 262*(1), 239–246.
88. Chuang, C.-H., Wu, P.-C., Tsai, T.-H., Fang, Y.-P., Tsai, Y.-H., Cheng, T.-C., et al. (2017). Development of pH-sensitive cationic PEGylated solid lipid nanoparticles for selective cancer-targeted therapy. *Journal of Biomedical Nanotechnology, 13*(2), 192–203.
89. Albanese, A., Tang, P. S., & Chan, W. C. (2012). The effect of nanoparticle size, shape, and surface chemistry on biological systems. *Annual Review of Biomedical Engineering, 14*(1), 1–16.
90. Kraft, J. C., Freeling, J. P., Wang, Z., & Ho, R. J. (2014). Emerging research and clinical development trends of liposome and lipid nanoparticle drug delivery systems. *Journal of Pharmaceutical Sciences, 103*(1), 29–52.
91. Yu, W., Zhang, N., & Li, C. (2009). Saccharide modified pharmaceutical nanocarriers for targeted drug and gene delivery. *Current Pharmaceutical Design, 15*(32), 3826–3836.
92. Zhang, N., Ping, Q., Huang, G., Xu, W., Cheng, Y., & Han, X. (2006). Lectin-modified solid lipid nanoparticles as carriers for oral administration of insulin. *International Journal of Pharmaceutics, 327*(1–2), 153–159.
93. Zhang, N., Ping, Q., Huang, G., Han, X., Cheng, Y., & Xu, W. (2006). Transport characteristics of wheat germ agglutinin-modified insulin-liposomes and solid lipid nanoparticles in a perfused rat intestinal model. *Journal of Nanoscience and Nanotechnology, 6*(9–10), 2959–2966.
94. Garcìa-Fuentes, M., Torres, D., & Alonso, M. J. (2005). New surface-modified lipid nanoparticles as delivery vehicles for salmon calcitonin. *International Journal of Pharmaceutics, 296*(1), 122–132.
95. Gupta, Y., Jain, A., & Jain, S. K. (2007). Transferrin-conjugated solid lipid nanoparticles for enhanced delivery of quinine dihydrochloride to the brain. *The Journal of Pharmacy and Pharmacology, 59*(7), 935–940.
96. Yu, W., Liu, C., Liu, Y., Zhang, N., & Xu, W. (2010). Mannan-modified solid lipid nanoparticles for targeted gene delivery to alveolar macrophages. *Pharmaceutical Research, 27*(8), 1584–1596.
97. Venishetty, V. K., Chede, R., Komuravelli, R., Adepu, L., Sistla, R., & Diwan, P. V. (2012). Design and evaluation of polymer coated carvedilol loaded solid lipid nanoparticles to improve the oral bioavailability: A novel strategy to avoid intraduodenal administration. *Colloids and Surfaces B: Biointerfaces, 95*(1), 1–9.
98. Varshosaz, J., Hassanzadeh, F., Sadeghi, H., & Khadem, M. (2012). Galactosylated nanostructured lipid carriers for delivery of 5-FU to hepatocellular carcinoma. *Journal of Liposome Research, 22*(3), 224–236.
99. Venishetty, V. K., Komuravelli, R., Kuncha, M., Sistla, R., & Diwan, P. V. (2013). Increased brain uptake of docetaxel and ketoconazole loaded folate-grafted solid lipid nanoparticles. *Nanomedicine: Nanotechnology, Biology, and Medicine, 9*(1), 111–121.
100. Resnier, P., LeQuinio, P., Lautram, N., André, E., Gaillard, C., Bastiat, G., et al. (2014). Efficient *in vitro* gene therapy with PEG siRNA lipid nanocapsules for passive targeting strategy in melanoma. *Biotechnology Journal, 9*(11), 1389–1401.
101. Fan, T., Chen, C., Guo, H., Xu, J., Zhang, J., Zhu, X., et al. (2014). Design and evaluation of solid lipid nanoparticles modified with peptide ligand for oral delivery of protein drugs. *European Journal of Pharmaceutics and Biopharmaceutics, 88*(2), 518–528.
102. Wang, W., Zhou, F., Ge, L., Liu, X., & Kong, F. (2014). A promising targeted gene delivery system: Folate-modified dexamethasone-conjugated solid lipid nanoparticles. *Pharmaceutical Biology, 52*(8), 1039–1044.
103. Ramalingam, P., & Ko, Y. T. (2015). Enhanced oral delivery of curcumin from N-trimethyl chitosan surface-modified solid lipid nanoparticles: Pharmacokinetic and brain distribution evaluations. *Pharmaceutical Research, 32*(2), 389–402.

104. Chapman, A. P. (2002). PEGylated antibodies and antibody fragments for improved therapy: A review. *Advanced Drug Delivery Reviews, 54*(4), 531–545.
105. Kim, C. H., Lee, S. G., Kang, M. J., Lee, S., & Choi, Y. W. (2017). Surface modification of lipid-based nanocarriers for cancer cell-specific drug targeting. *Journal of Pharmaceutical Investigation, 47*(3), 203–227.
106. Kuo, Y.-C., & Ko, H.-F. (2013). Targeting delivery of saquinavir to the brain using 83-14 monoclonal antibody-grafted solid lipid nanoparticles. *Biomaterials, 34*(20), 4818–4830.
107. Lu, Y.-m., Huang, J.-y., Wang, H., Lou, X.-f., Liao, M.-h., Hong, L.-j., et al. (2014). Targeted therapy of brain ischaemia using Fas ligand antibody conjugated PEG-lipid nanoparticles. *Biomaterials, 35*(1), 530–537.
108. Gandomi, N., Varshochian, R., Atyabi, F., Ghahremani, M. H., Sharifzadeh, M., Amini, M., et al. (2017). Solid lipid nanoparticles surface modified with anti-Contactin-2 or anti-Neurofascin for brain-targeted delivery of medicines. *Pharmaceutical Development and Technology, 22*(3), 426–435.
109. Ramishetti, S., Kedmi, R., Goldsmith, M., Leonard, F., Sprague, A. G., Godin, B., et al. (2015). Systemic gene silencing in primary T lymphocytes using targeted lipid nanoparticles. *ACS Nano, 9*(7), 6706–6716.
110. Katakowski, J. A., Mukherjee, G., Wilner, S. E., Maier, K. E., Harrison, M. T., DiLorenzo, T. P., et al. (2016). Delivery of siRNAs to dendritic cells using DEC205-targeted lipid nanoparticles to inhibit immune responses. *Molecular Therapy, 24*(1), 146–155.
111. Wong, P., Li, L., Chea, J., Delgado, M. K., Crow, D., Poku, E., et al. (2017). PET imaging of 64Cu-DOTA-scFv-anti-PSMA lipid nanoparticles (LNPs): Enhanced tumor targeting over anti-PSMA scFv or untargeted LNPs. *Nuclear Medicine and Biology, 47*(1), 62–68.
112. Abdolahpour, S., Toliyat, T., Omidfar, K., Modjtahedi, H., Wong, A. J., Rasaee, M. J., et al. (2018). Targeted delivery of doxorubicin into tumor cells by nanostructured lipid carriers conjugated to anti-EGFRvIII monoclonal antibody. *Artificial Cells, Nanomedicine, and Biotechnology, 46*(1), 89–94.
113. Zhang, H., Ma, Y., & Sun, X. L. (2010). Recent developments in carbohydrate-decorated targeted drug/gene delivery. *Medicinal Research Reviews, 30*(2), 270–289.
114. Nimje, N., Agarwal, A., Saraogi, G. K., Lariya, N., Rai, G., Agrawal, H., et al. (2009). Mannosylated nanoparticulate carriers of rifabutin for alveolar targeting. *Journal of Drug Targeting, 17*(10), 777–787.
115. Sahu, P. K., Mishra, D. K., Jain, N., Rajoriya, V., & Jain, A. K. (2015). Mannosylated solid lipid nanoparticles for lung-targeted delivery of Paclitaxel. *Drug Development and Industrial Pharmacy, 41*(4), 640–649.
116. Pinheiro, M., Ribeiro, R., Vieira, A., Andrade, F., & Reis, S. (2016). Design of a nanostructured lipid carrier intended to improve the treatment of tuberculosis. *Drug Design, Development and Therapy, 10*(1), 2467–2475.
117. Song, X., Lin, Q., Guo, L., Fu, Y., Han, J., Ke, H., et al. (2015). Rifampicin loaded mannosylated cationic nanostructured lipid carriers for alveolar macrophage-specific delivery. *Pharmaceutical Research, 32*(5), 1741–1751.
118. Costa, A., Sarmento, B., & Seabra, V. (2018). Mannose-functionalized solid lipid nanoparticles are effective in targeting alveolar macrophages. *European Journal of Pharmaceutical Sciences, 114*(1), 103–113.
119. Yang, X.-y., Li, Y.-x., Li, M., Zhang, L., Feng, L.-x., & Zhang, N. (2013). Hyaluronic acid-coated nanostructured lipid carriers for targeting paclitaxel to cancer. *Cancer Letters, 334*(2), 338–345.
120. Tran, T. H., Choi, J. Y., Ramasamy, T., Truong, D. H., Nguyen, C. N., Choi, H.-G., et al. (2014). Hyaluronic acid-coated solid lipid nanoparticles for targeted delivery of vorinostat to CD44 overexpressing cancer cells. *Carbohydrate Polymers, 114*(1), 407–415.
121. Shen, H., Shi, S., Zhang, Z., Gong, T., & Sun, X. (2015). Coating solid lipid nanoparticles with hyaluronic acid enhances antitumor activity against melanoma stem-like cells. *Theranostics, 5*(7), 755–771.

122. Mulik, R. S., Mönkkönen, J., Juvonen, R. O., Mahadik, K. R., & Paradkar, A. R. (2010). Transferrin mediated solid lipid nanoparticles containing curcumin: Enhanced *in vitro* anticancer activity by induction of apoptosis. *International Journal of Pharmaceutics, 398*(1), 190–203.
123. Shao, Z., Shao, J., Tan, B., Guan, S., Liu, Z., Zhao, Z., et al. (2015). Targeted lung cancer therapy: Preparation and optimization of transferrin-decorated nanostructured lipid carriers as novel nanomedicine for co-delivery of anticancer drugs and DNA. *International Journal of Nanomedicine, 10*(1), 1223–1233.
124. Yang, Z., Yu, B., Zhu, J., Huang, X., Xie, J., Xu, S., et al. (2014). A microfluidic method to synthesize transferrin-lipid nanoparticles loaded with siRNA LOR-1284 for therapy of acute myeloid leukemia. *Nanoscale, 6*(16), 9742–9751.
125. Singh, I., Swami, R., Pooja, D., Jeengar, M. K., Khan, W., & Sistla, R. (2016). Lactoferrin bioconjugated solid lipid nanoparticles: A new drug delivery system for potential brain targeting. *Journal of Drug Targeting, 24*(3), 212–223.
126. Kuo, Y.-C., & Cheng, S.-J. (2016). Brain targeted delivery of carmustine using solid lipid nanoparticles modified with tamoxifen and lactoferrin for antitumor proliferation. *International Journal of Pharmaceutics, 499*(1–2), 10–19.
127. Kuo, Y.-C., & Wang, I.-H. (2017). Using catanionic solid lipid nanoparticles with wheat germ agglutinin and lactoferrin for targeted delivery of etoposide to glioblastoma multiforme. *Journal of the Taiwan Institute of Chemical Engineers, 77*(1), 73–82.
128. Chen, Y., Yuan, L., Zhou, L., Zhang, Z.-h., Cao, W., & Wu, Q. (2012). Effect of cell-penetrating peptide-coated nanostructured lipid carriers on the oral absorption of tripterine. *International Journal of Nanomedicine, 7*(1), 4581–4591.
129. Skotland, T., Iversen, T. G., Torgersen, M. L., & Sandvig, K. (2015). Cell-penetrating peptides: Possibilities and challenges for drug delivery *in vitro* and *in vivo*. *Molecules, 20*(7), 13313–13323.
130. Bahrami, B., Hojjat-Farsangi, M., Mohammadi, H., Anvari, E., Ghalamfarsa, G., Yousefi, M., et al. (2017). Nanoparticles and targeted drug delivery in cancer therapy. *Immunology Letters, 190*(1), 64–83.
131. Deshayes, S., Morris, M., Divita, G., & Heitz, F. (2005). Cell-penetrating peptides: Tools for intracellular delivery of therapeutics. *Cellular and Molecular Life Sciences, 62*(16), 1839–1849.
132. Madani, F., Lindberg, S., Langel, Ü., Futaki, S., & Gräslund, A. (2011). Mechanisms of cellular uptake of cell-penetrating peptides. *Journal of Biophotonics, 2011*(1), 1–10.
133. Gao, W., Meng, T., Shi, N., Zhuang, H., Yang, Z., & Qi, X. (2015). Targeting and microenvironment-responsive lipid nanocarrier for the enhancement of tumor cell recognition and therapeutic efficiency. *Advanced Healthcare Materials, 4*(5), 748–759.
134. Asai, T., Tsuzuku, T., Takahashi, S., Okamoto, A., Dewa, T., Nango, M., et al. (2014). Cell-penetrating peptide-conjugated lipid nanoparticles for siRNA delivery. *Biochemical and Biophysical Research Communications, 444*(4), 599–604.
135. Mansour, A. M., Drevs, J., Esser, N., Hamada, F. M., Badary, O. A., Unger, C., et al. (2003). A new approach for the treatment of malignant melanoma: Enhanced antitumor efficacy of an albumin-binding doxorubicin prodrug that is cleaved by matrix metalloproteinase 2. *Cancer Research, 63*(14), 4062–4066.
136. Carradori, D., Saulnier, P., Préat, V., Des Rieux, A., & Eyer, J. (2016). NFL-lipid nanocapsules for brain neural stem cell targeting *in vitro* and *in vivo*. *Journal of Controlled Release, 238*(1), 253–262.
137. Liu, B., Han, L., Liu, J., Han, S., Chen, Z., & Jiang, L. (2017). Co-delivery of paclitaxel and TOS-cisplatin via TAT-targeted solid lipid nanoparticles with synergistic antitumor activity against cervical cancer. *International Journal of Nanomedicine, 12*(1), 955–968.
138. Gartziandia, O., Egusquiaguirre, S. P., Bianco, J., Pedraz, J. L., Igartua, M., Hernandez, R. M., et al. (2016). Nanoparticle transport across *in vitro* olfactory cell monolayers. *International Journal of Pharmaceutics, 499*(1), 81–89.

139. Zhang, Z., Lv, H., & Zhou, J. (2009). Novel solid lipid nanoparticles as carriers for oral administration of insulin. *International Journal of Pharmaceutics, 64*(9), 574–578.
140. Sperling, R. A., & Parak, W. (2010). Surface modification, functionalization and bioconjugation of colloidal inorganic nanoparticles. *Philosophical Transactions of the Royal Society a Mathematical Physical and Engineering Sciences, 368*(1915), 1333–1383.
141. Jain, A., & Cheng, K. (2017). The principles and applications of avidin-based nanoparticles in drug delivery and diagnosis. *Journal of Controlled Release, 245*(1), 27–40.
142. Neves, A. R., Queiroz, J. F., Weksler, B., Romero, I. A., Couraud, P.-O., & Reis, S. (2015). Solid lipid nanoparticles as a vehicle for brain-targeted drug delivery: Two new strategies of functionalization with apolipoprotein E. *Nanotechnology, 26*(49), 495103.
143. Neves, A. R., Queiroz, J. F., & Reis, S. (2016). Brain-targeted delivery of resveratrol using solid lipid nanoparticles functionalized with apolipoprotein E. *Journal of Nanbiotechnology, 14*(1), 27–38.
144. Zhang, J., Liu, M.-Q., Zhang, J.-L., Li, B.-A., Wang, X.-Y., & Han, P. (2015). Biotinylated epidermal growth factor surface modified lipid nanoparticles to enhance the targeting efficiency in liver cancer therapy. *Journal of Biomaterials and Tissue Engineering, 5*(2), 135–141.
145. Li, L., Wartchow, C. A., Danthi, S. N., Shen, Z., Dechene, N., Pease, J., et al. (2004). A novel antiangiogenesis therapy using an integrin antagonist or anti–Flk-1 antibody coated 90 Y-labeled nanoparticles. *International Journal of Radiation Oncology, 58*(4), 1215–1227.
146. Shuhendler, A. J., Prasad, P., Leung, M., Rauth, A. M., DaCosta, R. S., & Wu, X. Y. (2012). A novel solid lipid nanoparticle formulation for active targeting to tumor αvβ3 integrin receptors reveals cyclic RGD as a double-edged sword. *Advanced Healthcare Materials, 1*(5), 600–608.
147. Banerjee, I., De, K., Mukherjee, D., Dey, G., Chattopadhyay, S., Mukherjee, M., et al. (2016). Paclitaxel-loaded solid lipid nanoparticles modified with Tyr-3-octreotide for enhanced anti-angiogenic and anti-glioma therapy. *Acta Biomaterialia, 38*(1), 69–81.

Chapter 5
Stealth Properties of Nanoparticles Against Cancer: Surface Modification of NPs for Passive Targeting to Human Cancer Tissue in Zebrafish Embryos

Samson A. Adeyemi, Pradeep Kumar, Yahya E. Choonara, and Viness Pillay

Abstract Cancer as a noncommunicable disease remains the major cause of death globally. A major drawback for cancer therapeutics is the lack of specific delivery to disease sites which in turn accounts for adverse effects on healthy cells. Incorporation into nanoparticle (NP) and subsequent surface functionalization remain a preferred strategy to circumvent this limitation and to achieve optimal delivery of anticancer drugs. NPs can be coated with hydrophilic and positively charged surfaces that confer on them stealth characteristics that enhance long circulation times and internalization through receptor-mediated endocytosis within the biological systems. One way of achieving this is the coating of poly ethylene glycol onto the surfaces of NPs. In this way, opsonization is reduced and engulfment through reticuloendothelial system is avoided. Central to the concept of passive targeting of NPs is the unique microvasculature of tumor. Various regulatory factors that control the blood pressure as well as maneuvering of the vasculature may shift the equilibrium towards the more captivating tumor environment for NPs. Highlighted in this chapter is how PEGylation of NPs and exploration of the EPR effect could increase the circulation times of NPs while escaping immediate elimination by the immune systems and rapid renal clearance in vivo. Similarly, the stealth properties of NPs can be explored for enhance therapeutic effects through surface modification with other nonfouling hydrophilic polymers in order to cover for the PEG dilemma. Meanwhile, the zebrafish model provides a more promising alternative to detect, view, monitor, image and characterize the interactions between NPs and neoplastic tissues in real time.

Keywords Cancer · Nanoparticles · Surface functionalization · Stealth properties PEGylation

S. A. Adeyemi · P. Kumar · Y. E. Choonara · V. Pillay (✉)
Wits Advanced Drug Delivery Platform Research Unit, Department of Pharmacy and Pharmacology, School of Therapeutic Sciences, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa
e-mail: viness.pillay@wits.ac.za

Y. V Pathak (ed.), *Surface Modification of Nanoparticles for Targeted Drug Delivery*, https://doi.org/10.1007/978-3-030-06115-9_5

1 Cancer: Introduction, History, Geographical Distribution, and Causes

Cancer as a noncommunicable disease remains the major cause of death globally after cardiovascular disease [1]. The global burden of cancer as reported by the International Agency for Research on Cancer (IARC) showed that cancer incidence has occurred at a rate that is twice its previous occurrence over the past 30 years. Current prediction has it that by the end of year 2030, there will be an additional 21.4 million people living with cancer. With 17 million deaths yearly due to cancer, there will be an average of 75 million cancer patients within 5 years of diagnosis. The World Health Organization report showed that 8.2 million new cases of deaths were recorded globally in 2012 due to cancer, 60% of which were from Africa, Asia, Central and South America and only 30% of cancers could be prevented [2]. In the year 2014, a total of 4500 new cases of cancer were projected to be diagnosed daily in the United States amounting to an average of 1,665,540 of which 585,720 deaths was recorded yearly [3].

The term cancer originates from the word "karkinos," which was used by a Greek doctor called Hippocrates to mean carcinoma between 460 and 370 BC. Meanwhile, prior to his discovery, records of human bone cancer discovered in mummies in ancient Egypt were documented as far back as 1600 BC. Egyptian scientists in 1500 BC were the first group of researchers that discovered and recorded the first breast cancer case without any treatment but palliative option. Through surgical procedures, tumors on the body surface were excised in the same way it is carried out today [4]. Generically, the word cancer is used to describe a large group of diseases. This disease group has the potential to affect any part of the body and is defined by abnormal cell growth both in the organ or part of the body it resides in and beyond their usual boundaries. Many types of cancer have their origin in cells and natural mutations of the DNA. However external factors are also reported to facilitate the development of cancer, including environmental toxins, radiation, exposure to certain chemicals, and more.

2 Employing the Enhanced Permeability and Retention Effect in Cancer Nanomedicines

Passive and active targeting are the two principal alternatives by which nanoparticles (NPs) can be delivered to the tumor sites. Tumor microenvironments possess unique characteristic features within the tumor cells and vasculatures that are different from that of healthy cells. Passive targeting employs this unique feature inherent within cancer cells to facilitate the deposition of NPs into the tumor milieu [5]. The characteristic features of the tumor vasculature as well as the stealth property of the NP including its shape, size and surface charge are the principal determining parameters for the delivery of NPs to cancer [6]. Meanwhile, in active targeting, various

mechanisms have been employed to facilitate the selective delivery and uptake of NPs into the tumor cells. Tumor cells overexpress some biomolecules on their surfaces as opposed to healthy cells that could serve as molecular signatures. Thus, molecular biomarkers such as ligands and short homing peptides are attached to the surface of nanovectors to target these overexpressed biomolecules on the neoplastic cells [7].

3 Passive Targeting of NPs in Cancer Nanomedicines

A unique phenomenon in passive targeting is the preferential accumulation of biomolecules including NPs into tumor tissues due to the enhanced permeability and retention (EPR) effect as first reported by Meada and Matsumura [8]. Two intrinsic properties of the tumor tissues, that is, the leaky vascular and impaired lymphatic drainage and the nanometer size distribution of the NPs are principal factors that influence the EPR phenomenon [6].

During tumor growth, the rate of diffusion of NPs across neoplastic tissues becomes limited at a volume of 2 mm^3 or above [9]. The movement of nutritional intake, the delivery of oxygen and excretion of waste are all impaired by the diffusion limitation effect. Meanwhile, through the process of angiogenesis, increased microenvironment of the tumour vasculature assists to overcome the diffusion limitation [10]. In angiogenesis, the basement membrane is abnormal and the pericytes, which underline the endothelial cells, are absent [10]. As such, compromised tumor vasculature becomes leaky with gap sizes ranging between 100 nm to 2 μm based on the tumor type [11]. Also, the interstitial pressure at the circumference of tumors is less compared to the pressure at their centres due to the absence of a finely defined lymphatic system. Thus, increased pressure within the tumor results in an outflow of convective interstitial fluid, which limits the diffusion of drug to the center of the tumor. Howbeit, drug-loaded NPs that penetrated into the tumor possessed high retention times compared to normal tissues [12]. The combinatory effects of a leaky vasculature as well as a poor lymphatic drainage are termed as the Enhanced Permeation and Retention (EPR) effect. The overall surface charge of the NPs can also influence their passive targeting to the tumour. Positively charged liposomes were reported to bind preferentially through electrostatic interactions, to the negatively charged phospholipid surfaces expressed on endothelial cells of tumor [13, 14].

4 Factors Influencing the Enhanced Permeability and Retention Effect

Aberrant Structural Tumor Vessels and Blood Pressure The normal blood vessels have a smooth muscle layer that is used to facilitate a vasogenic response to vascular mediators and maintain a steady blood supply to the organs. Conversely, smooth muscle cells are absent in the tumor tissues' microvasculature. As such,

tumor microvessels are in permanently vasodilated and do not respond to physiological stimulus regulating blood circulation [15]. These abnormalities in tumor vasculatures account for the irregular transport dynamics of fluid and solutes across its vessels, an option that can be maximized to further enhance the EPR effects [16]. Report has shown that increasing the mean arterial blood pressure by infusing angiotensin II yields a ~5.7-fold preferential increase in blood flow in neoplastic tissues as opposed to normal tissues. More so, tumor tissue selectively accumulate drugs having molecular weight of ~80 kDa while a reduction of about 60–80% drug accumulation was recorded in healthy organs including kidney and bone marrow [17]. The open endothelial gap interphase with abnormal neoplastic blood vessels allow for an increased intratumoral blood flow in response to raise the blood pressure [18]. Similarly, aberrant leaky vasculature and increased blood supply result in the enhanced accumulation of macromolecular drugs in tissues of diverse solid tumors when angiotensin II was administered to induce hypertension [19]. In contrast to the low toxicity effects of macromolecular anticancer drugs, macromolecular drugs under hypertensive state showed a greater concentration of higher than a five-fold increase in the neoplastic tissue while the hypertension was monitored for approximately 20 min [20].

Vasogenic Mediators Several internal mediating factors regulate the tumor microvasculature. Notably among these are the vascular endothelial growth factor (VEGF), Matrix metalloproteinases (MMPs), bradykinin, prostaglandins (PGs), nitric oxide (NO), and peroxynitrite. Several studies have documented the influence of these mediators for the potential application of the EPR effect for enhanced drug targeting and delivery to neoplastic tissue [21]. A brief listing of their influence will be discussed in this section.

VEGF Previously referred to as vascular permeability factor (VPF), the role of VEGF in enhancing the EPR effect has been well documented [22]. Increased VEGF concentration up to 30-fold high has been reported in tumor tissues as opposed to that recorded in healthy tissues [23]. Meanwhile, an exception was observed in the lung. Increased vascular permeability and mitogenic property of VEGF [19] are central for endothelial cells extravasation of Evans blue dye in a dose-dependent rate through intradermal administration. In this way, the role of VEGF is pivotal for enhancing the EPR effect.

MMPs The growth and spread of solid tumor depends the degradation of their extracellular matrix thereby increasing angiogenesis. Meanwhile, MMPs are known to enhance cancer metastasis in this regard [24]. In an in vivo experiment using mice, MMPs increased the permeability of solid tumors vasculature while this effect was reduced by MMP inhibitors [25]. A number of factors have been itemized limiting the application of various MMP inhibitors that have been developed over the decades. Since neoplastic cells remain viable, they easily resume growth when treatment with anticancer chemotherapeutics are stopped vis-à-vis MMP inhibitors. Also, since MMPs are proteases, they are pivotal for cellular metabolism and a

higher dosage of MMP inhibitors results in toxicity. Owing to these negative consequences, the development of several anti-MMP based drugs has been terminated and clinically not applicable [19].

Bradykinin The kallikrein–kinin system is made up of a couple of proteases such as the Hageman factor XII of the coagulation cascade. Prior to the conversion of prekallikrein to kallikrein is the activation of the Hageman factor XII. Bradykinin is produced directly from kininogen through the activity of kallikrein [23]. Several research reports have identified bradykinin receptors in different types of animal and human tumors [26]. The bradykinin-producing cascade has been shown to be activated in cancer tissues [27]. Bradykinin is upregulated is both the pleural and peritoneal fluids of animals and human neoplastic tissues. Studies have also shown that bradykinin inhibits kallikrein in the extravasation of plasma components into either the pleural or peritoneal cavity [28]. As such, bradykinin plays a pivotal role as a mediator in regulating the EPR effect in tumor tissues [27]. Meanwhile, bradykinin also activates endothelial NO synthase (eNOS), which in turn triggers the production of NO [25]. The angiogenic characteristics of VEGF in human endothelial cells is enhanced by NO production [29]. The inhibition of angiotensin-converting enzyme (ACE), among other peptidases that degrade bradykinin [30], results in higher concentration of bradykinin thereby increases the permeability of neoplastic vasculature. Inhibitors of ACE including temocapril and enalapril, have been reported to enhance the EPR effect [31, 32]. Interestingly, ACE inhibitors enhance the delivery of macromolecular drugs to cancers at normal blood pressure [33]. Thus, ACE inhibitors may act preferentially at tumor sites in individuals with normal blood pressure as ACE inhibitors are only active in patients with high blood pressure, a condition that enhances the EPR effect [34].

NO The reaction between L-arginine and oxygen by three isoforms of NOS results in the production of NO the most active form of NOS is produced in macrophages and neutrophils which are readily present in neoplastic tissues [34]. NO is an established mediator of vasodilation, angiogenesis and extravasation [23]. It has been shown that NO mediates increased vascular permeability in solid tumours, a phenomenon that is hindered by NO scavengers and NO synthase inhibitors [28]. Due to its role in mediating the permeability of tumor vasculature, NO contributes immensely in facilitating the EPR effect in neoplastic tissues [30]. In addition to its direct influence on the EPR, the interaction between NO and the superoxide anion, mainly produced by leukocytes, results in the production of peroxynitrite. Peroxynitrite converts MMP precursors (proMMPs) into MMPs [35] which also play a vital role in the EPR effect [35]. Simultaneous systemic infusion of both isosorbide dinitrate—an NO-releasing agent and angiotensin II into the artery of a localized tumor resulted in an enhanced site-specific delivery of poly(styrene-co-maleic acid/anhydride) neocarzinostatin (SMANCS)-Lipiodol, corroborating the postulation that NO influences the EPR effect [36].

Prostaglandins (PGs) Among the PGs, PGE2 is a unique mediator of vascular permeability. It is produced through the activation of cyclooxygenase (COX) isozymes, like COX-2, which is highly expressed in tumors. Reports have shown that the vascular permeability in sarcoma 180 and other solid tumors are suppressed by COX inhibitors including indomethacin and salicylic acid [37]. This in turn further validates PGs as potent enhancers of vascular permeability and their subsequent role in the EPR effect. In another related report by Tanaka and colleagues [38], the systemic circulation half-life of a PG12 derivative, beraprost sodium, is significantly longer (>1 h) compared to PG12 which only lasts for a few seconds. Their finding showed that the EPR effect was increased up to about two- to three-fold by PG12 analogs which may provide an efficient delivery alternative for macromolecules.

5 Types of Nanoparticulate Systems Employed in Cancer Therapy

Various types of nanoparticulate systems are presently under investigation for the delivery of cancer drugs [12] including polymeric NPs [39], protein NPs [40], ceramic NPs [41], viral NPs [42], metallic NPs [43], carbon nanotubes [44], micelles [45], dendrimers [46] and liposomes [47]. The design and fabrication of each NP system is tailored towards its intrinsic material characteristics in order to facilitate efficient delivery to the tumor sites. NPs can be coated with hydrophilic and positively charged surfaces that confer on them stealth characteristics that enhance long circulation times and internalization through receptor-mediated endocytosis within the biological systems [48]. Quite often, serum proteins adhered to the surfaces of NPs through a process called opsonization [49]. This is turn exposes the NPs to resistance through the reticuloendothelial systems (RES) and thereby limits their circulation times within the biological systems [50]. A major way to circumvent this anomaly is to functionalize the surfaces of NPs in order to create a stealth surface from opsonization. As such, their circulation times are increased and the resistance through RES is avoided [51]. One way of achieving this is the incorporation of poly (ethylene glycol) (PEG) onto the surfaces of NPs. In this way, opsonization is reduced and engulfment through RES is avoided.

Advances in nanotechnology have given a major boost to research in the design and development of various nanoparticulate systems for cancer therapeutics [52]. Meanwhile, only few NP drug delivery systems have been approved till date by the US Federal Drug Administration and the European Agency for the treatment of cancer. Notable among those approved includes liposome–PEG doxorubicin (Doxil®, Ortho Biotech, and Caelyx®, Schering Plough), methoxy-PEG–poly(D,L-lactide) taxol (Genexol-PM®, Samyang), albumin-bound paclitaxel NPs (Abraxane®, Abraxis BioScience) [12]. Zhang and colleagues have reported a comprehensive review of the NP systems in preclinical and clinical developmental stages [53].

6 Stealth Properties of NPs and Their Possible Application in Cancer Nanomedicine

The stealth property of NP refers to the ability of a NP, through diverse modification processes, to by-pass detection and destruction by the immune systems thereby having a prolonged circulation time within the biological system and enhanced targeting potential to its site of action. This phenomenon is termed "stealth coating."

Study has shown that opsonization of NPs remained a major factor the influenced the clearance of NPs by RES within few minutes upon their intravenous administration into the blood [49]. The intrinsic chemical composition of the NP matrix [54], its shape/modulus [55], surface architecture [56], surface charge [57], and size [58] determine its circulation half-life within the biological system (Fig. 5.1). Research has shown that both hydrophobic and charged NPs possess shorter circulation times as a result of their ability to evade the opsonization process [60]. As such, coating the surfaces of NPs intended for systemic application with neutrally charged hydrophilic surface layer is a preferred alternative. Thereby, the circulation half-life of surface modified NPs is prolonged to more than 40 h by the stealth process [61].

The intrinsic material composition of NPs is directly linked to their ability to interact with their environment [62]. The rate at which NPs deliver their payloads at the site of interest can be engineered based on material degradation, or the diffusion through the NP matrix or pores [63]. To facilitate the level of control,

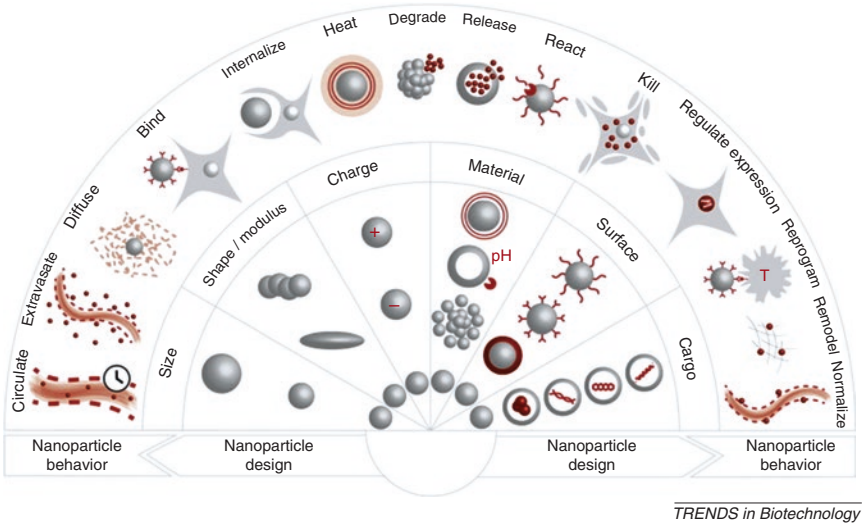


Fig. 5.1 Stealth properties of NPs. NPs' behavior are influenced by their size, shape, modulus, charge, material, and surface architecture. Reprinted under the permission of [59] (Copyright Elsevier Publishers, 2018)

materials can either be designed to release their payload at the action site or adopt new physical characteristics in response to internal stimuli in tumor microenvironments including changes in pH condition or enzymatic activities [64, 65]. External stimuli such as magnetic, sound or light waves can also be employed to activate materials which are responsive to energy for theranostics applications [66, 67].

The size effect on the circulation time, extravasation, internalization and diffusion into the cellular compartment has been widely reported [58]. NPs with smaller size below 5 nm possessed lower circulation half-life and gain rapid entrance into neoplastic cells and tissues [68]. Meanwhile, their disappearance from the tumor cells is rapid due to enhanced filtration by the kidney and rapid renal clearance from the urine. Conversely, NPs with larger size ranging between 5 nm and 500 nm have a higher circulatory retention time and accumulate in neoplastic tissues by exploiting the enhanced permeability and retention (EPR) effect [69]. Also, the size of NP influences its cellular internalization since different NP sizes are transported through diverse endocytic channels [70].

The shape of a NP also has a direct effect on its cellular uptake. Spherically shaped NPs showed increased cellular uptake into the tumor microenvironments. NPs that possessed high aspect ratios and have rigid shape were reported to accumulate more slowly in macrophages than those that have small and flexible morphology. This in turn enhances their retention time within the system and ultimately minimize their clearance time from the circulation [71].

The surface charge of NP influences its circulation time. Charged NPs are highly opsonized and are rapidly engulfed by the immune [72]. Meanwhile, positively charged NPs bind and are preferentially internalized by tumor cells than their uncharged pairs once they are in a tumor environment [73]. Hence, studies to shield NPs once they enter the tumor environment, and release their charged interiors and encapsulated payloads, as a result of the changes in pH or enzymatic activity within the tumor microenvironment, are the paradigm in cancer nanomedicines [74]. Therefore, passive coating, by using polyethylene glycol to confer neutral surface architecture, has been well documented to shield the charged surfaces of NPs, enhance their circulation time, and accumulation in tumor tissues [75]. Similarly, the threat faced by the phagocytes that engulf NPs are being eliminated by shielding NPs with “self-peptides” derived from the human CD47 receptor as later amplified in this review [52, 76].

In all, the potential of NPs to detect, navigate, and deliver their payloads in the body is influenced by their fabrication and interactions with their macro/microenvironments. Maneuvering the properties and subsequent behavior of NPs has become increasingly possible vis-à-vis the deepening knowledge expansion and understanding of molecular biology and NP transport. More important is the impressive nanotechnological techniques and toolbox available to bioengineers to modify NPs and biological systems.

7 Surface Modification and Optimization of NPs for Passive Targeting to Human Cancer Tissues

7.1 PEGylation Chemistry: Stealth Coating of NPs for Biological Application in Cancer Nanomedicines

The covalent attachment of the polyethylene glycol (PEG) chain unto specific given molecule for an enhanced systemic delivery is termed PEGylation. The stealth characteristics conferred on PEGylated molecules allow for their long circulation within the biological system as well as provide a "shield effect" from the RES systems and other blood phagocytes [77]. The first attempt by Abuchowski and colleagues using PEG coating on bovine serum albumin and bovine liver catalase significantly altered both their immunological properties and stability by covalently linking them to methoxy PEG (mPEG) using cyanuric chloride activation [78, 79]. Several other biomolecules including peptides, enzymes, liposomes, carbohydrates, nucleotides, antibody fragments, as well as small organic molecules and various nanoparticulate systems have all been modified using PEGylation chemistry [80–82]. While mPEG is mainly employed for the modification of polypeptides, several PEG variants having different molecular weights and structures including linear, branched, PEG dendrimers and of recent, multiarm PEGs are now being used in PEGylation chemistry [83]. The first approach in the PEGylation process is the activation of native PEG molecule through conjugation of its functional derivative at either one or both terminals of the PEG chain. The PEGylation conjugation process can either be by the first generation randomized technique or the second generation site-specific procedure [84]. Meanwhile, the focused has been given to second generation site-specific PEGylation because it produces well-defined conjugated products having improved product profiles more than those obtained using the randomized technique [77]. Similarly, both reversible and irreversible mechanisms are employed in PEGylation conjugation. However, irreversible PEG conjugation technique showed some negative effects on the biological activity of some therapeutics. As such, the reversible PGylation strategy has been adopted in order to reduce the loss of biological activity of potent therapeutics. In reversible PEGylation technique, drugs are attached to PEG variants through cleavable linkages. Thereafter, the drug is release through various cleaving agents including enzyme and hydrolytic cleavage, or degradation within the biological system at a predetermined release rate over a period of time (Fig. 5.2) [85].

A critical aim of most PEGylation conjugation process is to improve the circulation half-life of therapeutic biomolecules without affecting their activity. The unique advancement in PEG conjugation chemistry and the difference in both structural and molecular architectures of PEGs employed for the conjugation contribute immensely to the increasing demand for PEgylated products in the pharmaceutical industry. Surface modification of drugs and biomolecules enhance their therapeutic efficacy with several advantages over non-PEGylated products. Noteworthy among these systemic modifications are presented in Fig. 5.3. Increased circulation half-life of the PEGylated conjugated product in the blood is the major way to enhance

its therapeutic effect. PEGylation increases the circulation time of the PEG-modified therapeutics by reducing its renal clearance with increasing hydrophilicity [86]. PEGylation confers on the PEG-modified product protection from reticuloendothelial cells, degradation by proteolytic enzymes, reduced formation of neutralizing antibodies against the protein by hiding antigenic sites through the formation of a protective hydrophilic shield [87]. This in essence improves the pharmacokinetic profile of the PEGylated conjugates. Previous reports showed that the absorption half-life of therapeutics administered subcutaneously increased when PEGylated and showed reduced distribution volume [86].

As a nonbiodegradable polymer, the use of PEG is limited in its application. Previous reports showed that PEGs having molecular weight of about 20 kDa are easily cleared by the renal system while those with higher molecular weight were eliminated by fecal excretion [88]. Though PEGylation confers stealth property on conjugated therapeutics and biomolecules with prolong serum half-life, some hurdles were recorded on liposomes particular for the delivery of genes and nucleic acids in anticancer nanomedicines. The surface modification of lipoconjugates by PEG due to its hydrophilic shield decrease their cellular uptake with increasing stability of the lipid envelop, thereby results in lysosomal degradation of the conjugated vector due to poor endosomal escape through membrane fusion [89]. As such, PEG application

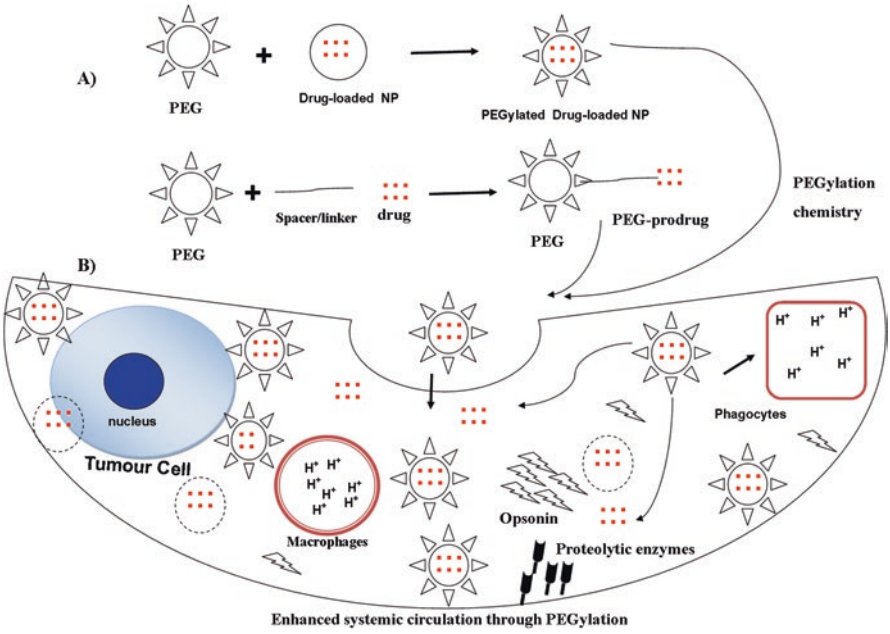


Fig. 5.2 Schematic diagram showing PEGylation and its influence on NP delivery (**a**) Surface modification using PEG on either preformed NPs loaded with drugs or attaching the PEG chain to drug through a linker (**b**) The stealth effect of PEGylation against blood barriers thereby enhancing prolonged systemic circulation of PEGylated drug-loaded NPs or PEG-prodrug as the case may be

in gene and nucleic acid delivery to tumor cells is termed “the PEG dilemma” [90]. Meanwhile, this limitation in “lipo-PEGylation” can be successively tackled by fabricating tumor-specific and pH-sensitive targeted PEG conjugated therapeutics [91, 92]. Nonsystemic delivery approach has also been shown to be efficient for the delivery of PEGylated NPs. In this way, PEGylated therapeutics is delivered locally rather than into the systemic circulation, thereby improving their efficacy while minimizing nontargeted side effects. A detail listing of various local administration strategies including vaginal delivery, pulmonary administration, gastrointestinal tract delivery, ocular and vaccine based delivery systems, of PEGylated NPs for improved delivery of their payloads has been well documented by Jung and coworkers [93].

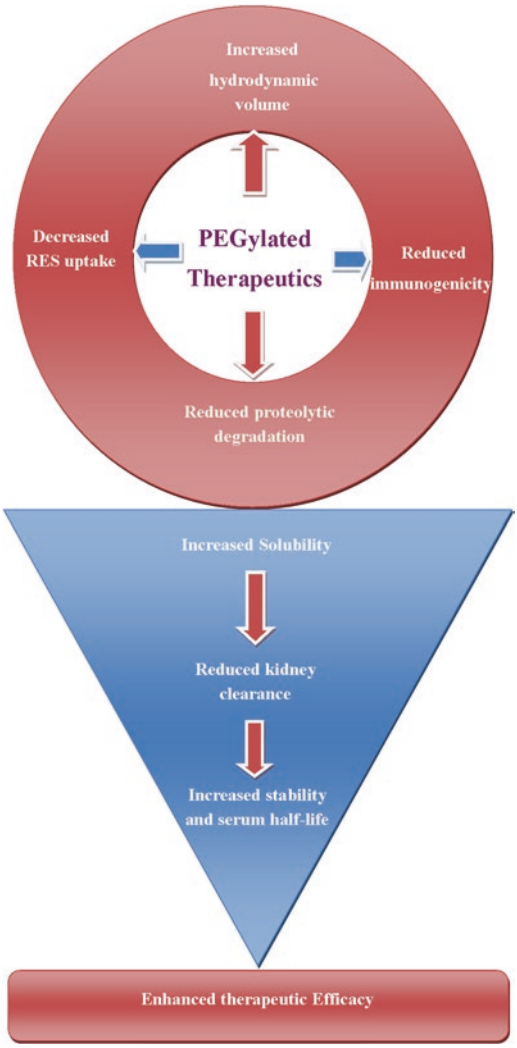


Fig. 5.3 Importance of PEGylated therapeutics

8 Surface Modification of Nanoparticles Using PEG Chains

The surface characteristics of NPs play a principal role as a determining factor that regulates the extent of their cellular internalization. Meanwhile, NPs surfaces can be engineered by the composition of the polymer to regulates either their hydrophobic or hydrophilic surface composition. The use of Polyethylene Glycol (PEG) is foremost in surface medication of these polymers in order to shield these nanosystems from opsonization and clearance by the RES as previously discussed [94]. Also, increased PEG molecular chains has been shown to enhance NPs circulation time as PEG chains provide a shielding effect particularly for negatively charged particles to protect them from immediate clearance in vivo [95].

Two major techniques that are employed in formulating PEGylated NPs including polymeric formulation, lipid-based NPs or micelle type NPs are the self-assembly of PEG-containing biomolecules, or surface modification of preformed NPs with PEG. It should be noted that the density of PEG on the surface of PEGylated NPs is a major factor that enhances their delivery in vivo [93]. In this chapter, effort will be focused on the surface modification of polymeric formulations using PEG chain.

A more assuring way to ascertain that a vast amount of the PEG molecules coated on NPs remained on their surfaces is to modify preformed NPs with PEG. One way to achieve this is through the adsorption technique. Using this method, PEG derivatives are dissolved in an aqueous phase and binds to the NP surface through hydrophobic and electrostatic interactions or ligand-binding. In polyethylene oxide–b-polypropylene oxide–b-polyethylene oxide (PEO-PPO-PEO) triblock copolymer popularly known as pluronics, the hydrophobic PPO component is surrounded by two molecules of PEO chains which are hydrophilic (PEO is a variant form of PEG) [96]. Using their hydrophobic PPO segment, pluronic molecules can interact with and adsorb on NP hydrophobic surfaces. In a report by Yang et al., PPO chain length was observed to contribute immensely in the interaction with NP surface. At a PPO chain length of ~3 kDa molecular weight (MW) pluronic molecules, a densely coated PEG surface that could enhance the diffusion of NP in human mucus was achieved [96]. In other developments, PEGylated phospholipids [97], fatty acid-PEG-esters [98], Vitamin-E-TPGS-PEG [99] among others can be engineered to absorb on polymeric NPs as well as inorganic NPs to form a PEG corona. Through electrostatic interactions, NPs with charged outer surfaces can coated with PEG-containing molecules having opposite charge. For instance, positively charged PEG-PLL and PEG-PEI block polymers can bind to negatively charged PLGA NPs [100]. This noncovalent adsorption technique is a weak interaction that allows for easy desorption of the PEG molecules from the NP surface. Furthermore, using ligand-interactions, a more stable biotinylated PEGylated avidin-functionalized NPs are formed as biotin–avidin interactions can be employed in attaching either ligands or drugs to NP surface for targeted delivery. Meanwhile, it is harder to separate and remove the nonadsorbed PEG molecules from the adsorbed ones using this procedure. Such unintended aftermaths on both delivery and translational mechanisms should be considered prior to preparation [101].

Chemical conjugation is another technique employed to PEGylate preformed NPs. This procedure is also termed grafting. PEGylated polystyrene (PS) NPs [102], PEGYlated gold NPs [103] as well as PEGylated dendrimers [104] have all been produced using this strategy. Meanwhile, a major setback using this approach is the steric hindrance that can limit the amount of PEG that can be coated (grafted) onto the surface of NP. In a comparison research, Nance et al. found that the amount of sufficiently dense mPEG-amine coating on PS NPs required to penetrate the human mucus is less as opposed to that required to penetrate the extracellular matrix of the brain tissue [105]. A limitation to this approach is the possible leakage of the encapsulated drug from the preformed NPs and variation from one batch to another.

The lipid bilayer of preformed liposomes can be explored in PEG-lipid conjugation. PEGylated lipid conjugates can be grafted preferentially to preformed liposomes using their hydrophobic lipid tail for interaction with the lipid bilayer. Attention must be given to the temperature and concentration of the lipids in the PEG-lipid solutions. While the temperature should be closer to the melting temperature of the lipid components and added at a slower rate, formation of micelles must be avoided by keeping the concentration of the PEG-lipid below the critical micellar concentration [106]. An interesting advantage of post-insertion technique as opposed to the self-assembly process is the allowance to modify the outer surface of liposome bilayer rather than the nonspecific insertion of PEG into the interior of liposome using the self-assembly strategy. As such, the post-insertion technique provides for the use of lesser PEG-lipid conjugate to achieve the same surface PEGylation compared to preinsertion method and provides for prolonged blood circulation half-life [107]. Another application using postinsertion conjugation technique is in the preparation of PEGylated liposomes when their surfaces are to be functionalized with targeting ligands. Tendered reaction conditions that underline the reactions between amino and carboxylic groups, pyridyldithiols and thiols, maleimide and thiols are fundamental for lipid PEGylation due to the fragile nature of liposomes [108]. A novel approach termed "click chemistry" has also been employed for PEG post-conjugation including interactions between the azide-modified PEG and the alkynyl groups on the surface of liposome [109, 110].

9 Alternative Polymers Used to Coat Nanoparticles

The paradigm in surface modification strategy employs PEGYlation—modifying the NPs' surfaces with PEG to reduce abrupt clearance of NPs from the systemic circulation. Notwithstanding, several reports have shown the some undesired negative influence PEGylation may presents on the performance of NPs as a vector in drug delivery. Meanwhile, alternative surface modification techniques, using other polymers to coat and provide stealth property on NPs, may offer possible solutions to the PEG dilemma [61]. Some of these alternative polymers for surface modification of NPs are highlighted below:

Polyoxazolines (POXs) POXs are group of potential alternative stealth polymers that have been explored in amphiphilic block copolymer as the hydrophilic components. POXs are generated through living cationic ring-opening polymerization (LCROP) of 2-oxazolines. They are versatile with a number of available variants with end-group and side-chains that could be functionalized [111]. POX has been used to coat proteins, micelle-based and liposomal formulations and have shown good nonbiofouling characteristics comparable to PEG in stealth effects [112]. More so, POXylated therapeutics have shown increased bioavailability compared to PEGylated formulations [113, 114] with more stability under physiological conditions [115]. For instance, poly(2-methyl-2-oxazoline) is more hydrophilic than PEG without amphiphilicity [116], having high biocompatibility with no cytotoxicity recorded at concentrations up to 20 g/L in cell culture [117] and up to 2 g/kg upon its intravenous administration in rats [113]. Poly(2-methyl-2-oxazoline) was coupled to poly(L-lysine) in exchange for PEG for nonviral gene delivery [118]. Poly(2-ethyl-2-oxazoline) was grafted to poly(caprolactone) [119], poly(1,3-trimethylene carbonate) [120], and poly(aspartic acid) [121], to produce polymeric micelles.

Polyglycerols Either in their linear of branched forms, polyglycerols which are also referred to as polyglycidols, are flexible and biocompatible hydrophilic aliphatic polyether polyols [122]. Highly branched polyglycerols possess antifouling properties with reduced susceptibility to oxidation or thermal stress that is comparable to PEG [123]. Also, the presence of multiple hydroxyl groups in polyglycerols allow for easy functionalization with other moieties [123]. The enhanced circulation half-life of hyper branched polyglycerols showed their potential as stealth polymer for surface modification of NPs [124]. Surface coated nanoliposomes were reported to show prolonged plasma circulation [125] and prevent opsonization to gold surface [123]. Recently, a multifactorial strategy in which both hyper branched polyglycerol and PEG were used as a block-copolymer to coat liposome was reported [126]. In this liposomal system, the polyglycerol moieties allowed for the multivalent functionalization of the liposome.

Poly (Amino Acids) Previous studies have reported the potential stealth characteristics of poly (amino acids) such as the poly hydroxyethyls of both L-asparagine and L-glutamine. Because poly (amino acids) are susceptible to protease degradation, there are limited chances that they will be accumulated in the biosystem [127]. Prolonged plasma circulation of NPs was observed using poly (amino acids) surface coating comparable to PEGylation [127]. Particularly, poly(hydroxyethyl-L-asparagine)-coated liposomes showed enhanced ABC resistance with higher stealth property than PEGylated liposomes after repeated low dose lipid administrations [128].

Polybetaines Zwitterionic molecules including sulfobetaine and carboxybetain as betaine derivatives interact with water molecules through electrostatic bonds [129] with stronger binding force as opposed to weak interaction via hydrogen bonding

[130]. Betaine-based polymers have been shown to decrease nontargeted protein adsorption [131], formation of biofilm [132], and bacterial adhesion on diverse surfaces. Interestingly, the carboxyl derivative of betaine (polycarboxybetaine) possess multiple functional groups which are responsive to multivalent conjugations thereby allows for multiple surface functionalization that could be explored in nanomedicines [133]. Based on these unique qualities, the use of polybetaines as alternative nonfouling materials for the surface medication of NPs has gained a lot of recognition. For instance, various nanomaterials including iron oxide [134], silica [135], gold [136], PLGA [137], and hydrogels have been modified using poly(carboxybetaine). These polybetaine-modified NPs exhibited enhanced size stability in protein solutions such as serum, which showed their potent resistance to nonspecific protein adsorption [134, 136].

Polysaccharides Another class of polymers that could provide excellent stealth property employed in surface medication of NPs are the polysaccharides. Surface engineered polysaccharide-coated NPs, having hydrophilic surfaces conferred on their surfaces, by polysaccharide derivatives such as chitosan [138], hyaluronic acid [139], dextran [140], and heparin [141] are well documented. The biodegradable, low immunogenic and less toxic properties of polysaccharides make them advantageous in surface medication chemistry [142]. Also, the presence of multiple functional groups that could be implored for conjugation with drugs molecules as well as cell-penetrating ligands allows for their application for surface modification. Reports have shown that polysaccharide-based NPs increase circulation half-lives of loaded therapeutics with enhanced accumulation in neoplastic tissues. For example, at lower acidic pH, chitosan molecules become positively charged and can facilitate cellular binding of coated NPs. This unique characteristic can be explored for direct targeting and delivery of anticancer drugs to the negatively charged surfaces of tumor tissues [143]. In another development, Papisov and colleagues employed acyclic hydrophilic polyacetals, a derivative of polycarbohydrates, instead of PEG for surface modification [144]. The high hydrophilicity and multiple modifiable functional groups of polyacetals make it more advantageous compared to PEG [144]. More importantly, the conjugation of polylysine to polyacetal produced a prolonged circulation time than a polylysine grafted dextran—a polysaccharide precursor for polyacetal. This was however attributed to changes in the rigid stereochemical structure of dextran [144].

10 The Rationale for Zebrafish as a Vertebrate Model for Human Disease

The completion of the human genome project as well as the complete sequencing of the zebrafish (ZF) genome allow for their comparative analyses in scientific research. Overall, ZF possess several unique features as a model organism in human disease

including their high fecundity, fast growth rate, easy and cost-effective maintenance, relatively small morphology having embryos and larvae that are optically translucent [145, 146]. Most importantly, a comparison between the ZF genome and that of human showed that over 70% of the human genes have their orthologs in the ZF genome [146]. ZF are readily available as transgenic lines having specialized marker genes for fluorescent macrophages, endothelial and the lymphatic systems. Meanwhile, diverse genetic tools have been developed for ZF mutation including zinc finger nucleases and knockdown using morpholino antisense oligonucleotides [147, 148]. These unique characteristics have popularized the increasing use of ZF as a model in scientific research of human disease including cancer over the past two decades [149].

11 Zebrafish as a Model for the Characterization of Human Cancer

Over the years, the ZF model has gained recognition as a diverse model for human diseases including cancer. Through transgenic techniques and gene-specific mutations in ZF cell lines, different models including that for melanomas, rhabdomyosarcoma and several other solid tumors are available [150]. There exists a large similarities between the growth of human cancer cell lines in ZF to the behavior of tumor xenografts in mammalian models like mice [151, 152]. Interestingly, tumors are developed in almost all the organs with similar histology to those found in human when carcinogens are used to induce tumor in ZF [153]. This is probably because many of the genes in human have at least an ortholog gene in ZF genome having the same cellular and molecular components which are involved in the disease initiation and development [146]. More importantly, the absence of any functional adaptive immune system during the early developmental stage in ZF until ~4 to 6 weeks old [154], allows for easy the formation of tumor within the model using either mouse or human cancer cells as there is nothing to actively suppress the immune system to avoid the rejection of injected human cells [154–156]. The transplanted tumors are established within 2 days post inoculation of the cancer cells. These properties make the ZF viable for cancer studies in vivo.

A more treasured and unique characteristic feature of the ZF model is the optical transparency of the embryo which allows for tissue imaging and monitoring down to the single cell level as a vertebrate research animal [151]. As such, the growth of tumors can be characterized by microscopic imaging at high resolution at time intervals in vivo in living organisms [149] which is a major advantage in cancer research. In time way, the growth of human cancer cells and the formation of solid tumor can be monitored as live phenomenon in real time with high spatial and transient resolution. Similarly, other fluorescent therapeutics such as NPs can be studied in vivo due to this optical transparency observed in ZF as further detailed in the next section.

12 NPs and the Zebrafish Model in Human Cancer Nanomedicines

Having highlighted the unique features of ZF as a model system for human diseases and tumor transplantation of human cancer cells into the ZF, the exceptional qualities of the ZF which allow for in vivo fluorescent microscopy coupled with its reduced immune system at the embryonic stage can be explored to study the mechanisms of action of cancer as well as to understand the possible treatment alternatives for cancer and other human diseases [157]. The importance of NPs both for diagnostic and drug delivery purposes is not easily understood in cancer nanomedicines. It is not readily easy to understand the dynamics of NP-host or NP-disease interactions within the biological system of most models after injection or other forms of delivery. Similarly, NPs' biodistribution and cellular internalization cannot be monitored easily in higher order vertebrate models such as mice and rats which require more complex and rigorous methods. Conversely, the ZF allows for an excellent view, monitoring and characterization of the interactions between NPs with the host as well as the disease in question [157]. In this way, both the benefits and potential complications of using NPs can be monitored. For instance, the undesirable characteristics exhibited by some NPs binding to the endothelial cells have been reported. Meanwhile, with very few exceptions, not many analyses have been done to understand the in vivo interactions of NPs with endothelial cells [158–160].

Usually, many of the studies that explored the use of nanotechnology and ZF mainly concentrate on the evaluation of the potential toxic effects of the NPs, with varied chemical makeup, have on normal systems [161, 162]. The report of Wagner and colleagues showed that prelabeled gold NPs with antibody specifically target and killed cancer cells when administered into the ZF embryo. Upon the application of heat through the laser pulse, the functionalized gold NPs generated a plasmonic nanobubble which killed the cancer cells preferentially [163]. However, in another development, previous report showed that NPs do not exhibit any toxic effect on the growth of cancer cells injected the ZF embryos [164]. Meanwhile, their reports do not validate the accumulation of NPs in the cancer tissues while the NPs were administered through bath-treatment.

13 Concluding Remarks

The increasing emergence of novel nanoparticulate systems has orchestrated the dire need of direct and accurate delivery of chemotherapeutics to tumor cells without harming the normal tissues. Central to the concept of passive targeting of NPs is the unique microvasculature of tumor. Various regulatory factors that control the blood pressure as well as maneuver the vasculature may shift the equilibrium towards the more captivating tumor environment for NPs. Based on the initial

success of an improved systemic delivery of proteins upon PEGylation, surface modification of NPs using PEG has also yield promising results for enhanced systemic delivery of therapeutic vectors. More importantly, we highlighted in this chapter how PEGylation of NPs and exploration of the EPR effect could increase the circulation times of NPs while escaping immediate elimination by the immune systems and rapid renal clearance in vivo. Similarly, the stealth properties of NPs can be explored for enhance therapeutic effects through surface modification with other nonfouling hydrophilic polymers in order to cover for the PEG dilemma. Furthermore, the zebrafish model presents a promising platform to monitor and characterize "magic" engineered nanocargoes for theranostic interventions. Overall, a matter of debate among the scientific community is that while passive targeting enhances the efficient accumulation of NPs in the neoplastic interstitium, it cannot facilitates their cellular uptake thereby requires the more promising and specific active targeting of NPs to overexpressed receptors on cancer cells. It is therefore our proposition that these two strategies be harnessed simultaneously in order to achieve an optimum therapeutic efficacy from prospective nanoengineered systems in cancer nanomedicines.

References

1. Masoudkabir, F., Sarrafzadegan, N., Gotay, C., Ignaszewski, A., Krahn, A. D., Davis, M. K., et al. (2017). Cardiovascular disease and cancer: Evidence for shared disease pathways and pharmacologic prevention. *Atherosclerosis, 263*, 343–351.
2. WHO|Cancer. WHO. Accessed November 24, 2014, from http://www.who.int/cancer/en/
3. Siegel, R., Ma, J., Zou, Z., & Jemal, A. (2014). Cancer statistics, 2014. *CA: a Cancer Journal for Clinicians, 64*(1), 9–29.
4. Sudhakar, A. (2009). History of Cancer, Ancient and Modern Treatment Methods. *Journal of Cancer Science and Therapy, 1*(2), 1–4.
5. Kreuter, J. (2007, February 22). Nanoparticles – A historical perspective. *International Journal of Pharmaceutics, 331*(1), 1–10.
6. Bazak, R., Houri, M., Se, A., Hussein, W., & Refaat, T. (2014). Passive targeting of nanoparticles to cancer: A comprehensive review of the literature. *Molecular and Clinical Oncology, 2*(6), 904–908.
7. Yu, X., Trase, I., Ren, M., Duval, K., Guo, X., & Chen, Z. (2016). Design of nanoparticle-based carriers for targeted drug delivery. *Journal of Nanomaterials*. https://doi.org/10.1155/2016/1087250.
8. Matsumura, Y., & Maeda, H. (1986). A new concept for macromolecular therapeutics in cancer chemotherapy: Mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. *Cancer Research, 46*(12 I), 6387–6392.
9. Byrne, J. D., Betancourt, T., & Brannon-Peppas, L. (2008). Active targeting schemes for nanoparticle systems in cancer therapeutics. *Advanced Drug Delivery Reviews, 60*(15), 1615–1626.
10. Jones, A., & Harris, A. L. (1998). New developments in angiogenesis: A major mechanism for tumor growth and target for therapy. *The Cancer Journal from Scientific American, 4*(4), 209–217.
11. Hobbs, S. K., Monsky, W. L., Yuan, F., Roberts, W. G., Griffith, L., Torchilin, V. P., et al. (1998). Regulation of transport pathways in tumor vessels: Role of tumor type and

microenvironment. *Proceedings of the National Academy of Sciences of the United States of America, 95*(8), 4607–4612.
12. Haley, B., & Frenkel, E. (2008). Nanoparticles for drug delivery in cancer treatment. *Urologic Oncology: Seminars and Original Investigations, 26*(1), 57–64.
13. Krasnici, S., Werner, A., Eichhorn, M. E., Schmitt-Sody, M., Pahernik, S. A., Sauer, B., et al. (2003). Effect of the surface charge of liposomes on their uptake by angiogenic tumor vessels. *International Journal of Cancer, 105*(4), 561–567.
14. Kunstfeld, R., Wickenhauser, G., Michaelis, U., Teifel, M., Umek, W., Naujoks, K., et al. (2003). Paclitaxel encapsulated in cationic liposomes diminishes tumor angiogenesis and melanoma growth in a "humanized" SCID mouse model. *The Journal of Investigative Dermatology, 120*(3), 476–482.
15. Kuruppu, D., Christophi, C., Maeda, H., & O'Brien, P. E. (2002). Changes in the microvascular architecture of colorectal liver metastases following the administration of SMANCS/lipiodol. *The Journal of Surgical Research, 103*(1), 47–54.
16. Greish, K. (2007). Enhanced permeability and retention of macromolecular drugs in solid tumors: A royal gate for targeted anticancer nanomedicines. *Journal of Drug Targeting, 15*(7–8), 457–464.
17. Li, C. J., Miyamoto, Y., Kojima, Y., & Maeda, H. (1993). Augmentation of tumour delivery of macromolecular drugs with reduced bone marrow delivery by elevating blood pressure. *British Journal of Cancer, 67*(5), 975–980.
18. Seymour, L. W., & Fisher, K. D. (2016). Under pressure: Elevated blood pressure enhances targeting of tumors by oncolytic viruses. *Molecular Therapy, 24*(2), 204–205.
19. Iyer, A. K., Khaled, G., Fang, J., & Maeda, H. (2006). Exploiting the enhanced permeability and retention effect for tumor targeting. *Drug Discovery Today, 11*(17–18), 812–818.
20. Wu, J., Akaike, T., & Maeda, H. (1998). Modulation of enhanced vascular permeability in tumors by a bradykinin antagonist, a cyclooxygenase inhibitor, and a nitric oxide scavenger. *Cancer Research, 58*(1), 159–165.
21. Maeda, H. (2001). The enhanced permeability and retention (EPR) effect in tumor vasculature: The key role of tumor-selective macromolecular drug targeting. *Advances in Enzyme Regulation, 41*, 189–207.
22. Stefanini, M. O., Wu, F. T. H., Mac Gabhann, F., & Popel, A. S. (2010). Increase of plasma VEGF after intravenous administration of bevacizumab is predicted by a pharmacokinetic model. *Cancer Research, 70*(23), 9886–9894.
23. Seki, T., Fang, J., & Maeda, H. (2009). Tumor-targeted macromolecular drug delivery based on the enhanced permeability and retention effect in solid tumor. In *Pharmaceutical perspectives of cancer therapeutics* (pp. 93–120). New York: Springer.
24. Chambers, A. F., & Matrisian, L. M. (1997). Changing views of the role of matrix metalloproteinases in metastasis. *Journal of the National Cancer Institute, 89*(17), 1260–1270.
25. Wu, J., Akaike, T., Hayashida, K., Okamoto, T., Okuyama, A., & Maeda, H. (2001). Enhanced vascular permeability in solid tumor involving peroxynitrite and matrix metalloproteinases. *Japanese Journal of Cancer Research, 92*(4), 439–451.
26. Wu, J., Akaike, T., Hayashida, K., Miyamoto, Y., Nakagawa, T., Miyakawa, K., et al. (2002). Identification of bradykinin receptors in clinical cancer specimens and murine tumor tissues. *International Journal of Cancer, 98*(1), 29–35.
27. Matsumura, Y., Kimura, M., Yamamoto, T., & Maeda, H. (1988). Involvement of the kinin-generating cascade in enhanced vascular permeability in tumor tissue. *Japanese Journal of Cancer Research, 79*(12), 1327–1334.
28. Wu, J., Akaike, T., & Maeda, H. (1998). Modulation of enhanced vascular permeability in tumors by a bradykinin antagonist, a cyclooxygenase inhibitor, and a nitric oxide scavenger. *Cancer Research, 58*(1), 159–165.
29. Papapetropoulos, A., García-Cardeña, G., Madri, J. A., & Sessa, W. C. (1997). Nitric oxide production contributes to the angiogenic properties of vascular endothelial growth factor in human endothelial cells. *The Journal of Clinical Investigation, 100*(12), 3131–3139.

30. Seki, T., Fang, J., & Maeda, H. (2009). Tumor-targeted macromolecular drug delivery based on the enhanced permeability and retention effect in solid tumor. In *Pharmaceutical perspectives of cancer therapeutics* (pp. 93–120). New York: Springer.
31. Hori, K., Saito, S., Takahashi, H., Sato, H., Maeda, H., & Sato, Y. (2000). Tumor-selective blood flow decrease induced by an angiotensin converting enzyme inhibitor, temocapril hydrochloride. *Japanese Journal of Cancer Research, 91*(2), 261–269.
32. Noguchi, A., Takahashi, T., Yamaguchi, T., et al. (1992). Enhanced tumor localization of monoclonal antibody by treatment with kininase II inhibitor and angiotensin II. *Japanese Journal of Cancer Research, 83*(3), 240–243.
33. Greish, K. (2010). Enhanced permeability and retention (EPR) effect for anticancer nanomedicine drug targeting. *Methods in Molecular Biology, 624*, 25–37.
34. Maeda, H. (2012). Vascular permeability in cancer and infection as related to macromolecular drug delivery, with emphasis on the EPR effect for tumor-selective drug targeting. *Proceedings of the Japan Academy. Series B, Physical and Biological Sciences, 88*(3), 53–71.
35. Okamoto, T., Akaike, T., Sawa, T., Miyamoto, Y., van der Vliet, A., & Maeda, H. (2001). Activation of matrix metalloproteinases by peroxynitrite-induced protein S-glutathiolation via disulfide S-oxide formation. *The Journal of Biological Chemistry, 276*(31), 29596–29602.
36. Iyer, A. K., Khaled, G., Fang, J., & Maeda, H. (2006). Exploiting the enhanced permeability and retention effect for tumor targeting. *Drug Discovery Today, 11*(17–18), 812–818.
37. Greish, K. (2007). Enhanced permeability and retention of macromolecular drugs in solid tumors: A royal gate for targeted anticancer nanomedicines. *Journal of Drug Targeting, 15*(7–8), 457–464.
38. Tanaka, S., Akaike, T., Wu, J., et al. (2003). Modulation of tumor-selective vascular blood flow and extravasation by the stable prostaglandin 12 analogue beraprost sodium. *Journal of Drug Targeting, 11*(1), 45–52.
39. Adeyemi, S. A., Choonara, Y. E., Kumar, P., Du Toit, C. L., & Pillay, V. (2017). Design and characterization of endostatin-loaded nanoparticles for in vitro antiangiogenesis in squamous cell carcinoma. *Journal of Nanomaterials, 2017*, 2539065. https://doi.org/10.1155/2017/2539065.
40. Tarhini, M., Greige-Gerges, H., & Elaissari, A. (2017). Protein-based nanoparticles: From preparation to encapsulation of active molecules. *International Journal of Pharmaceutics, 522*(1), 172–197.
41. Thomas, S. C., Harshita, Mishra, P. K., & Talegaonkar, S. (2015). Ceramic nanoparticles: Fabrication methods and applications in drug delivery. *Current Pharmaceutical Design, 21*(42), 6165–6188.
42. Cho, C. F., Sourabh, S., Simpson, E. J., Steinmetz, N. F., Luyt, L. G., & Lewis, J. D. (2014). Molecular targeted viral nanoparticles as tools for imaging cancer. *Methods in Molecular Biology, 1108*, 211–230.
43. Singla, R., Guliani, A., Kumari, A., & Yadav, S. K. (2016). Metallic nanoparticles, toxicity issues and applications in medicine. In *Nanoscale materials in targeted drug delivery, theragnosis and tissue regeneration* (pp. 41–80). Singapore: Springer.
44. Applications of Carbon Nanotubes. (2018). Accessed June 20, 2018, from https://www.azonano.com/article.aspx?ArticleID=4842
45. Sutton, D., Nasongkla, N., Blanco, E., & Gao, J. (2007). Functionalized micellar systems for cancer targeted drug delivery. *Pharmaceutical Research, 24*(6), 1029–1046.
46. Kukowska-Latallo, J. F., Candido, K. A., Cao, Z., Nigavekar, S. S., Majoros, I. J., Thomas, T. P., et al. (2005). Nanoparticle targeting of anticancer drug improves therapeutic response in animal model of human epithelial cancer. *Cancer Research, 65*(12), 5317–5324.
47. Allen, T. M., & Cullis, P. R. (2013). Liposomal drug delivery systems: From concept to clinical applications. *Advanced Drug Delivery Reviews, 65*(1), 36–48.
48. Byrne, J. D., Betancourt, T., & Brannon-Peppas, L. (2008). Active targeting schemes for nanoparticle systems in cancer therapeutics. *Advanced Drug Delivery Reviews, 60*(15), 1615–1626.

49. Owens, D. E., & Peppas, N. A. (2006). Opsonization, biodistribution, and pharmacokinetics of polymeric nanoparticles. *International Journal of Pharmaceutics, 307*(1), 93–102.
50. Gustafson, H. H., Holt-Casper, D., Grainger, D. W., & Ghandehari, H. (2015). Nanoparticle uptake: The phagocyte problem. *Nano Today, 10*(4), 487–510.
51. Gref, R., Lück, M., Quellec, P., et al. (2000). 'Stealth' corona-core nanoparticles surface modified by polyethylene glycol (PEG): Influences of the corona (PEG chain length and surface density) and of the core composition on phagocytic uptake and plasma protein adsorption. *Colloids and Surfaces. B, Biointerfaces, 18*(3), 301–313.
52. Adebowale, A. S., Choonara, Y. E., Kumar, P., du Toit, L. C., & Pillay, V. (2015). Functionalized nanocarriers for enhanced bioactive delivery to squamous cell carcinomas: Targeting approaches and related biopharmaceutical aspects. *Current Pharmaceutical Design, 21*(22), 3167–3180.
53. Zhang, L., Gu, F. X., Chan, J. M., Wang, A. Z., Langer, R. S., & Farokhzad, O. C. (2008). Nanoparticles in medicine: Therapeutic applications and developments. *Clinical Pharmacology and Therapeutics, 83*(5), 761–769.
54. Canelas, D. A., Herlihy, K. P., & DeSimone, J. M. (2009). Top-down particle fabrication: Control of size and shape for diagnostic imaging and drug delivery. *Wiley Interdisciplinary Reviews. Nanomedicine and Nanobiotechnology, 1*(4), 391–404.
55. Longmire, M. R., Ogawa, M., Choyke, P. L., & Kobayashi, H. (2011). Biologically optimized nanosized molecules and particles: More than just size. *Bioconjugate Chemistry, 22*(6), 993–1000.
56. Albanese, A., Sykes, E. A., & Chan, W. C. W. (2010). Rough around the edges: The inflammatory response of microglial cells to spiky nanoparticles. *ACS Nano, 4*(5), 2490–2493.
57. Yamamoto, Y., Nagasaki, Y., Kato, Y., Sugiyama, Y., & Kataoka, K. (2001). Long-circulating poly(ethylene glycol)-poly(D,L-lactide) block copolymer micelles with modulated surface charge. *Journal of Controlled Release, 77*(1–2), 27–38.
58. Dreaden, E. C., Austin, L. A., Mackey, M. A., & El-Sayed, M. A. (2012). Size matters: Gold nanoparticles in targeted cancer drug delivery. *Therapeutic Delivery, 3*(4), 457–478.
59. Hauert, S., & Bhatia, S. N. (2014). Mechanisms of cooperation in cancer nanomedicine: Towards systems nanotechnology. *Trends in Biotechnology, 32*(9), 448–455.
60. Sheng, Y., Yuan, Y., Liu, C., Tao, X., Shan, X., & Xu, F. (2009). In vitro macrophage uptake and in vivo biodistribution of PLA-PEG nanoparticles loaded with hemoglobin as blood substitutes: Effect of PEG content. *Journal of Materials Science. Materials in Medicine, 20*(9), 1881–1891.
61. Amoozgar, Z., & Yeo, Y. (2012). Recent advances in stealth coating of nanoparticle drug delivery systems. *Wiley Interdisciplinary Reviews. Nanomedicine and Nanobiotechnology, 4*(2), 219–233.
62. Zhu, L., & Torchilin, V. P. (2013). Stimulus-responsive nanopreparations for tumor targeting. *Integrative Biology, 5*(1), 96–107.
63. Sailor, M. J., & Park, J.-H. (2012). Hybrid nanoparticles for detection and treatment of cancer. *Advanced Materials, 24*(28), 3779–3802.
64. Olson, E. S., Jiang, T., Aguilera, T. A., Nguyen, Q. T., Ellies, L. G., Scadeng, M., et al. (2010). Activatable cell penetrating peptides linked to nanoparticles as dual probes for in vivo fluorescence and MR imaging of proteases. *Proceedings of the National Academy of Sciences of the United States of America, 107*(9), 4311–4316.
65. Poon, Z., Chang, D., Zhao, X., & Hammond, P. T. (2011). Layer-by-layer nanoparticles with a pH-Sheddable layer for in vivo targeting of tumor hypoxia. *ACS Nano, 5*(6), 4284–4292.
66. Yaari, Z., da, S. D., Zinger, A., et al. (2016). Theranostic barcoded nanoparticles for personalized cancer medicine. *Nature Communications, 7*, 13325.
67. McCarthy, J. R., & Weissleder, R. (2008). Multifunctional magnetic nanoparticles for targeted imaging and therapy. *Advanced Drug Delivery Reviews, 60*(11), 1241–1251.
68. Soo Choi, H., Liu, W., Misra, P., et al. (2007). Renal clearance of quantum dots. *Nature Biotechnology, 25*(10), 1165–1170.

69. Maeda, H., Nakamura, H., & Fang, J. (2013). The EPR effect for macromolecular drug delivery to solid tumors: Improvement of tumor uptake, lowering of systemic toxicity, and distinct tumor imaging in vivo. *Advanced Drug Delivery Reviews, 65*(1), 71–79.
70. Wang, J., Byrne, J. D., Napier, M. E., & DeSimone, J. M. (2011). More effective nanomedicines through particle design. *Small, 7*(14), 1919–1931.
71. Venkataraman, S., Hedrick, J. L., Ong, Z. Y., et al. (2011, November). The effects of polymeric nanostructure shape on drug delivery. *Advanced Drug Delivery Reviews, 63*(14–15), 1228–1246.
72. Owens, D. E., III, & Peppas, N. A. (2006). Opsonization, biodistribution, and pharmacokinetics of polymeric nanoparticles. *International Journal of Pharmaceutics, 307*(1), 93–102.
73. Marquez, M., Nilsson, S., Lennartsson, L., et al. (2004). Charge-dependent targeting: Results in six tumor cell lines. *Anticancer Research, 24*(3A), 1347–1352.
74. Romberg, B., Hennink, W. E., & Storm, G. (2008). Sheddable coatings for long-circulating nanoparticles. *Pharmaceutical Research, 25*(1), 55–71.
75. Petros, R. A., & DeSimone, J. M. (2010). Strategies in the design of nanoparticles for therapeutic applications. *Nature Reviews. Drug Discovery, 9*(8), 615–627.
76. Rodriguez, P. L., Harada, T., Christian, D. A., Pantano, D. A., Tsai, R. K., & Discher, D. E. (2013). Minimal "self" peptides that inhibit phagocytic clearance and enhance delivery of nanoparticles. *Science, 339*(6122), 971–975.
77. Mishra, P., Nayak, B., & Dey, R. K. (2016). PEGylation in anti-cancer therapy: An overview. *Asian Journal of Pharmaceutical Sciences, 11*(3), 337–348.
78. Abuchowski, A., van Es, T., Palczuk, N. C., & Davis, F. F. (1977). Alteration of immunological properties of bovine serum albumin by covalent attachment of polyethylene glycol. *The Journal of Biological Chemistry, 252*(11), 3578–3581.
79. Abuchowski, A., McCoy, J. R., Palczuk, N. C., van Es, T., & Davis, F. F. (1977). Effect of covalent attachment of polyethylene glycol on immunogenicity and circulating life of bovine liver catalase. *The Journal of Biological Chemistry, 252*(11), 3582–3586.
80. Matsushima, A., Kodera, Y., Hiroto, M., Nishimura, H., & Inada, Y. (1996). Bioconjugates of proteins and polyethylene glycol: Potent tools in biotechnological processes. *Journal of Molecular Catalysis B: Enzymatic, 2*(1), 1–17.
81. Giorgi, M. E., Agusti, R., & de Lederkremer, R. M. (2014). Carbohydrate PEGylation, an approach to improve pharmacological potency. *Beilstein Journal of Organic Chemistry, 10*, 1433–1444.
82. Riley, T, & Riggs-Sauthier, J. (2008) The benefits and challenges of PEGylating small molecules. Accessed July 2, 2018, from http://www.pharmtech.com/benefits-and-challenges-pegylating-small-molecules
83. Roberts, M. J., Bentley, M. D., & Harris, J. M. (2002). Chemistry for peptide and protein PEGylation. *Advanced Drug Delivery Reviews, 54*(4), 459–476.
84. Dozier, J. K., & Distefano, M. D. (2015). Site-specific PEGylation of therapeutic proteins. *International Journal of Molecular Sciences, 16*(10), 25831–25864.
85. Veronese, F. M., & Pasut, G. (2008). PEGylation: Posttranslational bioengineering of protein biotherapeutics. *Drug Discovery Today: Technologies, 5*(2–3), e57–e64.
86. Caliceti, P., & Veronese, F. M. (2003). Pharmacokinetic and biodistribution properties of poly(ethylene glycol)-protein conjugates. *Advanced Drug Delivery Reviews, 55*(10), 1261–1277.
87. Bailon, P., & Berthold, W. (1998). Polyethylene glycol-conjugated pharmaceutical proteins. *Pharmaceutical Science & Technology Today, 1*(8), 352–356.
88. Yamaoka, T., Tabata, Y., & Ikada, Y. (1994). Distribution and tissue uptake of poly(ethylene glycol) with different molecular weights after intravenous administration to mice. *Journal of Pharmaceutical Sciences, 83*(4), 601–606.
89. Remaut, K., Lucas, B., Braeckmans, K., Demeester, J., & De Smedt, S. C. (2007). Pegylation of liposomes favours the endosomal degradation of the delivered phosphodiester oligonucleotides. *Journal of Controlled Release, 117*(2), 256–266.

90. Hatakeyama, H., Akita, H., & Harashima, H. (2013). The polyethyleneglycol dilemma: Advantage and disadvantage of PEGylation of liposomes for systemic genes and nucleic acids delivery to tumors. *Biological & Pharmaceutical Bulletin, 36*(6), 892–899.
91. Sato, Y., Hatakeyama, H., Sakurai, Y., Hyodo, M., Akita, H., & Harashima, H. (2012). A pH-sensitive cationic lipid facilitates the delivery of liposomal siRNA and gene silencing activity in vitro and in vivo. *Journal of Controlled Release, 163*(3), 267–276.
92. Hatakeyama, H., Akita, H., & Harashima, H. (2011). A multifunctional envelope type nano device (MEND) for gene delivery to tumours based on the EPR effect: A strategy for overcoming the PEG dilemma. *Advanced Drug Delivery Reviews, 63*(3), 152–160.
93. Suk, J. S., Xu, Q., Kim, N., Hanes, J., & Ensign, L. M. (2016). PEGylation as a strategy for improving nanoparticle-based drug and gene delivery. *Advanced Drug Delivery Reviews, 99*. (Pt A, 28–51.
94. Vittaz, M., Bazile, D., Spenlehauer, G., et al. (1996). Effect of PEO surface density on long-circulating PLA-PEO nanoparticles which are very low complement activators. *Biomaterials, 17*(16), 1575–1581.
95. Alexis, F., Pridgen, E., Molnar, L. K., & Farokhzad, O. C. (2008). Factors affecting the clearance and biodistribution of polymeric nanoparticles. *Molecular Pharmaceutics, 5*(4), 505–515.
96. Yang, M., Lai, S. K., Wang, Y.-Y., et al. (2011). Biodegradable nanoparticles composed entirely of safe materials that rapidly penetrate human mucus. *Angewandte Chemie (International Ed. in English), 50*(11), 2597–2600.
97. Chan, J. M., Zhang, L., Yuet, K. P., et al. (2009). PLGA-lecithin-PEG core-shell nanoparticles for controlled drug delivery. *Biomaterials, 30*(8), 1627–1634.
98. Lin, J.-J., Chen, J.-S., Huang, S.-J., et al. (2009). Folic acid-Pluronic F127 magnetic nanoparticle clusters for combined targeting, diagnosis, and therapy applications. *Biomaterials, 30*(28), 5114–5124.
99. Mi, Y., Liu, Y., & Feng, S.-S. (2011). Formulation of Docetaxel by folic acid-conjugated d-α-tocopheryl polyethylene glycol succinate 2000 (Vitamin E TPGS(2k)) micelles for targeted and synergistic chemotherapy. *Biomaterials, 32*(16), 4058–4066.
100. Wang, H., Zhao, P., Su, W., et al. (2010). PLGA/polymeric liposome for targeted drug and gene co-delivery. *Biomaterials, 31*(33), 8741–8748.
101. Cu, Y., & Saltzman, W. M. (2009). Controlled surface modification with poly(ethylene) glycol enhances diffusion of PLGA nanoparticles in human cervical mucus. *Molecular Pharmaceutics, 6*(1), 173–181.
102. Nuruzatulifah, A. M., Nizam, A. A., & Ain, N. M. N. (2016). Synthesis and characterization of polystyrene nanoparticles with covalently attached fluorescent dye. *Materials Today Proceedings, 3*, S112–S119.
103. Perrault, S. D., & Chan, W. C. W. (2009). Synthesis and surface modification of highly monodispersed, spherical gold nanoparticles of 50–200 nm. *Journal of the American Chemical Society, 131*(47), 17042–17043.
104. Kim, A. J., Boylan, N. J., Suk, J. S., et al. (2013). Use of single-site-functionalized PEG dendrons to prepare gene vectors that penetrate human mucus barriers. *Angewandte Chemie (International Ed. in English), 52*(14), 3985–3988.
105. Nance, E. A., Woodworth, G. F., Sailor, K. A., et al. (2012). A dense poly(ethylene glycol) coating improves penetration of large polymeric nanoparticles within brain tissue. *Science Translational Medicine, 4*(149), 149ra119.
106. Uster, P. S., Allen, T. M., Daniel, B. E., Mendez, C. J., Newman, M. S., & Zhu, G. Z. (1996). Insertion of poly(ethylene glycol) derivatized phospholipid into pre-formed liposomes results in prolonged in vivo circulation time. *FEBS Letters, 386*(2–3), 243–246.
107. Nakamura, K., Yamashita, K., Itoh, Y., Yoshino, K., Nozawa, S., & Kasukawa, H. (2012). Comparative studies of polyethylene glycol-modified liposomes prepared using different PEG-modification methods. *Biochimica et Biophysica Acta, 1818*(11), 2801–2807.
108. Torchilin, V. P. (2005). Recent advances with liposomes as pharmaceutical carriers. *Nature Reviews. Drug Discovery, 4*(2), 145–160.

109. Cavalli, S., Tipton, A. R., Overhand, M., & Kros, A. (2006). The chemical modification of liposome surfaces via a copper-mediated [3+2] azide–alkyne cycloaddition monitored by a colorimetric assay. *Chemical Communications, 0*(30), 3193–3195.
110. Kumar, A., Erasquin, U. J., Qin, G., Li, K., & Cai, C. (2010). "Clickable", polymerized liposomes as a versatile and stable platform for rapid optimization of their peripheral compositions. *Chemical Communications, 46*(31), 5746–5748.
111. Manzenrieder, F., Luxenhofer, R., Retzlaff, M., Jordan, R., & Finn, M. G. (2011). Stabilization of virus-like particles with poly(2-oxazoline)s. *Angewandte Chemie, International Edition, 50*(11), 2601–2605.
112. Zhang, N., Pompe, T., Amin, I., Luxenhofer, R., Werner, C., & Jordan, R. (2012). Tailored poly(2-oxazoline) polymer brushes to control protein adsorption and cell adhesion. *Macromolecular Bioscience, 12*(7), 926–936.
113. Viegas, T. X., Bentley, M. D., Harris, J. M., et al. (2011). Polyoxazoline: Chemistry, properties, and applications in drug delivery. *Bioconjugate Chemistry, 22*(5), 976–986.
114. Tong, J., Yi, X., Luxenhofer, R., et al. (2013). Conjugates of superoxide dismutase 1 with amphiphilic poly(2-oxazoline) block copolymers for enhanced brain delivery: Synthesis, characterization and evaluation in vitro and in vivo. *Molecular Pharmaceutics, 10*(1), 360–377.
115. Pidhatika, B., Rodenstein, M., Chen, Y., et al. (2012). Comparative stability studies of poly(2-methyl-2-oxazoline) and poly(ethylene glycol) brush coatings. *Biointerphases, 7*(1), 1.
116. Foreman, M. B., Coffman, J. P., Murcia, M. J., et al. (2003). Gelation of amphiphilic lipopolymers at the air-water interface: 2D analogue to 3D gelation of colloidal systems with grafted polymer chains? *Langmuir, 19*(2), 326–332.
117. Luxenhofer, R., Schulz, A., Roques, C., et al. (2010). Doubly amphiphilic poly(2-oxazoline)s as high-capacity delivery systems for hydrophobic drugs. *Biomaterials, 31*(18), 4972–4979.
118. von Erlach, T., Zwicker, S., Pidhatika, B., et al. (2011). Formation and characterization of DNA-polymer-condensates based on poly(2-methyl-2-oxazoline) grafted poly(L-lysine) for non-viral delivery of therapeutic DNA. *Biomaterials, 32*(22), 5291–5303.
119. Cheon Lee, S., Kim, C., Chan Kwon, I., Chung, H., & Young Jeong, S. (2003). Polymeric micelles of poly(2-ethyl-2-oxazoline)-block-poly(epsilon-caprolactone) copolymer as a carrier for paclitaxel. *Journal of Controlled Release, 89*(3), 437–446.
120. Kim, C., Lee, S. C., Shin, J. H., Yoon, J.-S., Kwon, I. C., & Jeong, S. Y. (2000). Amphiphilic diblock copolymers based on poly(2-ethyl-2-oxazoline) and poly(1,3-trimethylene carbonate): Synthesis and micellar characteristics. *Macromolecules, 33*(20), 7448–7452.
121. Wang, C.-H., Wang, W.-T., & Hsiue, G.-H. (2009). Development of polyion complex micelles for encapsulating and delivering amphotericin B. *Biomaterials, 30*(19), 3352–3358.
122. Kainthan, R. K., & Brooks, D. E. (2007). In vivo biological evaluation of high molecular weight hyperbranched polyglycerols. *Biomaterials, 28*(32), 4779–4787.
123. Siegers, C., Biesalski, M., & Haag, R. (2004). Self-assembled monolayers of dendritic polyglycerol derivatives on gold that resist the adsorption of proteins. *Chemistry, 10*(11), 2831–2838.
124. Kainthan, R. K., Hester, S. R., Levin, E., Devine, D. V., & Brooks, D. E. (2007). *In vitro* biological evaluation of high molecular weight hyperbranched polyglycerols. *Biomaterials, 28*(31), 4581–4590.
125. Maruyama, K., Okuizumi, S., Ishida, O., Yamauchi, H., Kikuchi, H., & Iwatsuru, M. (1994). Phosphatidyl polyglycerols prolong liposome circulation in vivo. *International Journal of Pharmaceutics, 111*(1), 103–107.
126. Hofmann, A. M., Wurm, F., Hühn, E., Nawroth, T., Langguth, P., & Frey, H. (2010). Hyperbranched polyglycerol-based lipids via oxyanionic polymerization: Toward multifunctional stealth liposomes. *Biomacromolecules, 11*(3), 568–574.
127. Romberg, B., Metselaar, J. M., Baranyi, L., et al. (2007). Poly(amino acid)s: Promising enzymatically degradable stealth coatings for liposomes. *International Journal of Pharmaceutics, 331*(2), 186–189.

128. Romberg, B., Oussoren, C., Snel, C. J., Carstens, M. G., Hennink, W. E., & Storm, G. (2007). Pharmacokinetics of poly(hydroxyethyl-l-asparagine)-coated liposomes is superior over that of PEG-coated liposomes at low lipid dose and upon repeated administration. *Biochimica et Biophysica Acta, 1768*(3), 737–743.
129. Shao, Q., He, Y., White, A. D., & Jiang, S. (2010). Difference in hydration between carboxybetaine and sulfobetaine. *The Journal of Physical Chemistry. B, 114*(49), 16625–16631.
130. Chen, S., Chen, S., Jiang, S., et al. (2011). Study of zwitterionic sulfopropylbetaine containing reactive siloxanes for application in antibacterial materials. *Colloids and Surfaces. B, Biointerfaces, 85*(2), 323–329.
131. Zhang, Z., Vaisocherová, H., Cheng, G., Yang, W., Xue, H., & Jiang, S. (2008). Nonfouling behavior of polycarboxybetaine-grafted surfaces: Structural and environmental effects. *Biomacromolecules, 9*(10), 2686–2692.
132. Cheng, G., Li, G., Xue, H., Chen, S., Bryers, J. D., & Jiang, S. (2009). Zwitterionic carboxybetaine polymer surfaces and their resistance to long-term biofilm formation. *Biomaterials, 30*(28), 5234–5240.
133. Jiang, S., & Cao, Z. (2010). Ultralow-fouling, functionalizable, and hydrolyzable zwitterionic materials and their derivatives for biological applications. *Advanced Materials, 22*(9), 920–932.
134. Zhang, L., Xue, H., Gao, C., et al. (2010). Imaging and cell targeting characteristics of magnetic nanoparticles modified by a functionalizable zwitterionic polymer with adhesive 3,4-dihydroxyphenyl-l-alanine linkages. *Biomaterials, 31*(25), 6582–6588.
135. Jia, G., Cao, Z., Xue, H., Xu, Y., & Jiang, S. (2009). Novel zwitterionic-polymer-coated silica nanoparticles. *Langmuir, 25*(5), 3196–3199.
136. Yang, W., Zhang, L., Wang, S., White, A. D., & Jiang, S. (2009). Functionalizable and ultra stable nanoparticles coated with zwitterionic poly(carboxybetaine) in undiluted blood serum. *Biomaterials, 30*(29), 5617–5621.
137. Cao, Z., Yu, Q., Xue, H., Cheng, G., & Jiang, S. (2010). Nanoparticles for drug delivery prepared from amphiphilic PLGA zwitterionic block copolymers with sharp contrast in polarity between two blocks. *Angewandte Chemie (International Ed. in English), 49*(22), 3771–3776.
138. Kim, K., Kim, J. H., Park, H., Kim, Y.-S., Park, K., Nam, H., et al. (2010). Tumor-homing multifunctional nanoparticles for cancer theragnosis: Simultaneous diagnosis, drug delivery, and therapeutic monitoring. *Journal of Controlled Release, 146*(2), 219–227.
139. Choi, K. Y., Chung, H., Min, K. H., et al. (2010). Self-assembled hyaluronic acid nanoparticles for active tumor targeting. *Biomaterials, 31*(1), 106–114.
140. Li, Y.-L., Zhu, L., Liu, Z., et al. (2009). Reversibly stabilized multifunctional dextran nanoparticles efficiently deliver doxorubicin into the nuclei of cancer cells. *Angewandte Chemie (International Ed. in English), 48*(52), 9914–9918.
141. Hou, L., Fan, Y., Yao, J., et al. (2011). Low molecular weight heparin-all-trans-retinoid acid conjugate as a drug carrier for combination cancer chemotherapy of paclitaxel and all-trans-retinoid acid. *Carbohydrate Polymers, 86*(3), 1157–1166.
142. Kean, T., & Thanou, M. (2010). Biodegradation, biodistribution and toxicity of chitosan. *Advanced Drug Delivery Reviews, 62*(1), 3–11.
143. Adeyemi, S. A., Choonara, Y. E., Kumar, P., du Toit, L. C., & Pillay, V. (2017). Synthesis and in vitro characterization of a pH-responsive chitosan- polyethylenimine nanosystem for the delivery of therapeutic proteins. *Journal of Drug Delivery Science and Technology, 39*, 266–276.
144. Papisov, M. I. (2001). Biopolymers from polysaccharides and agroproteins. *American Chemical Society, 786*. https://doi.org/10.1021/bk-2001-0786.
145. Dahm, R., & Geisler, R. (2006). Learning from small fry: The zebrafish as a genetic model organism for aquaculture fish species. *Marine Biotechnology, 8*(4), 329–345.
146. Howe, K., Clark, M. D., Torroja, C. F., Torrance, J., Berthelot, C., Muffato, M., et al. (2013). The zebrafish reference genome sequence and its relationship to the human genome. *Nature, 496*(7446), 498–503.

147. Corey, D. R., & Abrams, J. M. (2001). Morpholino antisense oligonucleotides: Tools for investigating vertebrate development. *Genome Biology, 2*(5), 1015.1.
148. Meng, X., Noyes, M. B., Zhu, L. J., Lawson, N. D., & Wolfe, S. A. (2008). Targeted gene inactivation in zebrafish using engineered zinc-finger nucleases. *Nature Biotechnology, 26*(6), 695–701.
149. Zon, L. I., & Peterson, R. T. (2005). In vivo drug discovery in the zebrafish. *Nature Reviews. Drug Discovery, 4*(1), 35–44.
150. Etchin, J., Kanki, J. P., & Look, A. T. (2011). Zebrafish as a model for the study of human cancer. *Methods in Cell Biology, 105*, 309–337.
151. He, S., Lamers, G. E., Beenakker, J.-W. M., et al. (2012). Neutrophil-mediated experimental metastasis is enhanced by VEGFR inhibition in a zebrafish xenograft model. *The Journal of Pathology, 227*(4), 431–445.
152. Teng, Y., Xie, X., Walker, S., White, D. T., Mumm, J. S., & Cowell, J. K. (2013). Evaluating human cancer cell metastasis in zebrafish. *BMC Cancer, 13*, 453.
153. Amatruda, J. F., Shepard, J. L., Stern, H. M., & Zon, L. I. (2002). Zebrafish as a cancer model system. *Cancer Cell, 1*(3), 229–231.
154. Tobin, D. M., May, R. C., & Wheeler, R. T. (2012). Zebrafish: A see-through host and a fluorescent toolbox to probe host–pathogen interaction. *PLoS Pathogens, 8*(1), e1002349.
155. Yang, X., Cui, W., Gu, A., et al. (2013). A novel zebrafish xenotransplantation model for study of glioma stem cell invasion. *PLoS One, 8*(4), e61801.
156. Konantz, M., Balci, T. B., Hartwig, U. F., et al. (2012). Zebrafish xenografts as a tool for in vivo studies on human cancer. *Annals of the New York Academy of Sciences, 1266*, 124–137.
157. Fenaroli, F., Westmoreland, D., Benjaminsen, J., et al. (2014). Nanoparticles as drug delivery system against tuberculosis in zebrafish embryos: Direct visualization and treatment. *ACS Nano, 8*(7), 7014–7026.
158. Moghimi, S. M., Hunter, A. C., & Murray, J. C. (2001). Long-circulating and target-specific nanoparticles: Theory to practice. *Pharmacological Reviews, 53*(2), 283–318.
159. Coradeghini, R., Gioria, S., García, C. P., et al. (2013). Size-dependent toxicity and cell interaction mechanisms of gold nanoparticles on mouse fibroblasts. *Toxicology Letters, 217*(3), 205–216.
160. Palko, H. A., Fung, J. Y., & Louie, A. Y. (2010). Positron emission tomography: A novel technique for investigating the biodistribution and transport of nanoparticles. *Inhalation Toxicology, 22*(8), 657–688.
161. Rocco, L., Santonastaso, M., Mottola, F., et al. (2015). Genotoxicity assessment of TiO2 nanoparticles in the teleost *Danio rerio*. *Ecotoxicology and Environmental Safety, 113*, 223–230.
162. Gao, J., Sepúlveda, M. S., Klinkhamer, C., Wei, A., Gao, Y., & Mahapatra, C. T. (2015). Nanosilver-coated socks and their toxicity to zebrafish (*Danio rerio*) embryos. *Chemosphere, 119*, 948–952.
163. Wagner, D. S., Delk, N. A., Lukianova-Hleb, E. Y., Hafner, J. H., Farach-Carson, M. C., & Lapotko, D. O. (2010). The in vivo performance of plasmonic nanobubbles as cell theranostic agents in zebrafish hosting prostate cancer xenografts. *Biomaterials, 31*(29), 7567–7574.
164. Wu, Q., Deng, S., Li, L., et al. (2013). Biodegradable polymeric micelle-encapsulated quercetin suppresses tumor growth and metastasis in both transgenic zebrafish and mouse models. *Nanoscale, 5*(24), 12480–12493.

Chapter 6
Passive Targeting of Nanoparticles to Cancer

Jayvadan K. Patel and Anita P. Patel

Abstract Cancer is a leading cause of death globally. For the effectual treatment of cancer, it is crucial to advance our knowledge of the pathophysiology of cancer, discover novel anti-cancer agents, and expand new biomedical technology. A large number of possible barriers exist in the efficient delivery of small-sized drugs to solid tumors. After intravenous administration, many small-sized chemotherapeutic medicines have a larger volume of distribution, which is usually related to a narrow therapeutic index that is attributable to their elevated level of toxic effects in healthy tissues. A nanoparticle-based drug for targeting cancer is one of the auspicious advances to conquer the lack of tissue specificity associated with common chemotherapeutic drugs. Accordingly, the overall objectives are to lengthen a patient's lifespan, avoid recurrence of a cancer episode, and concurrently lessen the toxic effects of chemotherapeutic drugs. A range of approaches have been investigated for the nanoparticle-mediated targeting of drugs. Among them, a passive drug targeting approach has been the most commonly explored, and much preclinical learning has provided insight into its soundness. This approach is in accordance with the abnormality of tumor vasculatures, allowing nanoparticles the right of entry to tumors while avoiding distribution into healthy tissues. Thus, a passive drug targeting approach facilitates the advancement of a targeted nano-carrier structure loaded with chemotherapeutic agents for an improved effective profile with negligible toxic effects.

Keywords Cancer · Nanoparticles · Passive targeting · Tissue specificity

1 Introduction

Cancer is a leading cause of fatality worldwide, and the number of cancer-diagnosed patients is quickly rising [1]. According to estimates from the World Health Organization, approximately 84 million people died from cancer between 2005 and 2015 [2]; in 2012, approximately 14.1 million patients were diagnosed with cancer,

J. K. Patel (✉) · A. P. Patel
Nootan Pharmacy College, Faculty of Pharmacy, Sankalchand Patel University, Visnagar, Gujarat, India

Y. V Pathak (ed.), *Surface Modification of Nanoparticles for Targeted Drug Delivery*, https://doi.org/10.1007/978-3-030-06115-9_6

of which 8.2 million cases were incurable. This figure is predicted to increase to 19.3 million new cases of cancer by 2025 [3]. At present, the management of cancer is a very important aim of research [4]. For the effectual treatment of cancer, it is crucial to advance our understanding of cancer pathophysiology. Traditional chemotherapeutic drugs are tremendously inadequate in their treatment profiles because of their very poor solubility, inauspicious pharmacokinetic profiles, and unfocused distribution within the body, which eventually results in serious toxic effects [5].

Currently, the treatment of cancer is a multidisciplinary effort that necessitates a close relationship between doctors, biological researchers, and biomedical engineers to form a delivery method that is strong enough to resist the fair number of challenges in a multifaceted microenvironment. There are many possible barriers to the effectual release of an active-form drug in solid tumors. After intravenous administration, most small-sized chemotherapeutic medicines have a larger volume of distribution, specifically related to a narrow therapeutic index attributable to their elevated level of toxic effects in healthy tissues [6, 7]. Accordingly, the overall objectives are to lengthen a patient's lifespan, improve a patient's quality of living, avoid recurrence of a cancer episode, and concurrently lessen the toxic effects of chemotherapeutic drugs. The vasculature of a tumor has to be altered to counterbalance the negative effects of chemotherapeutic drugs. A drug-loaded nanocarrier system has been used to conquer the lack of specificity that is generally associated with chemotherapeutic agents [8].

The tissue selectivity of current anticancer medicines is of an amplitude that allows effective and harmless cancer chemotherapy. Against this background, nanoparticle-based medicine that targets the tumor has been developed as an exciting advance to overcome the inadequacy of tissue specificity of traditional chemotherapeutic agents. Nanoparticles can be used for submicron-sized drug delivery systems that can predictably enhance the bio-distribution of systemically delivered chemotherapeutic agents. By transporting pharmacologically active drugs more specifically to tumor tissues and/or directing them far away from healthy tissues, nanoparticulate-based medicines can strike a balance between efficacy and the toxic effects of systemically administered chemotherapeutic interventions.

A variety of approaches have been investigated for nanoparticle-mediated targeting of drugs. Among them, a passive drug targeting approach has been the most commonly explored, with preclinical learning providing insight into the soundness of the approach [9–11]. A drug delivery system with passive targeting is burdened by several challenges as well as restrictions [12]. The major challenge is to accurately recognize and then guide the chemotherapeutic agent to a specific target. This is generally caused by the very limited solubility of the majority of these chemotherapeutic drugs, which have a deprived pharmacokinetic profile along with a high toxicity potential. These limits can be overcome by loading these drugs into nanocarriers and permitting passive targeting to occur as a result of the compromised vasculature. Although the nanosystem is intended for active targeting, mostly passive targeting occurs initially, with subsequent active targeting [13]. This approach is rooted in the irregularities of tumor vasculatures, which permit nanoparticles to access tumors while circumventing distribution in normal healthy

tissues. At present, targeted anti-cancer drugs alone have established successes, with well-known examples that include Gleevec® (imatinib mesylate), Herceptin® (trastuzumab), and Iressa® (gefitinib) [14]. Thus, this approach facilitates the advancement of a targeted nano-carrier structure loaded with chemotherapeutic agents for an improved effective profile with negligible toxic effects.

2 Passive Targeting

Passive targeting deposits a drug or drug-carrier structure at a specific site as a result of physico-chemical or pharmacological factors [15, 16]. Solid tumors present more favorable situations for the gathering of macromolecular drugs as well as colloidal-size drug delivery systems resembling micellar systems, liposomes, polymeric-drug conjugates, and polymeric nanoparticles. The enlarged vascular permeability together with the defective lymphatic drainage of fast-growing tumors provides for an enhanced permeability and retention (EPR) effect of the nano-systems in the tumor [17, 18].

There are a small number of commonly used techniques for targeting tumors and tumor cells due to the large phenotypic variety of cancerous cells and tumors. Many tumors and vascularized solid tumors, in addition to a few vascularized metastatic tumor lumps, show signs of an EPR effect that can be used for the passive targeting of anti-tumor drugs [19]. This effect happens because many solid tumors have a permeable vasculature along with missing or damaged lymphatic drainage, which induces the buildup of higher molecular-weight compounds in addition to smaller particles (~20–500 nm diameter) inside the tumor tissue.

3 Passive Targeting by Nanoparticles

Passive targeting by nanoparticles occurs because of the uniqueness of solid tumors (i.e., leaky vasculature and defective lymphatic drainage), which permits nanoparticles to build up in the tumor. This observable fact, which was first reported in 1986 by Matsumura and Maeda, is called the EPR effect [20, 21].

3.1 Enhanced Permeability and Retention Effect

A nanoparticle that fulfills the size and surface requirements for evading reticulo-endothelial system capture has a greater ability to travel in the bloodstream, as well as a higher possibility of arriving at the targeted tumor tissues. In particularly, macromolecules comprising nanoparticles build up in tumor tissues as a result of the distinctive pathophysiologic individuality of tumor vessels [22]. The rapidly

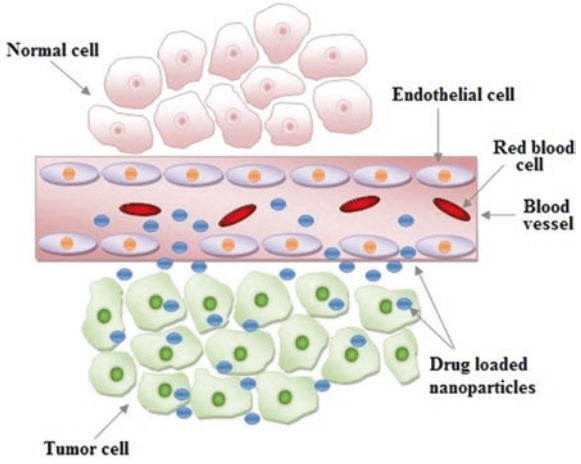


Fig. 6.1 Schematic drawing of the EPR effect

expanding cancerous cells require new vessels (neovascularization) or otherwise the diversion of existing vessels close to the tumor mass to provide them with oxygen and nutrients [23]. The resultant disparity of angiogenic regulators, such as growth factors, along with matrix metalloproteinases creates tumor vessels that are extremely disordered and enlarged, with abundant pores displaying expanded gap junctions among endothelial cells and compromised lymphatic drainage [23]. The EPR effect is an essential means through which macromolecules, together with nanoparticles, of a molecular weight greater than 50 kDa, are able to specifically build up in the tumor interstitium. A schematic drawing of the EPR effect is presented in Fig. 6.1, which illustrates the mechanism behind passive targeting [21].

The EPR effect can be used to overcome a dilemma affecting nearly every kind of cancer therapy currently in use: deficiency in tumor selectivity. However, the use of "selectivity" could be deceptive. Although nanoparticles are administered into the circulation, there is no "selectivity" with reference to the EPR effect. The nanoparticles are distributed all over the body, with the aim of "passive targeting" through the EPR effect to attain an inconsistent distribution of the nanoparticles by concentrating on the tumors.

The EPR effect is an effect of tumor vasculature irregularity, similar to the increased vascular permeability and hypervascularization [24]. Along with other growth factors, vascular endothelial growth factor (VEGF) causes the development of neo-vasculature and angiogenesis. This recently created tumor vasculature is irregular in structure and structural design, imparting fenestrations in the vessels that are induced by inadequately aligned endothelial cells, a deficit in smooth muscle, and raised levels of vascular permeability [25, 26]. In addition, the hyperproduction of vascular mediators plus VEGF, similar to nitric oxide, peroxynitrite, prostaglandins, and matrix metalloproteinases, result in better vascular permeability of the vasculature in tumor tissue [27, 28].

It is also feasible that a reduction in the boundary of the tumor-affected vasculature, which is a result of the tumor naturally pushing otherwise confining vessels, may possibly produce high hydrostatic pressure nearby. Researchers also revealed that an increase in fluid pressure in the vasculature increases the particle deposition on the endothelial cells ex vivo [29]. With this pressure-deposition system, it is possible that nanoparticles would comprise a high level of linkage to the tumor vasculature. As a result, nanoparticles may stick more regularly to endothelial cells in tumor-affected regions, which makes co-localization of the nanoparticle with tumors possible.

The therapeutic effectiveness of passively targeted nanoparticles is influenced by the heterogeneity of the EPR effect inside as well as among dissimilar tumors. An inconsistent endothelial gap (ranging from 1 to 100 nm) give rise to non-uniform extravasations of nanoparticles into the tumor [30]. The outside edge of the tumor is not as leaky as the hypoxic core, which suggests that nanoparticles extravasate more regularly at the core than the margin. However, numerous investigations have pointed out the opposite—that nanoparticles administered intravenously extravasate more often in the tumor margin [31, 32]. In addition, apart from the permeability, nanoparticle extravasation is also directed by perfusion, which has both spatial and temporal heterogeneity inside a tumor; this adds another point of complication to the scheme for nanoparticle extravasation [33]. Nanoparticle characteristics such as particle size, shape, and surface charge have an influence on the EPR effect, which in turn affects circulation time, penetration speed, and intracellular internalization [34, 35]. Furthermore, physiochemical properties such as size and shape also affect nanoparticle extravasation and accumulation [36, 37].

Nanoparticles that cross the vasculature and then extravasate into the tumor are hindered by the interstitial tumor milieu, which serves as an obstacle to their profound infiltration into tumor tissue. The diffusional obstruction can be considerably decreased by reducing the particle size, thus enhancing its diffusion into the interstitial milieu. Wong and co-workers suggested a multistage technique in which a 100-nm gelatin particle is condensed to 10 nm in size after its extravasation into the tumor tissue through degradation by tumor-associated matrix metalloproteinases (MMPs) [38]. Other groups have also reported comparable advances by diverse nanocarriers [39–41]. Passive targeting is mostly achievable during diffusion-mediated transport, in which size is the main factor. A size range of 40–200 nm is considered to be most favorable for an extended circulation time, augmented buildup inside the tumor mass, and decreased renal clearance [42].

Other physicochemical characteristics of nanoparticles, including elasticity, shape, and surface charge, can also influence the contact of nanoparticles with physiological barriers and the microenvironment of a tumor; as a result, they can be critical factors in the design of nanoparticles and the maximization of their biological function. Such as, a nanoparticle's shape is a vital feature in determining their blood circulation, ability to marginate in blood vessels, and their ability to intake through tumor cells and macrophages [43–46]. For microparticles with elliptical shapes, the macrophage may first contact these particles beside the major axis; the particles are then quickly internalized in less than 6 min. However, if the initial contact is beside

the minor axis, then the particles are not internalized for an extended time, up to 10 h. It is simply a result of their symmetry that these spherical particles are speedily internalized. In such cases, this effect of shape was separate from the size of particle. The only difference in response to the size of the particles was the degree of uptake, which was experiences only in particles having volumes that were considerably more than the volume of the cell [47]. In another investigation, it was reported that sphere-shaped particles were taken up at a rate that was approximately 5 times greater than that of rod-shaped particles, therefore indicating the importance of a nanoparticle's shape on the mechanism of uptake [48].

The elasticity of nanoparticles can also influence their biological effects, comprising blood circulation as well as tumor uptake. Softer nanoparticles (10 kPa) were associated with extended blood circulation in comparison with harder nanoparticles (3000 kPa) in an in vivo demonstration by Anselmo and co-workers [49].

In measurements of the magnitude of internalization of nanoparticles into cells, surface properties also have played an incredibly significant role. The surface characteristics can be somewhat customized by the polymer composition, which results in an additional degree of hydrophobicity or hydrophilicity for these particles. The surface modification of these polymers with the addition of polyethylene glycol (PEG) has been identified to defend the nanosystems from opsonization along with clearance by the reticulo-endothelial (RES) system [50]. Moreover, the circulation time of nanoparticles was extended by increasing the molecular weight of PEG chains. This PEG protection can provide greater defense against negatively charged nanoparticles and also put off instantaneous clearance of these particles. By modifying the nanoparticles' size, shape, or (in a few cases) surface dimensions, one can regulate the passive targeting.

Nanoparticles can remain in circulation for a long period of time, while not being able to infiltrate the tight endothelial junctions that exist in healthy vasculature and avoiding clearance via the mononuclear phagocyte system (MPS). This allows the EPR effect to bring similar passive targeting to solid tumors for anticancer drug-loaded nanoparticles. The tumor vasculature is therefore an important goal in cancer management by means of nanoparticles. Through tumor vasculature targeting, the tumor itself is targeted circuitously; otherwise, the tumors supply a line of nutrients and routes for metastasis that are able to be affected. In general, the physiochemical characteristics of nanoparticles can to a large extent affect their accretion, preservation, and permeation in tumors. Conversely, the optimization of physiochemical characteristics of nanoparticles is explicit to the target tumor's pathophysiology, as demonstrated by Sykes et al.; as a result, they should be customized to every type of tumor to exploit therapeutic efficacy [51].

3.2 Tumor Microenvironment

Another passive targeting approach uses the distinctive tumor environment in a system referred to as the tumor-activated therapy of prodrugs. Rapidly growing, hyperproliferative cancerous cells exhibit a higher metabolic rate; however, the delivery

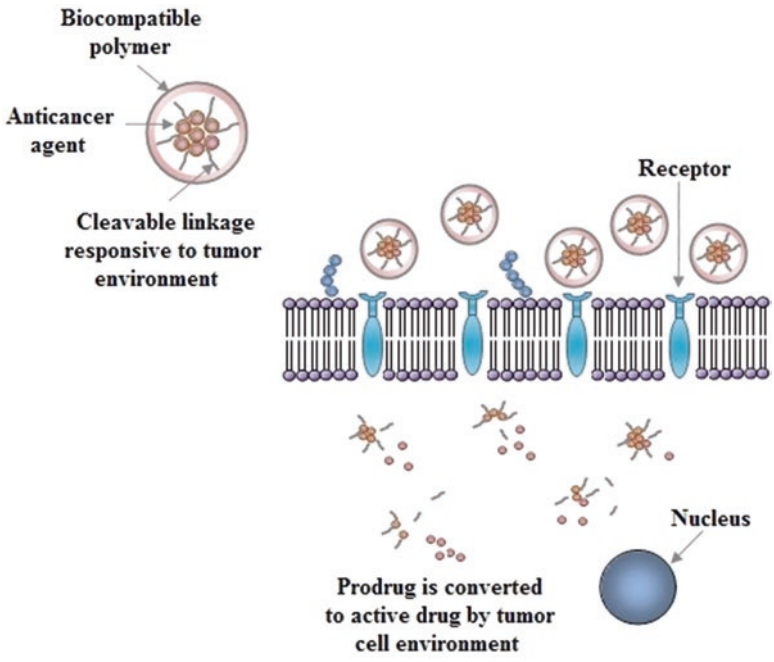


Fig. 6.2 Tumor-targeted delivery of a prodrug

of oxygen plus nutrients is generally not enough to support this. Consequently, tumor cells make use of glycolysis to obtain additional energy, resulting in an acidic milieu [52]. Furthermore, cancerous cells articulate and discharge distinctive enzymes, such as matrix metalloproteinases, which are involved in their motion as well as endurance mechanisms [53]. The drug is coupled to a tumor-specific particle and stays inert, waiting to arrive at the target (Fig. 6.2). The anticancer drug is coupled with a biocompatible polymer through an ester link. The association is hydrolyzed by a cancer-specific enzyme or through higher or lower pH at the site of tumor, at which point the nanoparticle delivers the drug [54].

For example, an albumin-bound structure of doxorubicin that integrated a matrix metalloproteinase-2-specific octapeptide series between the anticancer drug and the carrier was reported to be proficiently and specially cleaved by matrix metalloproteinase-2 in an in vitro examination [55].

3.3 Direct Local Delivery

Another passive targeting technique is the straight local release of anticancer agents to the tumor cells. This technique has the understandable benefit of excluding the drug from systemic circulation. However, administration can be extremely intrusive

because it entails injections or otherwise surgical procedures. For a few tumors that are not easy to access, such as lung cancers, this approach is almost impossible to employ [56].

4 Nanoparticle Delivery System for Cancer Through Passive Targeting

At present, numerous passively targeted nanoparticles are in clinical use, such as Genexol-PM™ in Korea and ProLindac™ and Opaxio™ in United States [57, 58]. Furthermore, a number of additional nanocarriers, including AZD2811, CPX-1, and NK911, have confirmed safety and/or therapeutic effectiveness in clinical investigations [59–61]. From a great number of nanosystems, only a small number of nanomedicines are accepted for use in cancer treatment, as depicted in Table 6.1 [58].

Even if most of these nanosystems change the pharmacokinetics, toxicological profile, or drug solubility, some have also demonstrated noteworthy endurance advantages and improved therapeutic effectiveness compared with the parent medicine in clinical investigations. Abraxane™ (nanoparticle albumin-bound paclitaxel) is one example that established considerably high response rates in comparison with standard paclitaxel in a phase III clinical trial of patients with metastatic breast cancer [62]. Likewise, the U.S. Food and Drug Administration (FDA)-approved CPX-351 (Vyxeos™) a liposomal preparation of cytarabine-daunorubicin combination, has demonstrated better endurance of 9.56 months compared with 5.95 months for cytarabine and daunorubicin delivered in their free forms in patients with recently identified vulnerable acute myeloid leukemia [63].

Our knowledge of EPR efficiency is restricted by the limited information gained from pre-clinical tumor models to precisely classify solid tumors in individuals. In reality, the most frequently used subcutaneous tumor xenografts are rapidly expanding, resulting in extremely high-EPR tumors that might provide an inaccu-

Table 6.1 Examples of passively-targeted nanosystems approved for anticancer therapy

Name	Type of formulation	Bioactive compound	Indication
DaunoXome®	Non-PEGylated liposomes	Daunorubicin	Kaposi sarcoma
Myocet®	Non-PEGylated liposomes	Doxorubicin	Breast cancer
Onco TCS®	Non-PEGylated liposomes	Vincristine	Non-Hodgkin lymphoma
Doxil®/ Caelyx®	PEGylated liposomes	Doxorubicin	Breast cancer, ovarian cancer, multiple myeloma, Kaposi sarcoma
Abraxane®	Albumin-based	Paclitaxel	Breast cancer
Oncaspar®	PEG-L-asparaginase	Asparagine specific enzyme	Acute lymphoblastic leukemia

rate assessment of the therapeutic advantages of nanocarriers in treatments that depend on EPR-based passive targeting [64]. Moreover, there is restricted patient-based investigational information on the EPR phenomenon itself in addition to its effects on the buildup of a drug in the tumor site, which can be interpreted as clinical effectiveness [64]. Additional research on the EPR effect in different human tumors as well as the progress of advanced preclinical models are therefore necessary for the design of nanoparticles with improved tumor penetration and therapeutic effects [8, 50].

The link between tumor vascularization and EPR-based passive targeting has been explored by Theek and co-workers in a subcutaneous tumor model [65]. Through the use of both contrast-enhanced ultrasound and computed tomography-fluorescence molecular systems, the authors established heterogeneous buildup of 10-nm near infrared-labeled polymeric nanocarriers (pHPMA-Dy750) inside and among the tumors (5–12%). In the same way, copper-64-loaded PEGylated liposomes were investigated by Hansen and his group. Moreover, the EPR effect of PEGylated liposomes was evaluated with a micro-positron emission tomography/computed tomography (PET/CT) imaging technique [66]. Evaluation of eleven dogs bearing spontaneous solid tumors revealed that the EPR effect is a predominant feature in a few solid tumors (e.g., carcinoma), resulting in a higher accumulation of liposomes; however, this might not be widespread to every solid tumor.

An FDA-approved 30-nm carboxymethyl dextran-coated magnetic nanoparticle (ferumoxytol) could be used as a substitute or companion element for intratumoral transfer, pharmacokinetics, and distribution of a therapeutic nanocarrier rooted in poly(D,L-lactic-co-glycolic acid)-*b*-poly(ethylene glycol) (PLGA-PEG), as demonstrated by Miller et al. [67]. Lee et al. developed 64Cu-labeled human epidermal growth factor receptor-2 (HER2) targeted liposomes along with PET/CT to measure the accumulation of s drug in 19 patients with HER2-positive metastatic breast cancer [68]. The maximum accumulation of liposomes was found at 24–48 h; patients were categorized in accordance with 64Cu-liposomal abrasion deposition by a cut-point that was similar to a response threshold as determined in preclinical investigations. Patients with elevated 64Cu-liposomal abrasion deposition were associated with additional positive therapy results. These investigations reveal that the use of imaging systems for the assessment and characterization of EPR may ultimately allow clinicians to preselect patients with higher-EPR tumors who are expected to respond to passively targeted nanosystems with enhanced therapeutic effects.

A meta-analysis of pre-clinical data based on a nano-carrier delivery system for tumors reported during the past 10 years found that a mean of approximately 0.7% of the injected dose of nanocarriers arrives at the target tumors [69]. This figure appears to be very small on the surface, elevating concerns about the competence of the EPR effect and the management of low-EPR tumors. However, a delivery effectiveness of approximately 0.7% for nanocarriers is considerably better than the delivery effectiveness of most chemotherapeutic preparations that are commonly used in hospitals, including docetaxel, doxorubicin, and paclitaxel [70–73]. In a preclinical investigation, van Vlerken et al. established the delivery effectiveness of 0.6% of the injected dose for paclitaxel-loaded nanocarriers in comparison with

0.2% of the injected dose for free paclitaxel [70]. This outcome is hopeful and indicates the benefits of nanocarrier systems for the tumor-targeted delivery of drugs.

Nevertheless, the delivery effectiveness of nanocarrier systems can be additionally enhanced to make the best use of their therapeutic advantages. Enhancing EPR effects with angiotensin II-induced hypertension or heat-based vasodilation might be an answer; however, such a system could make the clinical transformation of nanocarriers difficult. One more possible and comparatively adaptable solution, particularly for low-EPR tumors, is are the gracefully engineered delivery methods that make use of non-EPR advances for tumor targets. For example, injectable nanoparticle generators (iNPGs) that tackle the many physiological barriers were developed by Xu and co-workers [74]. An iNPG is a discoidal micrometer-sized nanoporous silicon particle that can be laden with drug polymer conjugates, controlling tumor growth because of normal tropism along with improved vascular dynamics. The iNPG delivers the drug polymer conjugate by self-assembling to make nanoparticles that are transferred to the perinuclear area, thus bypassing the drug efflux pump. Superior efficiency was observed with iNPG in MDA-MB-231 and 4 T1 mouse models of metastatic breast cancer compared with its individual components as well as other existing therapeutic dosage forms. The delivery effectiveness of nanocarriers can be appreciably enhanced by such realistically engineered systems.

The cell-mediated delivery of nanocarriers may be an additional EPR-free advance to improve tumor targeting in low-EPR tumors or certain metastatic tumor sites that are inaccessible to passive targeting. This method uses the aptitude of definite cell types to house or travel to such tumors [75]. Huang et al. bridled the innate capability of T-cells to travel throughout the lymphatic system via conjugating nanocapsules by encapsulating the topoisomerase I drug SN-38 to the surface of the cell [76]. The authors reported an approximately 90-fold increase in the concentration of SN-38 in lymph nodes found by cell-mediated delivery compared with the free drug when injected systemically at 10-fold high doses, as well as an extended median endurance by 35 days without toxic effects. In addition to targeting low-EPR tumors, the immune cell-mediated delivery of nanocarriers can also result in better tumor accumulation in dispersed tumors as well as metastases. This may possibly unlock novel opportunities for more secure targeted delivery of immune-modulating compounds, such as IFN-γ, which can encourage the segregation of tumor-promoting M2 macrophages to antitumor M1 macrophages. In addition, the use of tumor-infiltrating lymphocytes or chimeric antigen receptor T-cells for targeted delivery of immune-modulating agent-loaded nanocarriers may facilitate a synergetic dual-arm therapy, thus boosting anti-tumor immune responses by tumor targeting and/or intonation of immunosuppressive cells. However, this advance is restricted to medications with few toxic effects to common carrier cells.

The coating of nanoparticles can help to manage the interaction of nanoparticles with proteins in the bloodstream [77, 78]. Targeting approaches are being used to ensure that an adequate quantity of nanoparticles reach tumor cells. In a passive targeting approach, the individual receives the benefit of the elevated endocytic

uptake of cancer cells as well as the permeable vasculature in the region of tumors, which allows for high uptake of nanoparticles compared with healthy tissues [79].

As soon as the organism detects a foreign body in the bloodstream, specific serum proteins (opsonins) will adsorb on the surface of the body, tagging it for discharge from the body [80]. By attaching suitable molecules, such as PEG, on the surface of the nanoparticles, this response has been avoided [50, 81]. The PEG-coating of nanoparticles repulses the opsonins, un-labeling them to coat the surface as a result [82]. Although nanoparticles have a propensity to focus on tumor tissue as a result of the irregular blood vessel wall configuration around tumor tissues, in addition to a weakly urbanized lymphatic system that restricts discharge of macromolecules from tumor tissue [83], the EPR effect is useful in such cases. Coating a nanoparticle with PEG increases its blood circulation time, therefore resulting in high passive uptake because of the EPR effect. The ability of the coating layer to offer passive targeting circumstances is influenced by a number of factors, such as nanoparticle core size, length, and surface density of capping molecules, which has been previously studied both computationally and experimentally [84, 85]. Non-targeted strategies take advantage of passive targeting on the basis of the pathophysiological circumstances of the myeloma microenvironment for precise release of the medicine. Most nanoparticle delivery systems explored for myeloma have used non-targeted nanoparticles.

Liposomal bortezomib nanoparticles have 100-nm size ranges with great reproducibility and 80% encapsulation efficiency. For investigation of proteasome inhibition, apoptosis, and cell viability, in vitro studies have been performed. It was observed that liposomal bortezomib nanoparticle systems restrained proteasome action, encouraged apoptosis, and enhanced cytotoxicity on manifold myeloma cells. In the in vivo investigations, multiple myeloma cells were injected subcutaneously in the severe combined immune-deficient mice, processed by free drug or liposomal bortezomib nanoparticles intravenously on the first and fourth days at 1 mg/kg bortezomib equivalent dose, and examined for the succession of the tumor and systemic toxicities. The outcomes showed that liposomal bortezomib nanoparticles were effective in the suppression of tumor growth, in addition to lessening the systemic side effects, including body weight loss. The free drug group exhibited >20% weight loss and moribundity on the seventh day, which required sacrifice of the mice, whereas the liposomal bortezomib nanoparticle group exhibited <10% weight loss for the duration of the 2 weeks [86].

PEGylated liposomal doxorubicin was the initial nanoparticle delivery method approved by the FDA for medical use in multiple myeloma. It has been used with additional anti-myeloma agents, such as bortezomib or vincristine and dexamethasone. Patients with regression or refractory multiple myeloma received PEGylated liposomal doxorubicin (Tibotec Therapeutics) delivered on the fourth day at 30 mg/m^2 as well as bortezomib administered on days 1, 4, 8, and 11 at 0.90–1.50 mg/m^2. The time to progression (TTP) was considerably extended in the combining arm (median TTP = 9.3 months) in relation to bortezomib monotherapy (median TTP = 6.5 months) [87, 88].

Thymoquinone-encapsulated PLGA-PEG nanoparticles exhibited a size of approximately 200 nm with uniform distribution and 94% encapsulation efficiency. PLGA-PEG based thymoquinone nanoparticles had anti-proliferative effects on multiple myeloma cells; these nanoparticles were more effective than free drug-sensitizing leukemic cells to TNF-α as well as paclitaxel-induced apoptosis [89]. However, this investigation is very preliminary, with only in vitro studies reported. In vivo examinations are required to confirm the effectiveness and delivery for thymoquinone nanoparticles in myeloma. Other polymeric nanoparticles that are nanocolloids derived from *N*,*N*,*N*-trimethyl chitosan have been formulated to encapsulate camptothecin, a powerful anticancer drug with a plant source. There was no statistical dissimilarity observed between loaded nanocolloids and the free drug in the in vitro cytotoxicity study. Conversely, loaded nanocolloids more efficiently restrained growth of the tumor and extended survival time compared with the free drug in vivo [90].

Silica nanoparticles have been conjugated with snake venom derived from *Walterinnesia aegyptia*, a natural toxin that exhibits antitumor activity [91]. The obtained nanoparticles had 300-nm particle size. Silica nanoparticles containing snake venom were examined in the cells of five patients with myeloma, as well as a XG2 cell line. It was observed that this combination had the ability to decrease viability and encourage apoptosis [92]. Furthermore, iron oxide-based nanoparticles have been studied for multiple myeloma. Paclitaxel is an effectual anticancer medicine with poor aqueous solubility. However, Abraxane® (Celgene, Summit, NJ, USA), an albumin-bound paclitaxel-loaded iron oxide nanoparticle, is a water-soluble commercially available nanoparticle approved by the FDA for the management of metastatic breast cancer [93]. In myeloma-bearing mice, 7-nm paclitaxel iron oxide nanoparticles were used to treat CD138-CD34-tumor stem-like cells. The inhibition of tumor growth was greater with paclitaxel iron oxide nanoparticles (0.6–2 mg/kg once a week for 2 weeks) in comparison with nanoparticles only or paclitaxel only; in addition, they were found to encourage the apoptosis of cancer cells in treated mice [94].

In most passive targeting nanosystems, surface coating with PEG is performed for biocompatibility and "stealth" purposes [50, 95, 96]. Significantly, improved hydrophilicity on the surface of the nanoparticle can obstruct its uptake by cancerous cells, thus hindering the competent delivery of a drug to tumors with passive targeting nanoparticles [50, 97, 98]. Nevertheless, PEG-based block copolymers have been used in many passive targeting polymeric nanoparticles, including Genexol-PM, SP1049C and NK911. Among them, SP1049C is a pluronic-based polymeric micelle nanoparticle of doxorubicin. At present, it is being investigated in Phase II clinical trials for metastatic cancer of the esophagus versus the usual chemotherapeutic protocols [99]. Another polymeric micellar nanoparticle that acts through a passive targeting mechanism is NK911 containing PEG, doxorubicin, and poly(aspartic acid), which is currently being investigated in Phase II clinical trials for a variety of cancers [100]. Likewise, Opaxio™ and passively targeted paclitaxel/poly(L-glutamic acid) nano-construct are established as effectual in ovarian cancers [101, 102]. CRLX101 (previously IT-101), a camptothecin-cyclodextrin polymeric

conjugate, has shown better pharmacokinetic effectiveness in preclinical and clinical investigations [103]. NC-6004 is a cisplatin-incorporated PEG-poly(glutamic acid) block copolymer micellar nanotherapeutics, whereas ProLindac™ is a diaminocyclohexane-platinum hydroxypropylmethacrylamide prodrug, which are both in the final stage of clinical investigation [104].

A passive targeting lipid nanoparticle system is also moving to the progressive stages of clinical trials, and profound attempts are being made to put these methods into medical practice. Promising liposomal nanoparticles in clinical trials include Thermodox®, a thermosensitive liposomal doxorubicin nanoparticle that delivers the drug at approximately 39 °C; it is presently being examined in Phase III clinical investigations in addition to radiofrequency excision in hepatocellular carcinoma patients [105]. SPI-77 is a PEGylated liposomal cisplatin nanoparticle, which is currently in Phase II clinical investigations for patients with recurring epithelial ovarian tumors [106]. CPT-11, a nanosized liposomal irinotecan formulation, is in Phase I clinical investigations for patients with glial cell tumors [107]. A number of liposomal nanoformulations containing two dissimilar categories of anticancer drugs, such as cytarabine and daunorubicin, are also being investigated [108].

5 Conclusion

The recent advancements in novel nanoparticulate strategies have necessitated greater accuracy in delivering medicine to cancerous cells, while also sparing nearby healthy tissues. The tumor microvasculature is the focus of theories on the passive targeting of nanoparticles. Despite the widespread investigations and progress in nanotechnology, only a small number of nanoparticle-based drug delivery approaches have been approved and are in practice for cancer management. This is because the pathophysiological characteristics of the tumor microenvironment and the molecular mechanisms inherent in tumor angiogenesis are fairly diverse and are reliant on the nature of cancers. Hence, further investigations and greater knowledge on these features of tumor tissues are essential to ensure a successful future for EPR effect-based chemotherapy of cancer with a passive drug-targeting strategy.

References

1. BWKP, S., & Wild, C. P. (2015). *World cancer report 2014*. Lyon: International Agency for Research on Cancer.
2. Ogawara, K., Yoshizawa, Y., Un, K., Araki, T., Kimura, T., & Higaki, K. (2013). Nanoparticle-based passive drug targeting to tumors: Considerations and implications for optimization. *Biological & Pharmaceutical Bulletin, 36*(5), 698–702.
3. Center for Disease Control and Prevention. (2015, February 2). *Global cancer statistics*. Retrieved from http://www.cdc.gov/cancer/international/statistics.htm

4. Bazak, R., Houri, M., El Achy, S., Hussein, W., & Refaat, T. (2014). Passive targeting of nanoparticles to cancer: A comprehensive review of the literature. *Molecular and Clinical Oncology, 2*(6), 904–908.
5. Danhier, F., Feron, O., & Préat, V. (2010). To exploit the tumor microenvironment: Passive and active tumor targeting of nanocarriers for anti-cancer drug delivery. *Journal of Controlled Release, 148*(2), 135–146.
6. Singla, A. K., Garg, A., & Aggarwal, D. (2002). Paclitaxel and its formulations. *International Journal of Pharmaceutics, 235*(1–2), 179–192.
7. Zhang, S., Liu, X., Bawa-Khalfe, T., Lu, L. S., Lyu, Y. L., Liu, L. F., et al. (2012). Identification of the molecular basis of doxorubicin-induced cardiotoxicity. *Nature Medicine, 18*(11), 1639–1642.
8. Byrne, J. D., Betancourt, T., & Brannon-Peppas, L. (2008). Active targeting schemes for nanoparticle systems in cancer therapeutics. *Advanced Drug Delivery Reviews, 60*(15), 1615–1626.
9. Araki, T., Kono, Y., Ogawara, K., Watanabe, T., Ono, T., Kimura, T., et al. (2012). Formulation and evaluation of paclitaxel-loaded polymeric nanoparticles composed of polyethylene glycol and polylactic acid block copolymer. *Biological & Pharmaceutical Bulletin, 35*(8), 1306–1313.
10. Thigpen, J. T., Aghajanian, C. A., Alberts, D. S., Campos, S. M., Gordon, A. N., Markman, M., et al. (2005). Role of pegylated liposomal doxorubicin in ovarian cancer. *Gynecologic Oncology, 96*(1), 10–18.
11. Wakaskar, R. R. (2017). Challenges pertaining to adverse effects of drugs. *International Journal of Drug Development and Research, 9*(3), 1–2.
12. Sparreboom, A., Scripture, C. D., Trieu, V., Williams, P. J., De, T., Yang, A., et al. (2005). Comparative preclinical and clinical pharmacokinetics of a cremophor-free, nanoparticle albumin-bound paclitaxel (ABI-007) and paclitaxel formulated in Cremophor (Taxol). *Clinical Cancer Research, 11*(11), 4136–4143.
13. Bae, Y. H. (2009). Drug targeting and tumor heterogeneity. *Journal of Controlled Release, 133*(1), 2–3.
14. Wakaskar, R. R. (2017). Passive and active targeting in tumor microenvironment. *International Journal of Drug Development and Research, 9*(2), 37–41.
15. Garnette, M. C. (2001). Targeted drug conjugates: Principles and progress. *Advanced Drug Delivery Reviews, 53*(2), 171–216.
16. Kim, J. H., Kim, Y. S., Park, K., Lee, S., Nam, H. Y., Min, K. H., et al. (2008). Antitumor efficacy of cisplatin-loaded glycol chitosan nanoparticles in tumor-bearing mice. *Journal of Controlled Release, 127*(1), 41–49.
17. Lamprecht, A., Ubrich, N., Yamamoto, H., Schäfer, U., Takeuchi, H., Maincent, P., et al. (2001). Biodegradable nanoparticles for targeted drug delivery in treatment of inflammatory bowel disease. *The Journal of Pharmacology and Experimental Therapeutics, 299*(2), 775–781.
18. Chytil, P., Etrych, T., Konák, C., Sírová, M., Mrkvan, T., Boucek, J., et al. (2008). New HPMA copolymer-based drug carriers with covalently bound hydrophobic substituents for solid tumour targeting. *Journal of Controlled Release, 127*(2), 121–130.
19. Maeda, H., Wu, J., Sawa, T., Matsumura, Y., & Hori, K. (2000). Tumor vascular permeability and the EPR effect in macromolecular therapeutics: A review. *Journal of Controlled Release, 65*(1–2), 271–284.
20. Rosenblum, D., & Peer, D. (2014). Omics-based nanomedicine: The future of personalized oncology. *Cancer Letters, 352*(1), 126–136.
21. Matsumura, Y., & Maeda, H. (1986). A new concept for macromolecular therapeutics in cancer chemotherapy: Mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. *Cancer Research, 46*(12), 6387–6392.
22. Maeda, H. (2001). The enhanced permeability and retention (EPR) effect in tumor vasculature: The key role of tumor-selective macromolecular drug targeting. *Advances in Enzyme Regulation, 41*(1), 189–207.

23. Carmeliet, P., & Jain, R. K. (2000). Angiogenesis in cancer and other diseases. *Nature, 407*(6801), 249–257.
24. Greish, K. (2010). Enhanced permeability and retention (EPR) effect for anticancer nanomedicine drug targeting. In S. R. Grobmyer & B. M. Moudgil (Eds.), *Cancer nanotechnology. Methods in Molecular Biology (Methods and Protocols)* (Vol. 624, pp. 25–37). Totowa, NJ: Humana Press/Springer.
25. Gazit, Y., Baish, J. W., Safabakhsh, N., Leuniq, M., Baxter, L. T., & Jain, R. K. (1997). Fractal characteristics of tumor vascular architecture during tumor growth and regression. *Microcirculation, 4*(4), 395–402.
26. Narang, A. S., & Varia, S. (2011). Role of tumor vascular architecture in drug delivery. *Advanced Drug Delivery Reviews, 63*(8), 640–658.
27. Maeda, H., Fanq, J., Inutsuka, T., & Kitamoto, Y. (2003). Vascular permeability enhancement in solid tumor: Various factors, mechanisms involved and its implications. *International Immunopharmacology, 3*(3), 319–328.
28. Wu, J., Akaike, T., Hayashida, K., Okamoto, T., Okuyama, A., & Maeda, H. (2001). Enhanced vascular permeability in solid tumor involving peroxynitrite and matrix metalloproteinases. *Japanese Journal of Cancer Research, 92*(4), 439–451.
29. Mann, M. J., Gibbons, G. H., Hutchinson, H., Poston, R. S., Hoyt, E. G., Robbins, R. C., et al. (1999). Pressure-mediated oligonucleotide transfection of rat and human cardiovascular tissues. *Proceedings of the National Academy of Sciences of the United States of America, 96*(11), 6411–6416.
30. Chauhan, V. P., & Jain, R. K. (2013). Strategies for advancing cancer nanomedicine. *Nature Materials, 12*(11), 958–962.
31. Yuan, F., Dellian, M., Fukumura, D., Leunig, M., Berk, D. A., Torchilin, V. P., et al. (1995). Vascular permeability in a human tumor xenograft: Molecular size dependence and cutoff size. *Cancer Research, 55*(17), 3752–3756.
32. Lee, H., Hoang, B., Fonge, H., Reilly, R. M., & Allen, C. (2010). In vivo distribution of polymeric nanoparticles at the whole-body, tumor, and cellular levels. *Pharmaceutical Research, 27*(11), 2343–2355.
33. Ernsting, M. J., Murakami, M., Roy, A., & Li, S.-D. (2013). Factors controlling the pharmacokinetics, biodistribution and intratumoral penetration of nanoparticles. *Journal of Controlled Release, 172*(3), 782–794.
34. Chou, L. Y. T., Ming, K., & Chan, W. C. W. (2011). Strategies for the intracellular delivery of nanoparticles. *Chemical Society Reviews, 40*(1), 233–245.
35. Albanese, A., Tang, P. S., & Chan, W. C. W. (2012). The effect of nanoparticle size, shape, and surface chemistry on biological systems. *Annual Review of Biomedical Engineering, 14*(1), 1–16.
36. Cabral, H., Matsumoto, Y., Mizuno, K., Chen, Q., Murakami, M., Kimura, M., et al. (2011). Accumulation of sub-100 nm polymeric micelles in poorly permeable tumours depends on size. *Nature Nanotechnology, 6*(12), 815–823.
37. Kolhar, P., Anselmo, A. C., Gupta, V., Pant, K., Prabhakarpandian, B., Ruoslahti, E., et al. (2013). Using shape effects to target antibody-coated nanoparticles to lung and brain endothelium. *Proceedings of the National Academy of Sciences of the United States of America, 110*(26), 10753–10758.
38. Wong, C., Stylianopoulos, T., Cui, J., Martin, J., Chauhan, V. P., Jiang, W., et al. (2011). Multistage nanoparticle delivery system for deep penetration into tumor tissue. *Proceedings of the National Academy of Sciences of the United States of America, 108*(6), 2426–2431.
39. Tong, R., Hemmati, H. D., Langer, R., & Kohane, D. S. (2012). Photoswitchable nanoparticles for triggered tissue penetration and drug delivery. *Journal of the American Chemical Society, 134*(21), 8848–8855.
40. Tong, R., Chiang, H. H., & Kohane, D. S. (2013). Photoswitchable nanoparticles for in vivo cancer chemotherapy. *Proceedings of the National Academy of Sciences of the United States of America, 110*(47), 19048–19053.

41. Li, H. J., Du, J. Z., Du, X. J., Xu, C. F., Sun, C. Y., Wang, H. X., et al. (2016). Stimuli-responsive clustered nanoparticles for improved tumor penetration and therapeutic efficacy. *Proceedings of the National Academy of Sciences of the United States of America, 113*(15), 41640–44169.
42. Liechty, W. B., & Peppas, N. A. (2012). Expert opinion: Responsive polymer nanoparticles in cancer therapy. *European Journal of Pharmaceutics and Biopharmaceutics, 80*(2), 241–246.
43. Toy, R., Peiris, P. M., Ghaghada, K. B., & Karathanasis, E. (2014). Shaping cancer nanomedicine: The effect of particle shape on the in vivo journey of nanoparticles. *Nanomedicine (London, England), 9*(1), 121–134.
44. Smith, B. R., Kempen, P., Bouley, D., Xu, A., Liu, Z., Melosh, N., et al. (2012). Shape matters: Intravital microscopy reveals surprising geometrical dependence for nanoparticles in tumor models of extravasation. *Nano Letters, 12*(7), 3369–3377.
45. Barua, S., Yoo, J. W., Kolhar, P., Wakankar, A., Gokam, Y. R., & Mitragotri, S. (2013). Particle shape enhances specificity of antibody-displaying nanoparticles. *Proceedings of the National Academy of Sciences of the United States of America, 110*(9), 3270–3275.
46. Ananta, J. S., Godin, B., Sethi, R., Moriggi, L., Liu, X., Serda, R. E., et al. (2010). Geometrical confinement of gadolinium-based contrast agents in nanoporous particles enhances T1 contrast. *Nature Nanotechnology, 5*(11), 815–821.
47. Champion, J. A., & Mitragotri, S. (2006). Role of target geometry in phagocytosis. *Proceedings of the National Academy of Sciences of the United States of America, 103*(13), 4930–4934.
48. Chithrani, B. D., & Chan, W. C. (2007). Elucidating the mechanism of cellular uptake and removal of protein-coated gold nanoparticles of different sizes and shapes. *Nano Letters, 7*(6), 1542–1550.
49. Anselmo, A. C., Zhanq, M., Kumar, S., Vogus, D. R., Menegatti, S., Helgeson, M. E., et al. (2015). Elasticity of nanoparticles influences their blood circulation, phagocytosis, endocytosis, and targeting. *ACS Nano, 9*(3), 3169–3177.
50. Alexis, F., Pridgen, E., Molnar, L. K., & Farokhzad, O. C. (2008). Factors affecting the clearance and biodistribution of polymeric nanoparticles. *Molecular Pharmaceutics, 5*(4), 505–515.
51. Sykes, E. A., Dai, Q., Sarsons, C. D., Chen, J., Rocheleau, J. V., Hwang, D. M., et al. (2016). Tailoring nanoparticle designs to target cancer based on tumor pathophysiology. *Proceedings of the National Academy of Sciences of the United States of America, 113*(9), E1142–E1151.
52. Pelicano, H., Martin, D. S., Xu, R. H., & Huang, P. (2006). Glycolysis inhibition for anticancer treatment. *Oncogene, 25*(34), 4633–4646.
53. Deryugina, E. I., & Quigley, J. P. (2006). Matrix metalloproteinases and tumor metastasis. *Cancer Metastasis Reviews, 25*(1), 9–34.
54. Chari, R. V. (1998). Targeted delivery of chemotherapeutics: Tumor-activated prodrug therapy. *Advanced Drug Delivery Reviews, 31*(1–2), 89–104.
55. Mansour, A. M., Drevs, J., Esser, N., Hamada, F. M., Badary, O. A., Unger, C., et al. (2003). A new approach for the treatment of malignant melanoma: Enhanced antitumor efficacy of an albumin-binding doxorubicin prodrug that is cleaved by matrix metalloproteinase 2. *Cancer Research, 63*(14), 4062–4066.
56. Kim, G. J., & Nie, S. (2005). Targeted cancer nanotherapy. *Materials Today, 8*(8), 28–33.
57. Shi, J., Kantoff, P. W., Wooster, R., & Farokhzad, O. C. (2017). Cancer nanomedicine: Progress, challenges and opportunities. *Nature Reviews. Cancer, 17*(1), 20–37.
58. Sanna, V., Pala, N., & Sechi, M. (2014). Targeted therapy using nanotechnology: Focus on cancer. *International Journal of Nanomedicine, 9*(1), 467–483.
59. Awada, A., Bondarenko, I. N., Bonneterre, J., Nowara, E., Ferrero, J. M., Bakshi, A. V., et al. (2014). A randomized controlled phase II trial of a novel composition of paclitaxel embedded into neutral and cationic lipids targeting tumor endothelial cells in advanced triple-negative breast cancer (TNBC). *Annals of Oncology, 25*(4), 824–831.

60. Burris, H. A., Wang, J. S., Johnson, M. L., Falchook, G. S., Jones, S. F., Strickland, D. K., et al. (2017). A phase I, open-label, first-time-in-patient dose escalation and expansion study to assess the safety, tolerability, and pharmacokinetics of nanoparticle encapsulated Aurora B kinase inhibitor AZD2811 in patients with advanced solid tumours. *Journal of Clinical Oncology, 35*(15), TPS2608–TPS2608.
61. Batist, G., Sawyer, M., Gabrail, N., Christiansen, N., Marshall, J. L., Spigel, D. R., et al. (2008). A multicenter, phase II study of CPX-1 liposome injection in patients (pts) with advanced colorectal cancer (CRC). *Journal of Clinical Oncology, 26*(15), 4108–4108.
62. Gradishar, W. J., Tjulandin, S., Davidson, N., Shaw, H., Desai, N., Bhar, P., et al. (2005). Phase III trial of nanoparticle albumin-bound paclitaxel compared with polyethylated castor oil-based paclitaxel in women with breast cancer. *Journal of Clinical Oncology, 23*(31), 7794–7803.
63. Lancet, J. E., Uy, G. L., Cortes, J. E., Newell, L. F., Lin, T. L., Ritchie, E. K., et al. (2016). Final results of a phase III randomized trial of CPX-351 versus 7 + 3 in older patients with newly diagnosed high risk (secondary) AML. *Journal of Clinical Oncology, 34*(15), 7000.
64. Bogart, L. K., Pourroy, G., Murphy, C. J., Puntes, V., Pellegrino, T., Rosenblum, D., et al. (2014). Nanoparticles for imaging, sensing, and therapeutic intervention. *ACS Nano, 8*(4), 3107–3122.
65. Theek, B., Gremse, F., Kunjachan, S., Fokong, S., Pola, R., Pechar, M., et al. (2014). Characterizing EPR-mediated passive drug targeting using contrast-enhanced functional ultrasound imaging. *Journal of Controlled Release, 182*(1), 83–89.
66. Hansen, A. E., Petersen, A. L., Henriksen, J. R., Boerresen, B., Rasmussen, P., Elema, D. R., et al. (2015). Positron emission tomography based elucidation of the enhanced permeability and retention effect in dogs with cancer using copper-64 liposomes. *ACS Nano, 9*(7), 6985–6995.
67. Miller, M. A., Gadde, S., Pfirschke, C., Engblom, C., Sprachman, M. M., Kohler, R. H., et al. (2015). Predicting therapeutic nanomedicine efficacy using a companion magnetic resonance imaging nanoparticle. *Science Translational Medicine, 7*(314), 314ra183.
68. Lee, H., Shields, A. F., Siegel, B. A., Miller, K. D., Krop, I., Ma, C. X., et al. (2017). 64Cu-MM-302 positron emission tomography quantifies variability of enhanced permeability and retention of nanoparticles in relation to treatment response in patients with metastatic breast cancer. *Clinical Cancer Research, 23*(15), 4190–4202.
69. Wilhelm, S., Tavares, A. J., Dai, Q., Ohta, S., Audet, J., Dvorak, H. F., et al. (2016). Analysis of nanoparticle delivery to tumours. *Nature Reviews Materials, 1*(1), 16014.
70. van Vlerken, L. E., Duan, Z., Little, S. R., Seiden, M. V., & Amiji, M. M. (2008). Biodistribution and pharmacokinetic analysis of paclitaxel and ceramide administered in multifunctional polymer-blend nanoparticles in drug resistant breast cancer model. *Molecular Pharmaceutics, 5*(4), 516–526.
71. Cui, Y., Zhang, M., Zeng, F., Jin, H., Xu, Q., & Huang, Y. (2016). Dual-targeting magnetic PLGA nanoparticles for codelivery of paclitaxel and curcumin for brain tumor therapy. *ACS Applied Materials & Interfaces, 8*(47), 32159–32169.
72. Peer, D., & Margalit, R. (2004). Tumor-targeted hyaluronan nanoliposomes increase the antitumor activity of liposomal doxorubicin in syngeneic and human xenograft mouse tumor models. *Neoplasia, 6*(4), 343–353.
73. Shi, J., Xiao, Z., Kamaly, N., & Farokhzad, O. C. (2011). Self-assembled targeted nanoparticles: Evolution of technologies and bench to bedside translation. *Accounts of Chemical Research, 44*(10), 1123–1134.
74. Xu, R., Zhang, G., Mai, J., Deng, X., Segura-Ibarra, V., Wu, S., et al. (2016). An injectable nanoparticle generator enhances delivery of cancer therapeutics. *Nature Biotechnology, 34*(4), 414–418.
75. Levy, O., Brennen, W. N., Han, E., Rosen, D. M., Musabeyezu, J., Safaee, H., et al. (2016). A prodrug-doped cellular Trojan Horse for the potential treatment of prostate cancer. *Biomaterials, 91*(1), 140–150.

76. Huang, B., Abraham, W. D., Zheng, Y., Bustamante Lopez, S. C., Luo, S. S., & Irvine, D. J. (2015). Active targeting of chemotherapy to disseminated tumors using nanoparticle-carrying T cells. *Science Translational Medicine, 7*(291), 291ra294.
77. Monopoli, M. P., Åberg, C., Salvati, A., & Dawson, K. A. (2012). Biomolecular coronas provide the biological identity of nanosized materials. *Nature Nanotechnology, 7*(12), 779–786.
78. Krpetić, Ž., Anguissola, S., Garry, D., Kelly, P. M., & Dawson, K. A. (2014). Nanomaterials: Impact on cells and cell organelles. In G. D. Capco & Y. Chen (Eds.), *Nanomaterial. Impact on cell biology and medicine* (pp. 135–156). Dordrecht: Springer.
79. Barreto, J. A., O'Malley, W., Kubeil, M., Graham, B., Stephan, H., & Spiccia, L. (2011). Nanomaterials: Applications in cancer imaging and therapy. *Advanced Materials, 23*(12), 18–40.
80. Malam, Y., Loizidou, M., & Seifalian, A. M. (2009). Liposomes and nanoparticles: Nanosized vehicles for drug delivery in cancer. *Trends in Pharmacological Sciences, 30*(11), 592–599.
81. Illés, E., Szekeres, M., Kupcsik, E., Tóth, I. Y., Farkas, K., Jedlovszky-Hajdú, A., et al. (2014). PEGylation of surfacted magnetite core-shell nanoparticles for biomedical application. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 460*(1), 429–440.
82. Thierry, B., & Griesser, H. J. (2012). Dense PEG layers for efficient immunotargeting of nanoparticles to cancer cells. *Journal of Materials Chemistry, 22*(18), 8810–8819.
83. Ranganathan, R., Madanmohan, S., Kesavan, A., Baskar, G., Krishnamoorthy, Y. R., Santosham, R., et al. (2012). Nanomedicine: Towards development of patient-friendly drug-delivery systems for oncological applications. *International Journal of Nanomedicine, 7*(1), 1043–1060.
84. Walkey, C. D., Olsen, J. B., Guo, H., Emili, A., & Chan, W. C. W. (2012). Nanoparticle size and surface chemistry determine serum protein adsorption and macrophage uptake. *Journal of the American Chemical Society, 134*(4), 2139–2147.
85. Haume, K., Mason, N. J., & Solov'yov, A. (2016). Modeling of nanoparticle coatings for medical applications. *European Physical Journal D: Atomic, Molecular, Optical and Plasma Physics, 70*(9), 181.
86. Ashley, J. D., Stefanick, J. F., Schroeder, V. A., Suckow, M. A., Kiziltepe, T., & Bilgicer, B. (2014). Liposomal bortezomib nanoparticles via boronic ester prodrug formulation for improved therapeutic efficacy *in vivo*. *Journal of Medicinal Chemistry, 57*(12), 5282–5292.
87. Orlowski, R. Z., Nagler, A., Sonneveld, P., Bladé, J., Hajek, R., Spencer, A., et al. (2007). Randomized phase III study of pegylated liposomal doxorubicin plus bortezomib compared with bortezomib alone in relapsed or refractory multiple myeloma: Combination therapy improves time to progression. *Journal of Clinical Oncology, 25*(25), 3892–3901.
88. Voorhees, P. M., Orlowski, R. Z., Mulkey, F., Watson, P., Geyer, S., Sanford, B. L., et al. (2015). Long-term outcomes for newly-diagnosed multiple myeloma patients treated with pegylated liposomal doxorubicin and bortezomib: Final results of CALGB (Alliance) 10301, a multicentre phase II study. *British Journal of Haematology, 171*(3), 373–377.
89. Ravindran, J., Nair, H. B., Sung, B., Prasad, S., Tekmal, R. R., & Aggarwal, B. B. (2010). Thymoquinone poly (lactide-co-glycolide) nanoparticles exhibit enhanced anti-proliferative, anti-inflammatory, and chemosensitization potential. *Biochemical Pharmacology, 79*(11), 1640–1647.
90. Li, Z., Li, X., Cao, Z., Xu, Y., Lin, H., Zhao, Y., et al. (2012). Camptothecin nanocolloids based on N, N, N-trimethyl chitosan: Efficient suppression of growth of multiple myeloma in a murine model. *Oncology Reports, 27*(4), 1035–1040.
91. Badr, G., Al-Sadoon, M. K., El-Toni, A. M., & Daghestani, M. (2012). *Walterinnesia aegyptia* venom combined with silica nanoparticles enhances the functioning of normal lymphocytes through PI3K/AKT, NFkappaB and ERK signaling. *Lipids in Health and Disease, 11*(1), 27.
92. Sayed, D., Al-Sadoon, M. K., & Badr, G. (2012). Silica nanoparticles sensitize human multiple myeloma cells to snake (*Walterinnesia aegyptia*) venom-induced apoptosis and growth arrest. *Oxidative Medicine and Cellular Longevity, 2012*, 386286.

93. Elsadek, B., & Kratz, F. (2012). Impact of albumin on drug delivery–new applications on the horizon. *Journal of Controlled Release, 157*(1), 4–28.
94. Yang, C., Wang, J., Chen, D., Chen, J., Xiong, F., Zhang, H., et al. (2013). Paclitaxel-Fe_3O_4 nanoparticles inhibit growth of CD138(−) CD34(−) tumor stem-like cells in multiple myeloma-bearing mice. *International Journal of Nanomedicine, 8*(1), 1439–1449.
95. Otsuka, H., Nagasaki, Y., & Kataoka, K. (2003). PEGylated nanoparticles for biological and pharmaceutical applications. *Advanced Drug Delivery Reviews, 55*(3), 403–419.
96. Avgoustakis, K. (2004). Pegylated poly(lactide) and poly(lactide-co-glycolide) nanoparticles: Preparation, properties and possible applications in drug delivery. *Current Drug Delivery, 1*(4), 321–333.
97. Knop, K., Hoogenboom, R., Fischer, D., & Schubert, U. S. (2010). Poly(ethylene glycol) in drug delivery: Pros and cons as well as potential alternatives. *Angewandte Chemie (International Ed. in English), 49*(36), 6288–6308.
98. Hatakeyama, H., Akita, H., & Harashima, H. (2011). A multifunctional envelope type nanodevice (MEND) for gene delivery to tumours based on the EPR effect: A strategy for overcoming the PEG dilemma. *Advanced Drug Delivery Reviews, 63*(3), 152–160.
99. Valle, J. W., Armstrong, A., Newman, C., Alakhov, V., Pietrzynski, G., Brewer, J., et al. (2011). A phase 2 study of SP1049C, doxorubicin in P-glycoprotein-targeting pluronics, in patients with advanced adenocarcinoma of the esophagus and gastroesophageal junction. *Investigational New Drugs, 29*(5), 1029–1037.
100. Matsumura, Y., Hamaguchi, T., Ura, T., Muro, K., Yamada, Y., Shimada, Y., et al. (2004). Phase I clinical trial and pharmacokinetic evaluation of NK911, a micelle-encapsulated doxorubicin. *British Journal of Cancer, 91*(10), 1775–1781.
101. Tong, R., & Cheng, J. (2007). Anticancer polymeric nanomedicines. *Polymer Reviews, 47*(3), 345–381.
102. Singer, J. W. (2005). Paclitaxel poliglumex (XYOTAX, CT-2103): A macromolecular taxane. *Journal of Controlled Release, 109*(1–3), 120–126.
103. Young, C., Schluep, T., Hwang, J., & Eliasof, S. (2011). CRLX101 (formerly IT-101)-a novel nanopharmaceutical of camptothecin in clinical development. *Current Bioactive Compounds, 7*(1), 8–14.
104. Lammers, T., Kiessling, F., Hennink, W. E., & Storm, G. (2012). Drug targeting to tumors: Principles, pitfalls and (pre-) clinical progress. *Journal of Controlled Release, 161*(2), 175–187.
105. Tagami, T., Ernsting, M. J., & Li, S. D. (2011). Efficient tumor regression by a single and low dose treatment with a novel and enhanced formulation of thermosensitive liposomal doxorubicin. *Journal of Controlled Release, 152*(2), 303–309.
106. Seetharamu, N., Kim, E., Hochster, H., Martin, F., & Muggia, F. (2010). Phase II study of liposomal cisplatin (SPI-77) in platinum-sensitive recurrences of ovarian cancer. *Anticancer Research, 30*(2), 541–545.
107. Clinical Trials.gov. *A phase I trial of nanoliposomal CPT-11 (NL CPT-11) in patients with recurrent high-grade gliomas*. Retrieved from http://clinicaltrials.gov/show/NCT00734682
108. Tardi, P., Johnstone, S., Harasym, N., Xie, S., Harasym, T., Zisman, N., et al. (2009). *In vivo* maintenance of synergistic cytarabine: Daunorubicin ratios greatly enhances therapeutic efficacy. *Leukemia Research, 33*(1), 129–139.

Chapter 7
Surface Modification of Magnetic Nanoparticles in Biomedicine

Viroj Wiwanitkit

Abstract Nanobiotechnology is proven for its advantage in several applications including applied biomedicine. The application of nanobiotechnology is possible in many aims such as drug and diagnostic test developments. Many new nanoparticles are developed and applied at present to serve those purposes. In this specific chapter, the author will focus on the surface modification of magnetic nanoparticles which pose specific properties of nanoparticle and magnetic property.

The application of surface modification of magnetic nanoparticles for pharmaceutical process as well as diagnostic test development will be summarized and presented in this article. Summary on important reports on the mentioned specific topics is also given in this article.

Keywords Nanobiotechnology · Surface · Modification · Magnetic · Nanoparticle

1 Introduction

Nano means the very small level at 10^{-9}. At this level, the substance will have many interesting nanoproperties, which can be useful for usage. At present, nanobiotechnology is proven for its advantage in several applications including applied biomedicine. In general, nanomaterials that are very small will have significant increased surface area compared to the same material in big size. Also, the nanomaterials have specific change of electric and biochemical properties. In addition, the extremely small size lets the particles to be able to freely cross the cellular barrier into intracellular environment and further affect the cell. It is proven that the

V. Wiwanitkit (✉)
DY Patil University, Pune, India

Joseph Ayobabalola University, Ilara-Mokin, Nigeria

Faculty of Medicine, University of Nis, Nis, Serbia

Hainan Medical University, Haikou, China

Y. V Pathak (ed.), *Surface Modification of Nanoparticles for Targeted Drug Delivery*, https://doi.org/10.1007/978-3-030-06115-9_7

nanoparticle can successfully enter in several cells including white blood cell [1], lymphocyte [2], or renal cells [3]. The change of the cells after getting nanomaterials inside is the important physiological change, and this is the principle of application of nanomaterials in nanomedicine. In biomedicine, the application of nanobiotechnology is possible in many aims such as drug and diagnostic test developments. For pharmaceutical purpose, the nanomaterials can help in drug transfer and targeting the pathological site. In diagnostic medicine, the nanoparticles can help stimulate diagnostic reaction and help analytical process.

Many new nanoparticles are developed and applied at present to serve those purposes. Of several presently available nanoparticles, magnetic nanoparticles are specific nanoparticles which have specific dual properties of nanoparticle and magnetic property. This dual property is very interesting and becomes very useful for application in biomedicine. The application of surface modification of magnetic nanoparticles for pharmaceutical process and well as diagnostic test development will be summarized and presented in this chapter. Summary on important reports on the mentioned specific topics is also given in this article.

2 What Is a Magnetic Nanoparticle?

Before a further discussion on using magnetic nanoparticle in biomedicine, the details about this kind of nanomaterial should be mentioned. Generally, a magnetic substance means a substance that can present magnetic property. Due to the intrinsic electric current and magnetic moment, the ferromagnetism is the basic characteristic of a magnetic substance. Basic interaction test for magnetic property can be done and helps confirm the magnetic property of a substance. The examples of magnetic materials are iron, nickel, and cobalt. It is no doubt that there are some extremely small magnetic substances and if the substance is at nanolevel, it will be called a nanomagnetic substance or magnetic nanoparticle.

Magnetic nanoparticle is a specific group of nanoparticle that has magnetic property and can be manipulated by magnetic fields. There are usually two main components of a magnetic nanosubstance, the magnetic part and nonmagnetic part. Hence, the magnetic nanosubstance is usually a nanomaterial complex. In that complex, the magnetic parts might be a magnetic material such as iron or cobalt or other specifically designed chemical component with magnetic property. Since the magnetic nanoparticles are usually complex, the size of the complex is usually larger than a simple nanoparticle. The average size of the magnetic nanoparticle is usually 50–100 nm. The nanomagnetic complex is sometimes presented in the form of magnetic nanobead that is an important nanosubstance in the present day. The magnetic nanobeads become widely used in several purposes such as catalysts in industry or applications in biomedicine. For biomedical application, the magnetic nanosubstance can be used for both diagnostic and therapeutic purpose. Similar to other nanosubstances, the usefulness of application of magnetic nanosubstance is usually for the disease that is very hard to manage in clinical practice such as cancer [4].

3 How Can a Magnetic Nanoparticle Be Applied in Biomedicine?

An important basic question in nanomedicine is "How can a magnetic nanoparticle be applied?" This question can be easily answered in the following. First, there must be a magnetic nanoparticle that is appropriate for application. For using in pharmaceutical process, the specific magnetic nanoparticle must have specific biochemical property that can be useful in disease management. The developed magnetic nanoparticle will be conjugated with drug to form the nanodrug. That finalized magnetic nanoparticle is hereby called a nanodrug. The nanodrug usually has a very small size to allow its biochemical reaction on cells when it is applied for pharmacological purpose. The second step that requires for completing the usage of magnetic nanoparticle as drug is the nanoparticle administration. Generally, there are many ways for nanoparticle administrations of drug such as oral intake, inhalation, dermatological application, or direct injection into blood vascular or body space. This is a requirement for using any nanodrug. If the administration of the nanodrug into the body is not possible, it will be useless. Hence, there must be a good preparation of the magnetic nanoparticle in order to allow feasibility of administration. Similar to any drugs, the next concern is on the bioavailability of the magnetic nanosubstance. There must be a trial to confirm that the newly developed magnetic nanosubstance-based drug can be effectively absorbed, distributed, and targeted at the drug target (Fig. 7.1).

For using in diagnostic medicine, the similar first step is there must be the appropriate magnetic nanoparticle. The nanoparticle must be adaptable to be used in the diagnostic tool formation. The next concern is the durability of nanoparticle. The good nanoparticle should last long, and there should be no decreasing of properties after usage. In addition, there must be no or very little interference on using the nanoparticle. These are basic concerns in development of any diagnostic tools in investigative medicine. The next step for consideration is the actual application of the nanosubstance to be a composition of the newly developed nanodiagnostic tool. The attachment of the nanoparticle must be easy, and there should be very little loss during the process. The standard method for evaluation of the new medical diagnostic test is needed for verifying the acceptability of any new nanodiagnostic tool [5–6]. The standard evaluation for diagnostic properties (threshold sensitivity, accuracy,

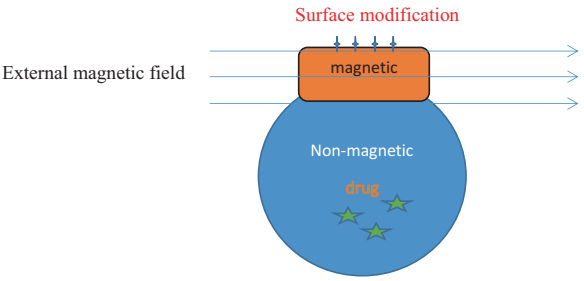


Fig. 7.1 Figure showing a magnetic nanoparticle complex (graphic drawing by Wiwanitkit V, 2018)

precision, interference, reproducibility, etc.) and clinical application properties (diagnostic sensitivity, specificity, false positive, false negative, etc.) is needed.

4 Preparation of Magnetic Nanomaterials for Use as Nanodrug

As already mentioned, magnetic nanomaterials are considered as useful nanoparticles for application of the clinical therapy [7, 8]. The main required pharmacological reaction of a magnetic nanosubstance is its magnetic property. This action is aimed at the target cell. The effect of external magnetic field is used as important part in therapeutic process [7]. The remote control of the nanoparticles accumulation by mean of an external magnetic field is the basic concept [7]. For targeting the pathological cell for treatment, the magnetic drug targeting (MDT) by effect of an external magnetic field to target is the main process [7]. Additional technology can also be used to help increase effectiveness in drug targeting. The good example is the controlled-release technology for allowing the magnetic nanodrug to have its pharmacological action at the diseased site. Nevertheless, there is still an important precaution on the unwanted adverse effect of the newly designed nanoparticle. There are some toxicological concerns including unpredictable cellular responses and induction of signaling pathways, induction of unwanted oxidative stress, aberrant gene expression profiles, and disturbance in biometal homeostasis [9]. Hence, there must be the good modification of the developed particle to get the final nontoxic magnetic nanosubstance for nanodrug development. For modifying, the surface modification is proven effective. The surface modification might be by several techniques. However, there are only three main processes, increasing the surface structure by adding additional parts (such as antibody conjugation, surfactant coating, polymer coating, ligand anchoring, adsorption), decreasing the surface structure by removing some parts, and substitution of the old structure with the new one. Nevertheless, the most widely used approach is the increasing structure parts. The modification might be by chemical, biological, or physical reaction. The advantages from modification include decreased unwanted side effect and increased stability or addition of new desired property of the molecule (such as ligand binding or immunological linkage).

With surface modification, the adverse effect due to magnetic property of nonmodified magnetic nanoparticle can be managed. For example, Strehl et al. recently proposed for a cross-linkage to reduce the unwanted cytokine induction due to magnetic nanoparticles [10]. The hybrid polymeric-magnetic nanoparticles resulting from surface modification is proposed as the present safe generation of magnetic nanoparticle [11] (Table 7.1).

The good examples of magnetic nanoparticles that are used in therapeutic process are metal oxide nanoparticles. A widely used nanoparticle includes superparamagnetic iron oxide nanoparticle (SPION). Structurally, a SPION has an iron oxide core, which is usually coated with organic materials such as polysaccharides, fatty

Table 7.1 Some important hybrid polymeric-magnetic nanoparticles

Hybrids	Details
Dendrimer-based magnetic iron oxide nanoparticle [12]	Dendrimer-based magnetic iron oxide nanoparticle is a nanohybrid that has both functional organic and inorganic properties. It can be used in several biomedical applications, such as magnetic resonance (MR) imaging, drug and gene delivery, and protein immobilization [12]
Multifunctional magnetic iron oxide nanoparticles/ mitoxantrone-loaded liposome [13]	The multifunctional magnetic iron oxide nanoparticles/ mitoxantrone-loaded liposome is a new hybrid that is used for both diagnosis and treatment (such as for both MR imaging and targeted cancer therapy). This hybrid can serve the need in present new concept of theranostics [13]

acids, or polymers. The coating is aimed at increasing colloidal stability and reducing separation between particle and carrier medium [7].

Some important reports in development of SPION are presented in Table 7.2.

There are many interesting reports on preparation of magnetic nanomaterials for using nanodrugs. The important application in several fields of clinical medicine will be hereby summarized.

(a) Reports in oncology

The development of new magnetic nanodrug usually aims at management of presently untreatable diseases, especially for cancers. The development of the magnetic nanomaterial for management of cancer is widely done at present. For example, Klein et al. reported the use of magnetite and cobalt ferrite nanoparticles for enhancing reactive oxidative stress formation in radiation cancer therapy [18]. Zhang and Song reported the use of combination therapy using an injectable, biodegradable, and thermosensitive polymeric hydrogel. In this system, a complex based on positively charged tumor necrosis factor-related apoptosis-inducing ligand and hydrophobic SPIONs complexed with negatively charged poly(organophosphazene) polymers via ionic and hydrophobic interactions is used in hyperthermia cancer therapy [19]. Based on the given examples, it might conclude that the magnetic nanomaterials are effective option for cancer management. The good design of magnetic nanodrug is the important determinant to achieve safe and effective magnetic anticancer nanodrug.

Table 7.2 Some recent publication on development of superparamagnetic iron oxide nanoparticle

Authors	Details
Singh et al. [14]	Singh et al. reported on development of SPIONs via direct conjugation with ginsenosides [14]
Miranda et al. [15]	Miranda et al. reported on development of inhalable SPIONs in microparticulate system for antituberculosis drug delivery [15]
Silva et al. [16]	Silva et al. reported on development of a new antitumor compound based on rhodium(II) succinate associated with SPIONs coated with lauric acid/albumin hybrid [16]
Muzio et al. [17]	Muzio et al. reported on development of a new SPIONs coated with silica and conjugated with linoleic acid which has an antitumor property [17]

(b) Reports in neurology

There are also some interesting reports on preparation of magnetic nanomaterials as new nanodrugs in neurology. Most applications are also the same as the already mentioned one in the topic of clinical oncology, the treatment of brain malignancy. For example, Babincová et al. reported on application of albumin-embedded magnetic nanoheaters for release of etoposide in combined hyperthermia and chemotherapy of glioma [20].

In non-oncological neurological disorder, the magnetic nanoparticles are also applicable for management of degenerative diseases such as Alzheimer's disease. For example, Do et al. reported on using magnetic nanocontainers for treatment of Alzheimer's disease with use of electromagnetic, targeted drug-delivery actuator [21]. Nevertheless, most present applications for neurodegenerative disease are usually on imaging diagnosis.

(c) Reports in infectious medicine

There are also some interesting reports on the preparation of magnetic nanomaterials as new nanodrugs in infectious medicine. The good example is the use in management of tuberculosis. As earlier mentioned, Miranda et al. developed a new inhalable SPIONs in microparticulate system for antituberculosis drug delivery [15]. Another good example is the use in HIV management. Williams et al. recently proposed a new approach for radication of latently infected HIV-positive cells by using magnetic field hyperthermia and SPIONs [22].

The efficacy of magnetic nanomaterials in treatment of microbial infection is proposed to be due to the similar process as that seen in malignant cell management.

The main actions of the nanoparticles are damaging the cell wall or by generating reactive oxygen species [23].

Based on the mentioned application in clinical medicine, it can be summarized that the present trend of using magnetic nanomaterial preparation is for the management of pathology at deep hard-to-access organs or of the disease that can be presently cured by classical therapy. The magnetic nanocomplex is usually designed and prepared to serve the different specific needs in treatment of different clinical problems. Several attempts on clinical trials have been done for many years, and it is aimed at finding new nanodrugs based on magnetic nanomaterials. Although there is still no commercially available magnetic nanodrug, it might be available within the next few years.

5 Preparation of Magnetic Nanomaterials for Use as Nanodrug and Theranostics

As already mentioned, the application of magnetic nanomaterials in diagnostic process is possible. The interesting concept is the combined diagnosis and treatment. This is called theranostics. For single use in diagnosis, the application in

imaging investigation is well described. The magnetic nanoparticle is widely applied for MR imaging at present [24]. In addition to basic MR imaging, the advanced technology also allows the use of magnetic nanoparticles as tools for helping molecular imaging. The imaging of cells is presently possible with use of magnetic nanoparticles [25].

As a substance with magnetic property, it is no doubt that manipulation of magnetic nanoparticle during MR imaging is possible. Also, if the magnetic nanodrug is well prepared (including ligands design for targeted delivery and fluorescent or other chemical tagging) and used, the final theranostic property can be achieved [26].

6 Conclusion

The magnetic nanomaterials can be served as important basic materials for developments of new nanodrugs and nanodiagnostic tools. The application of magnetic nanomaterials is proven useful for management of several medical problems. The design of magnetic nanomaterials is an important step for further application. This process must be carefully performed, and there must be specific consideration during formation to focus on the desired final product and prevent possible unwanted toxicity. The effective nanoformulation can be useful in both pharmaceutical and laboratory diagnostic developments. For formation of an effective nanodrug, the well-designed magnetic nanomaterials can support nanodrug administration, nanodrug delivery, targeting, and pharmacological acting. The magnetic nanomaterial-based nanodrug becomes the present hope for management of uncurable disease (such as malignancies) and effective approach to hard-to-access pathological sites (such as central nervous system, ocular and joint spaces). The good magnetic nanomaterials are stable in extracellular environment and have the preferable pharmacological reaction in the targeted cells. There are many compositions of the new magnetic nanomaterial complexes that are proven for different advantages. Some newly developed magnetic nanomaterials are already registered and used as the main composition of the new commercially available drug for clinical usage. Similarly, the application of the magnetic nanomaterials in the development of new medical diagnostic analyzer is based on good design and formation of the nanomaterials. The good magnetic nanomaterials are stable and give accurate diagnostic result when applied for diagnostic purpose. Some newly developed magnetic nanomaterials are already registered and used as the main composition of the new commercially available medical diagnostic tool. Although there are many reports on the development and use of magnetic nanomaterials in clinical medicine, further researches and developments in this area are still necessary.Conflict of InterestNone.

References

1. Wiwanitkit, V., Sereemaspun, A., & Rojanathanes, R. (2009). Effect of gold nanoparticle on the microscopic morphology of white blood cell. *Cytopathology, 20*(2), 109–110.
2. Wiwanitkit, V., Sereemaspun, A., & Rojanathanes, R. (2009). Identification of gold nanoparticle in lymphocytes: A confirmation of direct intracellular penetration effect. *Turkish Journal of Haematology, 26*(1), 29–30.
3. Sereemaspun, A., Rojanathanes, R., & Wiwanitkit, V. (2008). Effect of gold nanoparticle on renal cell: An implication for exposure risk. *Renal Failure, 30*(3), 323–325.
4. Brigger, I., Dubernet, C., & Couvreur, P. (2002). Nanoparticles in cancer therapy and diagnosis. *Advanced Drug Delivery Reviews, 54*(5), 631–651.
5. Azzazy, H. M., Mansour, M. M., & Kazmierczak, S. C. (2006). Nanodiagnostics: A new frontier for clinical laboratory medicine. *Clinical Chemistry, 52*(7), 1238–1246.
6. Jain, K. K. (2003). Nanodiagnostics: Application of nanotechnology in molecular diagnostics. *Expert Review of Molecular Diagnostics, 3*(2), 153–161.
7. Tietze, R., Zaloga, J., Unterweger, H., Lyer, S., Friedrich, R. P., Janko, C., et al. (2015). Magnetic nanoparticle-based drug delivery for cancer therapy. *Biochemical and Biophysical Research Communications, 468*(3), 463–470.
8. Vallabani, N. V. S., & Singh, S. (2018). Recent advances and future prospects of iron oxide nanoparticles in biomedicine and diagnostics. *Biotech, 8*(6), 279.
9. Laurent, S., Saei, A. A., Behzadi, S., Panahifar, A., & Mahmoudi, M. (2014). Superparamagnetic iron oxide nanoparticles for delivery of therapeutic agents: Opportunities and challenges. *Expert Opinion on Drug Delivery, 11*(9), 1449–1470.
10. Strehl, C., Maurizi, L., Gaber, T., Hoff, P., Broschard, T., Poole, A. R., et al. (2016). Modification of the surface of superparamagnetic iron oxide nanoparticles to enable their safe application in humans. *International Journal of Nanomedicine, 11*, 5883–5896.
11. Bonilla, A. M., & Gonzalez, P. H. (2017). Hybrid polymeric-magnetic nanoparticles in cancer treatments. *Current Pharmaceutical Design, 23*(35), 5392–5402.
12. Sun, W., Mignani, S., Shen, M., & Shi, X. (2016). Dendrimer-based magnetic iron oxide nanoparticles: Their synthesis and biomedical applications. *Drug Discovery Today, 21*(12), 1873–1885.
13. He, Y., Zhang, L., Zhu, D., & Song, C. (2014). Design of multifunctional magnetic iron oxide nanoparticles/mitoxantrone-loaded liposomes for both magnetic resonance imaging and targeted cancer therapy. *International Journal of Nanomedicine, 9*, 4055–4066.
14. Singh, H., Du, J., Singh, P., Mavlonov, G. T., & Yi, T. H. (2018). Development of superparamagnetic iron oxide nanoparticles via direct conjugation with ginsenosides and its in-vitro study. *Journal of Photochemistry and Photobiology. B, 185*, 100–110.
15. Miranda, M. S., Rodrigues, M. T., Domingues, R. M. A., Costa, R. R., Paz, E., Rodríguez-Abreu, C., et al. (2018). Development of inhalable superparamagnetic iron oxide nanoparticles (SPIONs) in microparticulate system for antituberculosis drug delivery. *Advanced Healthcare Materials, 23*, e1800124. https://doi.org/10.1002/adhm.201800124
16. Silva, M. O. D., Carneiro, M. L. B., Siqueira, J. L. N., Báo, S. N., & Souza, A. R. (2018). Development of a promising antitumor compound based on rhodium(II) succinate associated with iron oxide nanoparticles coated with lauric acid/albumin hybrid: synthesis, colloidal stability and cytotoxic effect in breast carcinoma cells. *Journal of Nanoscience and Nanotechnology, 18*(6), 3832–3843.
17. Muzio, G., Miola, M., Ferraris, S., Maggiora, M., Bertone, E., Puccinelli, M. P., et al. (2017). Innovative superparamagnetic iron-oxide nanoparticles coated with silica and conjugated with linoleic acid: Effect on tumor cell growth and viability. *Materials Science & Engineering. C, Materials for Biological Applications, 76*, 439–447.
18. Klein, S., Kızaloğlu, M., Portilla, L., Park, H., Rejek, T., Hümmer, J., et al. (2018). Enhanced in vitro biocompatibility and water dispersibility of magnetite and cobalt ferrite nanoparticles employed as ROS formation enhancer in radiation cancer therapy. *Small, 14*(21), e1704111.

19. Zhang, Z. Q., & Song, S. C. (2017). Multiple hyperthermia-mediated release of TRAIL/SPION nanocomplex from thermosensitive polymeric hydrogels for combination cancer therapy. *Biomaterials, 132*, 16–27.
20. Babincová, M., Vrbovská, H., Sourivong, P., Babinec, P., & Durdík, Š. (2018). Application of albumin-embedded magnetic nanoheaters for release of etoposide in integrated chemotherapy and hyperthermia of U87-MG glioma cells. *Anticancer Research, 38*(5), 2683–2690.
21. Do, T. D., Ul Amin, F., Noh, Y., Kim, M. O., & Yoon, J. (2016). Guidance of magnetic nanocontainers for treating Alzheimer's disease using an electromagnetic, targeted drug-delivery actuator. *Journal of Biomedical Nanotechnology, 12*(3), 569–574.
22. Williams, J. P., Southern, P., Lissina, A., Christian, H. C., Sewell, A. K., Phillips, R., et al. (2013). Application of magnetic field hyperthermia and superparamagnetic iron oxide nanoparticles to HIV-1-specific T-cell cytotoxicity. *International Journal of Nanomedicine, 8*, 2543–2554.
23. de Toledo, L. A. S., Rosseto, H. C., & Bruschi, M. L. (2018). Iron oxide magnetic nanoparticles as antimicrobials for therapeutics. *Pharmaceutical Development and Technology, 23*(4), 316–323.
24. Magro, M., Baratella, D., Bonaiuto, E., de A Roger, J., & Vianello, F. (2018). New perspectives on biomedical applications of iron oxide nanoparticles. *Current Medicinal Chemistry, 25*(4), 540–555.
25. Seth, A., Park, H. S., & Hong, K. S. (2017). Current perspective on in vivo molecular imaging of immune cells. *Molecules, 22*(6), E881. https://doi.org/10.3390/molecules22060881.
26. Belyanina, I., Kolovskaya, O., Zamay, S., Gargaun, A., Zamay, T., & Kichkailo, A. (2017). Targeted magnetic nanotheranostics of cancer. *Molecules, 22*(6), E975.

Chapter 8
A Novel Strategy for the Surface Modification of Superparamagnetic (Fe_3O_4) Iron Oxide Nanoparticle for Lung Cancer Imaging

Mayank Bhushan and Yogesh Kumar

Abstract Superparamagnetic iron oxide nanoparticles (SPIONs) have recently gained interest due to their low toxicity, biocompatibility, magnetic potential, and catalytic nature. Their biocompatible nature makes them suitable candidate for biomedical applications. SPIONs are considered as inert and used in different areas such as imaging, targeted drug delivery and biosensors. Their surfaces can be modified using different coating and labeling agents which has broaden their role in diagnosis and nanomedicine applications. SPIONs have got wide range of applications in biomedical and healthcare industry such as drug-delivery vehicle for chemotherapeutic drugs, an agent to induce heat mediated killing of cancer cells (hyperthermia) and as contrast agent in magnetic resonance imaging (MRI). More often SPIONs are used for simultaneous image guided delivery of chemotherapeutic drugs to the tumor cells and hyperthermia is used synergistically to enhance the overall lethal effect of the whole treatment process toward tumor cells. In this chapter, we discuss different strategies for surface modification of SPIONs and their applications for diagnosis and treatment of lung cancer.

Keywords SPIONs · Surface modification · Lung cancer imaging · Bioimaging

M. Bhushan (✉)
Department of Nanotechnology, North Eastern Hill University, Shillong, Meghalaya, India

Y. Kumar
Department of Biochemistry and Molecular Biology, Pondicherry University, Puducherry, India

Y. V Pathak (ed.), *Surface Modification of Nanoparticles for Targeted Drug Delivery*, https://doi.org/10.1007/978-3-030-06115-9_8

1 Introduction: Superparamagnetic (Fe_3O_4) Iron Oxide Nanoparticle

Iron oxide nanoparticles (IONPs) are well known for their potential application in biomedicine, imaging, drug delivery, hyperthermia, magnetic separation, and cell proliferation activity. The superparamagnetic nanoparticles aggregate when magnetic field is applied but again forms stable suspension on removal of magnetic field. The most studied IONPs are magnetite (Fe_3O_4), hematite (α-Fe_2O_3), and maghemite (γ-Fe_2O_3). The presence of both Fe^{2+} and Fe^{3+} ions in Fe_3O_4 NPs differentiate them from hematite and maghemite. In Fe_3O_4, Fe^{2+} ions occupy the octahedral sites and Fe^{3+} ions are divided between tetrahedral and octahedral sites. The α-Fe_2O_3 contains only Fe^{2+} ions which are located at octahedral sites. The γ-Fe_2O_3 contains only Fe^{3+} ions which are distributed among octahedral sites and tetrahedral sites. The presence of electron hopping nature in these IONPs make them suitable candidate for the biological and technical applications. The surfaces of these nanoparticles can further be functionalized by coating them with suitable surface coating agents like polymers, bioactive molecules and metal oxides to increase the application specific functionality of the nanoparticles.

The major challenge in using IONPs for biomedical applications is to keep them suspended in water at pH 7.0. Several methods are used to synthesize IONPs like thermal decomposition, coprecipitation, sol–gel, microemulsion, hydrothermal, sonochemical, microwave, electrochemical, and biosynthesis. But the most common method is coprecipitation method. In traditional way SPIONs were prepared by long-term grinding of magnetite in the presence of stabilizing agent [1]. However most of the properties of these NPs depend on their shape and size. To use IONPs in vivo they must comply with features such as low toxicity, biocompatibility, long retention time and magnetism to make their application in site directed delivery. IONPs are used as a probe in magnetic resonance imaging (MRI), positron emission tomography (PET), near-infrared fluorescence (NIRF) imaging and in biosensors for detection of biomolecules like glucose, proteins, urea, and uric acid. Surface modification of superoxide nanoparticles with markers for tumor receptor such as transferrin, folate, and Her-2/neu has enabled them to specifically bind with the tumor cells. Tagging with contrasting agents can make the SPIONs suitable for the following: early detection of the disease, personalized treatment with more accuracy, effective monitoring the treatment, and the understanding of cellular interaction with the environment in the living beings. This could improve imaging in laboratory and clinic.

The presence of aligned unpaired spin of electrons in ferromagnetic materials gives rise to their magnetic properties which are not dependent on external filed. However, the magnetic properties of nonmagnetized ferromagnetic materials are dependent on the presence of external field. This happens due to presence of aligned unpaired electrons at short distance but, at long distance it is antialigned. The short distance alignment in nonferromagnetic material is also called Weiss domains. The transition between these two aligned and antialigned domain forms Bloch wall; this gives rise to single domain crystals which are thermodynamically unstable. This is

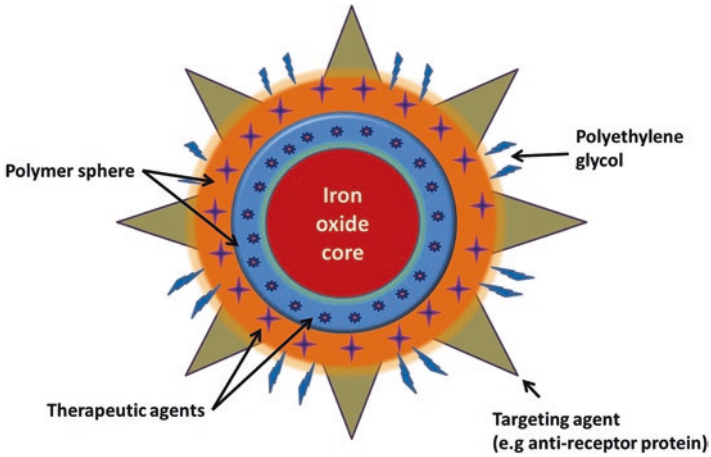


Fig. 8.1 Schematic of drug loaded and functionalized core-shell SPION

characteristic of nanoparticles with vary small size range and their characteristic magnetic behavior is known as superparamagnetism. The magnetic resonance (MR) is not the intrinsic properties of the SPIONs. This is generated by rapid dephasing of surrounding protons in the surrounding nuclei that leads to detectable MR signal. In fact, when the SPIONs are subjected under magnetic field; their momentums align in the direction of the magnetic field. This leads to increase in magnetic flux that causes dephasing of surrounding protons. The SPIONs under magnetic field relax the longitudinal and transverse field. The ability of SPIONs to generate the MR contrast is based on reduction in spin–spin relaxation (T2) of surrounding nuclei. The SPIONs are also able to generate the T1 contrast for biomedical applications. They possess both R1 and R2 relaxivities. A schematic of drug loaded and functionalized core-shell SPION is given in Fig. 8.1.

2 Synthesis of SPIONs

The most popular methods for synthesizing SPIONs are coprecipitation and microemulsion. However, other approaches are also used to synthesize SPIONs with uniform diameter with desirable properties.

Coprecipitation Method This is the most common method to prepare magnetic nanoparticles. This involves the coprecipitation of ferrous and ferric salts in an alkaline medium. The surface complexing agent such as dextran, polyethylene glycol (PEG), or polyvinyl alcohol (PVA) is often used to provide stability and biocompatibility to the nanoparticles. Some of the surface complexing agents are listed in Table 8.1. The SPIONs are synthesized by adding concentrated base to a divalent and trivalent ferrous salt solution. The precipitate formed is isolated by magnetic

Table 8.1 Surface coating and complexing agents

Core nanoparticle	Surface coating	Modifier	Drug	Cell	Mechanism	References
SPION			Ursolic methyl ester	Drug-resistant human leukemia KA cells	Apoptotic staining, DNA fragmentation,	[2]
Fe_3O_4			Daunorubicin	Glioma, an aggressive brain tumor	Apoptosis DNA, reactive oxygen species, DNA topoisomerase I and II, metal ions, and induction of apoptosis	[3]
Fe_3O_4	Graphene oxide		Doxorubicin (DOX)	HeLa cells	Hyperthermia	[4]
Fe_3O_4	Polydopamine (PDA)-PEG	Epidermal growth factor receptor (EGFR) antibody	Doxorubicin (DOX)		Photothermal ablation and near-infrared light-triggered DOX release	[5]
Fe_3O_4	Poly (D,L-lactic-co-glycolic acid) (PLGA) polymers copolymer PLGA-PEG		Doxorubicin			[6]
Fe_3O_4	Diblock copolymer folate-poly(ethylene glycol)-b-poly(glycerol monomethacrylate) (FA-PEG-bPGMA), and triblock copolymer methoxy poly(ethylene glycol)-b-poly(2-(dimethylamino) ethyl methacrylate)-b-poly(glycerol monomethacrylate) (MPEG-b-PDMA-b-PGMA)	Folate was conjugated onto the surface of the nanocarriers	Cisplatin	HeLa cells		[7]
Fe_3O_4	PEG and PEI polymers	Folic acid	Doxorubicin	MCF-7		[8]

Fe_3O_4	Glutamic acid	Hyaluronic acid		Targeting of CD44-overexpressing tumor cells	Receptor-meditated endocytosis	[9]
Fe_3O_4	Amine-functionalized PEG silane	Chlorotoxin (CTX)			Deactivation of membrane bound matrix metalloproteinase 2 (MMP-2) and increased internalization of lipid rafts containing surface-expressed MMP-2	[10]
Fe_3O_4	Poly (D,L-lactic-co-glycolic acid) poly (ethylene glycol) (PLGA-PEG)		Doxorubicin			[11]
Fe_3O_4	Dextran	Folic acid		KB cells		[12]

decantation or centrifugation process. To prepare bare nanoparticles by acidic method, the precipitate is treated with nitric or perchloric acid followed by centrifugation and then dispersion in water. For alkaline method, tetra methyl ammonium can be used. The obtained nanoparticles will be polydispersed, having size distribution below 10 nm and will have nearly spherical shape. The formed SPIONs can be coated with monomers and polymers through nonspecific adsorption. Many factors such as pH, heat, magnetite ratio, and polymer ratio can be used to control the size, stability, biocompatibility, and magnetic properties to make SPIONs more useful. Silica is one of the coating agents which are inert and biocompatible that can be further doped with other agent. This procedure is simple and therefore widely used at industrial level, but the nanoparticles formed are of polydispersed.

Microemulsion Method This method is adopted over coprecipitation method in order to adjust size and shape of the nanoparticles. In this method, the nanoparticles are synthesized in microemulsions which are oil in water and water in oil. This approach is generally used for the synthesis of biocompatible nanoparticle. This is an efficient way to synthesize nanoparticles of size range between 2 and 12 nm. The dispersion affinity of the nanoparticles in water or organic solvents can be finely tuned by adopting this synthesis approach. The coatings are suitable to make SPIONs dispersed in water can be used to prevent agglomeration.

3 Biocompatibility

In order to use the nanoparticles in-vivo they must be able to get cleared from the excretory system of body. Ideally, nanoparticles should have cell response with no or less cytotoxicity. Spleen and liver is most important organ to sequester the large particles. Generally particles of size 200 nm in diameter get sequestered by the spleen and liver. So, the particles like SPIONs which are of size around 10 nm easily get removed through extravasation and renal clearance. Amphipathic coatings are used to increase the half-life of SPIONs in plasma. Thus, extending their circulation time and hence effectiveness. Polymer coated SPIONs have not shown any cytotoxicity or alteration in cell adhesion behavior. PEG-coated SPIONs and dextran-coated SPIONs labeled with the Tat-internalizing peptide have not shown any effect on cell viability. Some studies have shown increase in SPIONs uptake by macrophage cells but there was no evidence of its activation because there was no interleukin-1 release.

Tumor metastasis results in leaky vasculature. It is also evident that SPIONs get accumulated at the tumor site due to the leaky vasculature and increased macrophage uptake at tumor sites. Targeted delivery of SPIONs to tumor site has been studied. The conjugation of SPIONs with specific antibodies/proteins enables them to bind directly to the cancer cells expressing that particular marker. This also facilitates the receptor-mediated internalization of SPIONs. Transferrin and Hep 2 tumor markers are most common cell surface markers expressed in cancer cells.

4 SPIONs in Cancer Diagnosis and Treatment

The strategy for cancer diagnosis and treatment using nanoparticles should follow the following points: high uptake by cancer cells; no or less side effect; targeted delivery; contrast between cancer and healthy cells [13].

The SPIONs are composed of an iron oxide nanoparticle core, linked with an amine-functionalized PEG silane and a small peptide, chlorotoxin (CTX) for tumor cell-specific binding of the nanoparticle. The cellular uptake of CTX bound nanoparticle was higher (98%) than the CTX unbound nanoparticle (45%). The study showed deactivation of membrane bound matrix metalloproteinase 2 (MMP-2) and increased internalization of lipid rafts containing surface-expressed MMP-2. Increased activity of MMP-2 is associated with cancer of breast, colon, skin, lung, prostate, and ovaries; the use of CTX bound nanoparticle can be for noninvasive diagnosis and treatment [10]. SPIONs and doxorubicin were encapsulated into poly (D, L-lactic-co-glycolic acid) poly (ethylene glycol) (PLGA-PEG) for local treatment. The drug loading capacity was maximum for PLGA:PEG 4000 triblock copolymer in comparison to PLGA:PEG 2000 and PLGA:PEG 3000 triblock copolymers [11]. SPIONs as MR contrasting agent were used in targeted delivery to cancer cells. KB cells (a human nasopharyngeal epidermal carcinoma cell line expressing surface receptors for folic acid) were used as positive control and A549 cells (a human lung carcinoma cell line which lacks folate receptors) were used as negative control. Dextran-coated maghemite nanoparticles prepared through precipitation method was coated with *N*-hydroxysuccinimide-folate and fluorescence isothiocyanate (FITC). The study showed that 97.5% uptake of SPION by KB cells within 1 h. And in vivo study it was delivered to the tumor site with three time efficiency than nontumor site [12].

SPIONs linked with dextran and conjugated with bisphosphonate have been synthesized for osteoporosis treatment. Osteoporosis results due to higher rate of bone resorption by osteoclast than its formation by osteoblast. The idea is to reduce the activity of osteoclast cells by incorporating nanoparticles to it and their lysis by inducing heat [14]. It has been found that, Fe_3O_4 nanoparticles alter the intracellular ice formation that helps to improve freezing capability. This increases the efficiency to destroy the cancer tissues. This is very helpful in cryosurgery to destroy the capillaries at the tumor edges [15].

SPIONs coated with PEG and PEI polymers and conjugated with folic acid (FA) via EDC/NHS method to target doxorubicin loaded FA-SPION at cancer site is reported. The rate of doxorubicin release was high in low pH. In vivo and in vitro treatment with DOX@FA-SPIONs to MCF-7 cancer showed tumor inhibiting effect. Aggregation of nanoparticles was monitored by magnetic resonance imaging (MRI). The nanoparticle did not show any significant toxicity on liver, lung, kidney, and heart in mice as indicated by histological examinations [8]. The SPIONs were linked with glutamic acid and conjugated with hyaluronic acid (HA). The process includes oxidation of nanoparticle surface with H_2O_2, followed by activation of hydroxyl group and reacting with glutamic acid as intermediate group. This is fol-

lowed by linking of HA. The study showed high survival of cancer cells and increased uptake of HA-SPIONs compared to noninteracting agarose-coated SPIONs (AgA-SPIONs) [9].

5 SPIONs in Lung Cancer Imaging

Lung cancer is prevalent type of cancer in developed countries like the USA. Its survival rate is less than 15%. Cancer patient survival can be increased if it is detected and diagnosed early. Currently, surgical removal of localized cancer, radiation therapy, and chemotherapy are in use. Computed tomography (CT) and positron emission tomography (PET) are also used for the lung cancer diagnosis. But, CT is associated with false positive rates and PET has low spatial resolution. SPIONs have been used to increase the spatial resolution of MRI and as T2 contrasting agent. Several formulations are already used for the liver and gastrointestinal tract imaging. An approach has been developed to combat cancer by targeting a peptide, H2009. It was successfully attached with the SPIONs with the help of PEG-cysteine moiety. The developed SPIONs have shown specificity toward α v β 6 of human H2009 lung cancer cells. But, they did not show specificity to α v β 6-negative H460 control as indicated by Prussian blue staining and T 2-weighted MR imaging. The SPIONs coated with hydrophobic surfactants resulted into stable single monodispersed particles [16]. The use of bidentate chelation and disulfide cross-linking stabilizes the surface attachment of biological molecules.

Folate conjugated poly(ethylene glycol) (FA-PEG) was linked with aminosilane-immobilized SPIONs to form FA-PEG-SPIONs. Then it was labeled with Cy5.5 to form FA-PEG-SPIONs-Cy5.5. The study carried out in KB cells and lung cancer model showed receptor-mediated endocytosis in KB cells and strong optical imaging in lung cancer model mice [17]. EGFR-targeted, inhalable SPION nanoparticles were developed for non-small cell lung tumor ablation. The principle lies in the hyperthermia to generate heat by magnetic SPIONs. The result showed retention of SPION nanoparticles in the tumor which significantly inhibited in vivo lung tumor growth [18]. Cis-diamminedichloroplatinum (II) is a chemotherapeutic agent that has some side effects specially affecting kidney and its distribution is nonspecific. A magnetic CDDP-encapsulated nanocapsule (CDDP-PAA-NC) with CDDP-polyacrylic acid (PAA) core in amphiphilic polyvinyl alcohol/superparamagnetic iron oxide nanoparticles shell was developed which significantly reduce toxicity and exhibit anticancer activity in A549-tumor bearing mice with negligible side effects [19]. Endothelial progenitor cells (EPCs) can be utilized for cancer ablation and to repair vascular injury. EPCs were labeled with *N*-alkyl-polyethylenimine 2 kDa (PEI2k)-stabilized superparamagnetic iron oxide (SPIO) to facilitate magnetic resonance imaging (MRI) of EPCs in a mouse lung carcinoma xenograft model. It was injected intravenously and subcutaneously (mixed with A549 cells). The extension of labeled EPCs was found inside tumor cells, as identified by MRI. This showed excellent biocompatibility and MRI sensitivity [20]. A folate

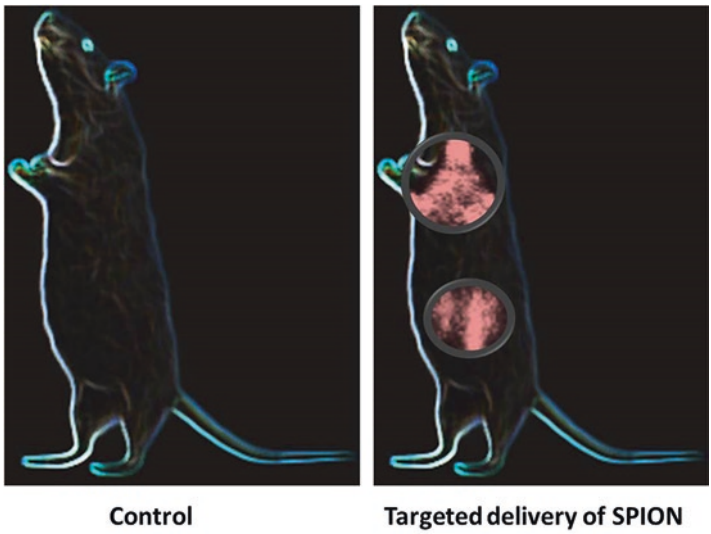


Fig. 8.2 Schematic of targeted delivery of SPIONs at lung cancer site and their subsequent accumulation in liver and kidney in mice

receptor-targeting multifunctional dual drug-loaded nanoparticle (MDNP) has been developed that contains a poly(*N*-isopropylacrylamide)-carboxymethyl chitosan shell and poly(lactic-co-glycolic) acid (PLGA) core for enhancing localized chemo-radiotherapy to effectively treat lung cancers. This showed controlled release of encapsulated therapeutic compounds (NU7441—a potent radiosensitizer, and gemcitabine). The study showed the use of nanovesicle in dual-mode simultaneous chemotherapy and radiation sensitization for lung cancer treatment [21]. EGFR targeted anticancer gene, plasmid-survivin/shRNA (pshRNA) albumin SPION complex were designed for the monoclonal antibody-dependent gene targeting in lung cancer therapy [22]. A schematic of targeted delivery of SPIONs in mice cancerous lung and their subsequent accumulation in liver and kidney is given in Fig. 8.2.

6 Conclusions

Magnetic resonance imaging plays a vital role in diagnosis of internal disease and injury. Because of their good contrast agent characteristics, SPIONs have become a means for MR imaging of tissues, cells and even of intracellular structures/molecules. The bioconjugation chemistry has pushed the boundaries of usability of SPIONs beyond molecular imaging for detection and quantification of genes, cell tracking, molecular and enzymatic interactions, etc. Effective imaging approaches such as MR, optical imaging using semiconductor quantum dots and nuclear are in

large demand because of the ongoing discovery of new class of genes and proteins in oncology. This requires study of ever-increasing genes, protein molecules and associated molecular pathways in order to provide diagnosis and treatment of cancer. However, the lack of techniques for effective, accurate, and noninvasive imaging of molecular interactions in biological systems prevents us to exploit the biological knowledge at full potential for the treatment of disease. With persistent advancement in SPIONs as molecular imaging probes in the areas like cost reduction, non-complex conjugation chemistry, enhanced target affinity, and as a means for MRS to minimize sequestration in lysosomes, the magnetite nanoparticles continue to display their significant potential as a means to probe further into biological systems.

References

1. Thorek, D. L., Chen, A. K., Czupryna, J., & Tsourkas, A. (2006). Superparamagnetic iron oxide nanoparticle probes for molecular imaging. *Annals of Biomedical Engineering, 34*(1), 23–38.
2. Huilan, Y., Gangyi, Z., Xiaohang, L., Congyang, L., Tingting, Z., Yanduo, T., Yanjun, K., Jiacheng, S., Yin, T., Bin, L., Xin, Z., & Gen, Z. (2016). Fe_3O_4 nanoparticles with ursolic methyl ester induce apoptosis of multidrug-resistant leukemia KA cell: In vitro evaluation. *Journal of Nanoscience and Nanotechnology, 16*(7), 7140–7144.
3. Xuhua, M., Zhanyun, R., Bin, H., Maomao, P., Haojie, L., Yina, D., Qiufan, X., Yihua, Z., Changliang, Z., Zhe, L., Yuxin, C., & Yanping, Z. (2016). Daunorubicin loaded Fe_3O_4 nanoparticles induce apoptosis of glioma cells and disrupt tight junction at blood–brain barrier. *Journal of Nanoscience and Nanotechnology, 16*(12), 12356–12361.
4. Gupta, J., Prakash, A., Jaiswal, M. K., Agarrwal, A., & Bahadur, D. (2018). Superparamagnetic iron oxide-reduced graphene oxide nanohybrid—A vehicle for targeted drug delivery and hyperthermia treatment of cancer. *Journal of Magnetism and Magnetic Materials, 448*, 332–338.
5. Ebrahimi, E., Khandaghi, A. A., Valipour, F., Babaie, S., Asghari, F., Motaali, S., Abbasi, E., Akbarzadeh, A., & Davaran, S. (2016). In vitro study and characterization of doxorubicin-loaded magnetic nanoparticles modified with biodegradable copolymers. *Artificial Cells, Nanomedicine, and Biotechnology, 44*(2), 550–558.
6. Mu, X., Zhang, F., Kong, C., Zhang, H., Zhang, W., Ge, R., Liu, Y., & Jiang, J. (2017). EGFR-targeted delivery of DOX-loaded Fe_3O_4@ polydopamine multifunctional nanocomposites for MRI and antitumor chemo-photothermal therapy. *International Journal of Nanomedicine, 12*, 2899–2911.
7. Zhang, Y., Wang, X. J., Guo, M., Yan, H. S., Wang, C. H., & Liu, K. L. (2014). Cisplatin-loaded polymer/magnetite composite nanoparticles as multifunctional therapeutic nanomedicine. *CJPS, 32*(10), 1329–1337.
8. Huang, Y., Mao, K., Zhang, B., & Zhao, Y. (2017). Superparamagnetic iron oxide nanoparticles conjugated with folic acid for dual target-specific drug delivery and MRI in cancer theranostics. *Materials Science and Engineering. C, Materials for Biological Applications, 70*, 763–771.
9. Yu, K. S., Lin, M. M., Lee, H. J., Tae, K. S., Kang, B. S., Lee, J. H., Lee, N. S., Jeong, Y. G., Han, S. Y., & Kim, D. K. (2016). Receptor-meditated endocytosis by hyaluronic acid@superparamagnetic nanovetor for targeting of CD44-overexpressing tumor cells. *Nanomaterials, 6*(8), 149.

10. Veiseh, O., Gunn, J. W., Kievit, F. M., Sun, C., Fang, C., Lee, J. S., & Zhang, M. (2009). Inhibition of tumor-cell invasion with chlorotoxin-bound superparamagnetic nanoparticles. *Small, 5*(2), 256–264.
11. Akbarzadeh, A., Mikaeili, H., Zarghami, N., Mohammad, R., Barkhordari, A., & Davaran, S. (2012). Preparation and in vitro evaluation of doxorubicin-loaded Fe_3O_4 magnetic nanoparticles modified with biocompatible copolymers. *International Journal of Nanomedicine, 7*, 511–526.
12. Choi, H., Choi, S. R., Zhou, R., Kung, H. F., & Chen, I. W. (2004). Iron oxide nanoparticles as magnetic resonance contrast agent for tumor imaging via folate receptor-targeted delivery. *Academic Radiology, 11*(9), 996–1004.
13. Pernal, S., Wu, V. M., & Uskoković, V. (2017). Hydroxyapatite as a vehicle for the selective effect of superparamagnetic iron oxide nanoparticles against human glioblastoma cells. *ACS Applied Materials & Interfaces, 9*(45), 39283–39302.
14. Lee, M. S., Su, C. M., Yeh, J. C., Wu, P. R., Tsai, T. Y., & Lou, S. L. (2016). Synthesis of composite magnetic nanoparticles Fe_3O_4 with alendronate for osteoporosis treatment. *International Journal of Nanomedicine, 11*, 4583–4594.
15. Ye, P., Kong, Y., Chen, X., Li, W., Liu, D., Xie, Y., Zhou, Y., Zou, H., Chang, Z., Dai, H., Kong, X., & Liu, P. (2017). Fe_3O_4 nanoparticles and cryoablation enhance ice crystal formation to improve the efficiency of killing breast cancer cells. *Oncotarget, 8*(7), 11389–11399.
16. Huang, G., Zhang, C., Li, S., Khemtong, C., Yang, S. G., Tian, R., Minna, J. D., Brown, K. C., & Gao, J. A. (2009). Novel strategy for surface modification of superparamagnetic iron oxide nanoparticles for lung cancer imaging. *Journal of Materials Chemistry, 19*, 6367–6372.
17. Yoo, M. K., Park, I. K., Lim, H. T., Lee, S. J., Jiang, H. L., Kim, Y. K., Choi, Y. J., Cho, M. H., & Cho, C. S. (2012). Folate-PEG-superparamagnetic iron oxide nanoparticles for lung cancer imaging. *Acta Biomaterialia, 8*(8), 3005–3013.
18. Sadhukha, T., Wiedmann, T. S., & Panyam, J. (2013). Inhalable magnetic nanoparticles for targeted hyperthermia in lung cancer therapy. *Biomaterials, 34*(21), 5163–5171.
19. Chiang, C. S., Tseng, Y. H., Liao, B. J., & Chen, S. Y. (2015). Magnetically targeted nanocapsules for PAA-cisplatin-conjugated cores in PVA/SPIO shells via surfactant-free emulsion for reduced nephrotoxicity and enhanced lung cancer therapy. *Advanced Healthcare Materials, 4*(7), 1066–1075.
20. Chen, C., Yu, H., Xia, R., Wang, L., Ai, H., Liu, S., Xu, Z., Xiao, X., & Gao, F. (2014). Magnetic resonance tracking of endothelial progenitor cells labeled with alkyl-polyethylenimine 2 kDa/superparamagnetic iron oxide in a mouse lung carcinoma xenograft model. *Molecular Imaging, 13*. https://doi.org/10.2310/7290.2014.00030.
21. Menon, J. U., Kuriakose, A., Iyer, R., Hernandez, E., Gandee, L., Zhang, S., Takahashi, M., Zhang, Z., Saha, D., & Nguyen, K. T. (2017). Dual-drug containing core-shell nanoparticles for lung cancer therapy. *Scientific Reports, 7*, 13249.
22. Hou, X., Zhang, H., Li, H., & Zhang, D. (2016). Magnetic albumin immuno-nanospheres as an efficient gene delivery system for a potential use in lung cancer: Preparation, in vitro targeting and biological effect analysis. *Journal of Drug Targeting, 24*(3), 247–256.

Chapter 9
Biodegradable Nanoparticles for Drug Delivery and Targeting

Viroj Wiwanitkit

Abstract The application of nano-biotechnology in pharmacology is very interesting. The nano-biotechnology can be applied in many steps including drug delivery and targeting. Several new nanoparticles are developed to serve those purposes. In this specific chapter, the author will focus on the biodegradable nanoparticles which can be automatic degraded. The application of biodegradable nanoparticles for drug delivery and targeting will be summarized and presented in this article. Summary on important reports on this topic is also provided in this article.

Keywords Nano-biotechnology · Pharmacology · Biodegradable nanoparticle Drug · Delivery · Targeting

1 Introduction

Nano-biotechnology is the novel biotechnology dealing with extremely small scale at nano-level (10^{-9}). The application of nano-biotechnology in pharmacology is very interesting. The basic concept is based on the biophysical property of the nanomaterials. In general, nanomaterials are usually very small. The extremely small size, at nanoscale, results in several specific properties of nanoparticles. The increased interaction surface and change in biophysical properties (such as electrical charge) can be observed. In addition, the extremely small size also implies the increased chance for passing to the passage. It is no doubt that the nanoparticles can pass or penetrate thorough many cellular pores and enter into the intracellular environment. There are many early evidences in nano-medicine studies confirming that nanoparticle can enter into several human cells such as leukocyte [1], lymphocyte

V. Wiwanitkit (✉)
DY Patil University, Pune, India

Joseph Ayobabalola University, Ilara-Mokin, Nigeria

Faculty of Medicine, University of Nis, Nis, Serbia

Hainan Medical University, Haikou, China

Y. V Pathak (ed.), *Surface Modification of Nanoparticles for Targeted Drug Delivery*, https://doi.org/10.1007/978-3-030-06115-9_9

[2], or renal cells [3]. As a consequence, the change of the mentioned cells after the invasion of the nanoparticle is also observable [1–3].

This means the nanoparticles can have effect within cells. Hence, if the nanoparticle is a kind of drug having pharmacological properties, it can be used as a very effective drug for management of illness in medicine. To achieve this purpose, the construction of the drug at the nano-level is the important step. This becomes the new area for pharmacological research and development. In general, the nanobiotechnology can be applied in many steps including drug delivery and targeting. Several new nanoparticles are developed to serve those purposes. In this specific chapter, the author will focus on the biodegradable nanoparticles which can be automatic degraded. The application of biodegradable nanoparticles for drug delivery and targeting will be summarized and presented in this article. Summary on important reports on this topic is also provided in this article.

2 How Can a Biodegradable Nanoparticle Act as Drug and Has Pharmacological Effect?

An important basic question in nano-medicine is "How can a nanoparticle be used as drug?" This question might be answered according to the already mentioned concept. First, there must be the drug molecule. This means there must be a specific nanoparticle that posed specific biochemical property that can be useful in disorder prevention or management. Then that specific nanoparticle will be called a nanodrug. The nanodrug usually has a very small size, and this very small molecular usually has more effectiveness in acting on cells. The second step that requires for completing the usage of nanoparticle as drug is the administration of the drug into the body. In general pharmacology, drug will not be useful if it is not administered into the body. For usage in human, there are many ways for administrations of drug such as intake by ingestion, inhalation, absorption via skin after dermatological application, absorption after dropping into the eye or ear, suppository into anal or vaginal canals, implantation, or injection (into blood vessel, subcutaneous fat, muscle, vertebral canal, or ocular cavity). This is also the necessary steps for using nanodrug. Nanodrug will be useless if it is not administered into the body. Hence, an important consideration of any newly developed nanodrug is the feasibility of administration. The specific method of administration of different drug is usually different. Focusing on nanodrug, the administration is usually not problematic. Due to the fact that nanodrug is very small, it can naturally be absorbed via normal skin barrier or directly inhaled and passed thorough lung alveoli.

The next step is similar to other drugs. The concern is usually on the bioavailability, pharmacostatics, pharmacokinetics, and pharmacodynamics. The general considerations on the newly available nanodrugs are on drug distribution and drug entering into the target cells. This is according to standard concept in pharmacology. A good nanodrug must be easily administered, absorbed, delivered into the target

cell, and induced desired pharmacological reaction. The described simple principles are applicable for any nanodrug including the specific nanodrug that is produced from biodegradable nanomaterial.

3 What Is a Biodegradable Nanomaterial?

Before, a further discussion on using biodegradable nanodrug, the details about degradable nanomaterial should be mentioned. Generally, degradable substance means a substance that can be degraded. The degrading can result in change of molecule. Erosion of the molecular structure during degradation and change of molecular property occur. Change in size, change and strength occur during degradation process. Finally, the complete disappearance of the molecule might be expected. A nanomaterial that can be degraded will be called a degradable nanomaterial. Automatic degradation is the expected property in using any degradable substance including to degradable nanosubstance. As already mentioned, the degradable process is similar to the catabolism in metabolic pathway. It will result in downing of the molecule and can finally result in ending of existence. Hence, the degradable nanomaterial will disappear without leftover; hence, it might be considered environmental-friendly substance.

There are many ways that a substance can undergo degradation. The mechanical degradation, the chemical degradation, or the biological degradation are the common types of degradation. The nanomaterials might be degraded by one the mentioned type of degradation. Nevertheless, in biological condition in the body, the spontaneous biological degradation is the most preferable type of degradation. This process is termed biodegradation. In biomedicine, biodegradation is a useful process that helps biotransform, recycle, and detoxify; hence, the substance with biodegradable property is more preferable than that one without this property. The biodegradation might be generated by enzymatic system within the body or by the microbial (such as bacteria and fungi) reaction. The proper might be aerobic and anaerobic condition, and it might occur intracellularly or extracellularly. In pharmacological use of nanomaterial, the autobiodegradation by the intracellular enzymatic process is the most preferable biodegradation. In cellular level, the interference of external factors such as temperature, water, or oxygen level is usually controlled, and the smooth biodegradation bioprocess can occur. This specific degradation is considered safe and does usually not induce any unwanted adverse effect.

The important consideration of biological degradation of a nanodrug is the time and place that the biodegradation occurs. A too early or tool late biodegradation is not preferable. In addition, the biodegradation must occur at the mot proper place, the target pathological site for allowing pharmacological reaction against the medical problem.

The degradability of the nanomaterial might be helpful in controlling of the releasing of the nanomaterial. In case that the nanodrug is designed in shell form, the degradability of the external shell core can be useful in controlling of the

releasing of the druggable part inside [4]. This is a very useful concept for new nanodrug design. The use of degradable nanocarrier is proven helpful in pharmaceutical process. How to prepare a good biodegradable nanomaterial is a basic question in nano-biotechnology. There are many factors that affect the preparation of degradable nanomaterials. Those factors include size of desired resulted nanoparticle, functionality and surface properties, releasing property of the outcome product, biocompatibility and degradability degree, and delivery material property [5].

4 Preparation of Biodegradable Nanomaterials for Use as Nanodrug

In preparation of degradable nanomaterials should focus on absorption, pharmacokinetics, and disposition [5–8]. The good absorption is usually the target. The preparation should result in the outcome with a favorable pharmacokinetics properties [5–8]. A less mentioned consideration in biodegradable nanomaterial preparation is on the toxicity. Jian et al. noted that biodegradable nanoscale preparations were commonly done at present, and there should be the concern on the toxicity [9]. The toxicity in nanoscale preparations is possible and strongly related to the preparation methodology [9]. Jian et al. concluded that there very many factors determining the toxicity of the biodegradable nanoscale preparations including to the particle size, shape, and surface structure [9]. The examples of the presently widely prepared degradable nanomaterials are liposome, micelle, and solid lipid nanoparticle (SLN) (Table 9.1).

Table 9.1 Some widely prepared degradable nanomaterials and details regarding preparation

Nanomaterials	Details
Liposome	Liposome is widely used nanomaterial at present. A liposome molecule has spherical shape and appears as a vesicle with at least one lipid bilayer. A liposome has a hydrophilic head group and hydrophobic hydrocarbon tail, hence, presents both hydrophobic and hydrophilic properties [10]. This bi-property helps ease distribution of liposome. It is considered as a useful soft nanocarrier. Barani and Montazer noted that the best character of liposomes was energy saving due to reduction in temperature of process [10]. Also, I considered the liposome environmental friendly since it is a degradable nanomaterial
SLN	SLN has a sufficient affinity with the biomembranes that can improve absorption by several administration routes (oral, transdermal, pulmonary, nasal, ocular, rectal, etc.) [8]. Qi et al. concluded that SLN was food for colloidal drug delivery systems due to the fact that SLN posed the nature of both the “soft” carriers (such as emulsions and liposomes) and non-soft polymeric nanoparticles [8]. Qi et al. noted that an oral SLN could enhance lymphatic absorption by either the chylomicron-association pathway or the M cell uptaking pathway [8]

As earlier mentioned, the preparation of biodegradable is complex and has many considerations during preparation. Due to the desired property, degradability, several biodegradable nanomaterials are produced and used in pharmacology at present. Dhiman et al. noted that different preparation techniques for biodegradable nanomaterials have different advantages and disadvantages [11]. Dhiman et al. found that biodegradable polymer usually had uncertainty in the absorption pathway in gastrointestinal tract [11]. Dhiman et al. also noted that there might be some harmful toxic by-products after metabolism occurred [11]. Dhiman et al. suggested that the use of synthetic or semisynthetic polymeric nanoparticles which had a defined structure might resolve the problem due to the molecule that was still in intact form till absorption in gastrointestinal tract [11]. Concerning the toxicity, the preparation of degradable nanomaterials with lipid composition might help decrease then problem [12]. The lipid nanoparticle is usually considered bioacceptable, and the high biodegradable nature results in little toxicity [12].

There are many interesting reports on preparation of biodegradable nanomaterials for using as nanodrugs. The important reports will be hereby summarized.

(a) Reports on in Oncology

The development of new nanodrug usually aims at management of presently untreatable diseases. The common focus is usually malignancy. The development of the degradable nanomaterial for management of cancer is widely done at present. Several newly developed biodegradables for use as new nanodrugs in oncology are proposed. For example, Cerqueira et al. developed biodegradable poly-lactic-co-glycolic acid (PLGA) nanoparticles surface engineered with hyaluronic acid for targeted delivery of paclitaxel to triple negative breast cancer cells [13]. Cerqueira et al. found that HA–PLGA nanoparticles could increase cellular uptake, when compared to non-coated PLGA nanoparticles, due to interaction of HA with CD44 receptors that result in a receptor-mediated endocytosis [13]. In another report by Zhang et al., the effect of autophagy inhibitors on drug delivery using biodegradable polymer nanoparticles in cancer treatment was investigated [14]. Zhang et al. found a new a new biological mechanism of malignant cells to have PLGA NPs captured and degraded by auto-lysosomes [14]. In another study, Kapoor et al. studied on intracellular delivery of peptide cargos using polyhydroxybutyrate-based biodegradable nanoparticles [15]. In this work, antitumor efficacy of BCL-2 converting peptide, NuBCP-9 was assessed, and it was concluded that this formulation might be a good alternative for tumor management [15]. In another recent publication, Aluri and Jayakannan reported on development of L-tyrosine-based enzyme-responsive amphiphilic poly(ester-urethane) nanocarriers for multiple drug delivery to neoplastic cells [16]. Aluri and Jayakannan mentioned for the specific advantage of this formulation that the drug-loaded L-tyrosine nanoparticles were stable extracellularly but enzymatic-biodegradable intracellular, which results in specific targeting on intracellular releasing of the drugs [16].

Based on the given examples, it might conclude that the biodegradable nanomaterials are effective option for cancer management. As concluded by

Zhao et al. [17], the biodegradable polymeric nanoparticles could be effectively used as a nanodrug delivery carrier [17]. The art on design of nanopolymer is the important determinant for the efficacy of the antitumor nanomolecule.

(b) Reports on in Ophthalmology

There are also some interesting reports on preparation of biodegradable nanomaterials as new nanodrugs in ophthalmology. For example, Bisht et al. reported on using nanoparticle-loaded biodegradable light-responsive in situ forming injectable implants for effective drug delivery to the posterior segment of the eye [18]. This study can confirm the usefulness of the degradable nanosubstance for help access to the hard-to-access part within the body. In another article, Salama et al. reported on success in using PLGA nanoparticles as subconjunctival injection for management of glaucoma in rabbit model [19]. In another report, Prakash and Dhesingh reported the success in using nanoparticle-modified drug-loaded biodegradable polymeric contact lenses for sustainable ocular drug delivery [20]. Finally, Salehi et al. recently reported the use of poly (glycerol sebacate)-poly (ε-caprolactone) (PEG–PCL) blend nanofibrous scaffold as intrinsic bio- and immunocompatible system for corneal repair [21]. These reports also confirm the usefulness of biodegradable nanomaterials in ocular disease management. Similar to the case of oncology, designing of a good nanopolymer is the important determinant for the success.

(c) Reports on in Neurology

There are also some interesting reports on preparation of biodegradable nanomaterials as new nanodrugs in neurology. For example, Mastorakos et al. reported on treatment of malignant brain tumor by biodegradable brain-penetrating DNA nanocomplexes [22]. This application is the same as the already mentioned one in the topic of clinical oncology. Ruan et al. reported another development on matrix metalloproteinase triggered size-shrinkable gelatin-gold fabricated nanoparticles for tumor microenvironment-sensitive penetration and diagnosis of glioma [23]. Similarly, this application is also the same as the already mentioned one in the topic of clinical oncology.

In fact, the application in non-oncology neurology is also reported in the medical literature. The good example is the report by Bi et al. on intranasal delivery of rotigotine to the brain with lactoferrin-modified PEG–PLGA nanoparticles for Parkinson's disease treatment [24]. The success use of same degradable nanomaterials in management of Parkinson's disease is also reported in another study by Hu et al. [25]. Focusing on another important neurodegenerative disorder, Alzheimer's disease, the role of degradable nanomaterials as nanodrugs is also reported [26–28]. A recent report by Sánchez-López et al. is the good example [26]. Sánchez-López et al. proposed for using pegylated biodegradable dexibuprofen nanospheres administration to APPswe/PS1dE9 as new potential strategies for Alzheimer's disease prevention [26]. Herran et al. also reported the observation on enhanced hippocampal neurogenesis in APP/Ps1 mouse model of Alzheimer's disease after implantation of VEGF-loaded PLGA nanospheres which implies the possible role of the mentioned nanomaterial in treatment of

Alzheimer's disease [27]. Finally, the development of PLGA-functionalized quercetin nanoparticles as possible effective nanomaterials for management of Alzheimer's disease is also reported by Sun et al. [28].

In fact, the use of degradable nanomaterials for management of degenerative neurological disorder is the new concept in clinical neurology. It is also the new hope to use degradable nanomaterials combining with gene therapy as an effective novel treatment of the previously untreatable neurodegenerative disorder [29, 30].

(d) Reports on in HIV Medicine

Human immunodeficiency virus (HIV) infection is still the global public health problem at present. There is still no effective treatment that can cure HIV infection. The use of nanomedical technology is the new hope for management of HIV. There are some interesting reports on using degradable nanomaterials in HIV medicine. For example, Mohideen et al. reported on development of degradable bioadhesive nanoparticles for prolonged intravaginal delivery and retention of elvitegravir [31]. In another study, Caizhen et al. reported on using zirconium phosphatidylcholine-based nanocapsules as an in vivo degradable drug delivery system of a momordica anti-HIV protein, MAP 30 [32].

(e) Reports on in Obstetrics and Gynecology

There are also some interesting reports on preparation of biodegradable nanomaterials as new nanodrugs in obstetrics and gynecology. For example, Luo et al. observed the efficient inhibition of ovarian cancer by degradable nanoparticle-delivered survivin T34A gene [33]. This application is also the same as the already mentioned one in the topic of clinical oncology. For non-oncology problem, the use of degradable nanomaterials to promote the efficacy of anti-HIV drug in HIV-infected mother as earlier mentioned is a good example.

(f) Reports on Infectious Medicine

There are also some interesting reports on preparation of biodegradable nanomaterials as new nanodrugs in infectious medicine. Apart from the already mentioned reports in HIV infection, the good example is the report by Zhang et al. Zhang et al. reported on the use of degradable polyphosphoester-based silver-loaded nanoparticles as therapeutics for bacterial lung infections in complicated case with underlying cystic fibrosis [34]. In another study, Wang et al. reported the development of an anti-infection nano-hydroxyapatite drug delivery microsphere and its drug release in vitro [35]. Wang et al. found favorable drug release and observed good efficacy of the nanoformulation in management of osteomyelitis, the deep bone infection [35].

Based on the mentioned application in clinical medicine, it can summarize that the present trend of using degradable nanomaterial preparation is for the management of the disease that is non-curable by presently available method or management of the pathology sit at hard-to-access position. The complex biodegradable is usually designed and prepared to serve the different specific needs in treatment of different clinical problems. This is the specific property of polymeric nanocomplex

that is superior to moneric nanocomplex in using as nanodrugs for management of clinical disorder in medicine. The use of advance nanotechnology for preparation or nanoformation of the drug is widely studied in the present day. Several new techniques are developed. The good example is the nanoprecipitation and nanoencapsulation techniques that help assemble the hydrophobic and hydrophilic compartments within molecule to form the final biodegradable nanomaterial product [36].

5 Drug Delivery by Biodegradable Nanoparticles

Drug delivery is an important step that determines the success of pharmacotherapy. The effective drug delivery is required for any treatment. Delivery of the drug should be focused since it is administered into the body until it reaches the target site. The issue on drug delivery by biodegradable nanomaterial substance is very interesting. The smooth delivery without any loss on the way transporting to the target site is the ideal concept. To achieve the successful delivery, the design of good nanodrug complex is very important. The good designed nanodrug should be small, easily transported in vivo, resistant to external insult during transportation, and nontoxic to the in vivo environment during the in vivo biotransportation process to the pharmacological target. The confirmation for these desirable properties of any newly developed nanomaterials is required.

The nanoformulation that is widely used for drug delivery includes the use of nanodisks, high-density lipoprotein (HDL) nanostructures, liposomes, and gold nanoparticles [37]. With the advancement in nanomaterial complex design, the next important step of modification in drug delivery is the integrating of other therapeutic stimulation systems such as such as triggered release, multicomponent therapies, theranostics, or gene delivery system [38]. The apparent advantages of nanoparticles for drug delivery are ability to carry delivery of water-insoluble substances, ability to target the pathological site, ability to co-deliver more than one drug for pharmacological combination therapy, and visualization of the drug delivery site by combining nanoimaging system [36]. Hence, the use of the nanodrug delivery system technology can increase the effectiveness of drug transportation to the pharmacological target with reduction on the loss of drug during the transportation pathway and can help solve the problem of multidrug resistance and increase drug targeting, which means increased efficacy and effectiveness of pharmacological treatment [37]. The research and development of the new nanocompositions and nanoformulation technology is useful for further development of new modalities for management of several untreatable medical disorders such as cancerous diseases, neurodegenerative disease, and HIV infection (Table 9.2).

Table 9.2 Some widely used compositions of nanomaterial polymer for drug delivery

Compositions	Details
PEG–PCL [38]	PEG–PCL can help increase drug accumulation at the site of therapeutic action; therefore, PEG–PCL use results in decreased off-target effects [38]. The example of drug delivery that this system is used is the delivery of doxorubicin [39]
PCL–PEG–PCL [40]	PCL–PEG–PCL is an important composition for forming core cross-linked micelle for drug delivery [40]. The example of drug delivery that this system is used is the delivery of doxorubicin [40] and honokiol [41]
PEG–PCL–PEG [40]	PEG–PCL–PEG or PECE is another important composition for forming core cross-linked micelle for drug delivery [40]. PECE is proven safe and can be used intravenously [40]. The PECE becomes the new composition for the new anticancerous drug development at present [40]
PEG-block-poly(D,L-lactic acid) (PEG-*b*-PLA) [42]	PEG-block-poly(D,L-lactic acid) (PEG-*b*-PLA) is another important composition for forming core cross-linked micelle for drug delivery aiming at transportation of poorly water-soluble drug [42]. It is presently used for development of anticancerous drug [42]
Poly(D,L-lactic-co-glycolic acid)-block-PEG-block-poly(D,L-lactic-co-glycolic acid) (PLGA-*b*-PEG-*b*-PLGA) [42]	PLGA-*b*-PEG-*b*-PLGA is another important composition for forming core cross-linked micelle for drug delivery aiming at transportation of both poorly water-soluble and water-soluble drugs [42]. The ability to deliver both water-soluble and water-non-soluble drug makes PLGA-*b*-PEG-*b*-PLGA superior to PEG-*b*-PLA [42, 43]. It is presently used for development of anticancerous drug [42]
Poly-lactic acid–PEG (PLA–PEG) [44]	Similar to PEG–PLC, PLA-PEG is another important composition that can help increase drug accumulation at the site of therapeutic action; therefore, PLA–PCL use results in decreased off-target effects [44]
PLA-D-α-tocopheryl polyethylene glycol 1000 succinate (PLA–TPGS) [44]	PLA–TPGS or PLA–vitamin E is another important composition that can help increase drug accumulation at the site of therapeutic action; therefore, PLA–TPGS use results in decreased off-target effects [44]
PCL–TPGS [45]	PCL–TPGS is another important composition that is under study regarding its efficacy in increased cytotoxic effect to the target malignant cells [45]

6 Targeting by Biodegradable Nanoparticles

The important step that determines the success of pharmacotherapy is the attacking to the problematic site. The ideal aim is the specific correctly hit to the pathological site. This means there must be both precision and accuracy in treatment. The targeting becomes the new concept in modern pharmacotherapy. Using the nanomedical technology, increasing accuracy and specificity of drug management can be expected.

The last step for finalizing the overall process is the biodegradation of the nanodrug within the target cell. The good nanodrug must be auto-degraded when it reaches the final destination in the pathological site. The favorable environmental-friendly degradation process is the biological process. This is usually a simple biochemical enzymatic reaction that occurs intracellularly. The biodegradation process should be simple, easily to occur and cause no toxic by-product or problematic adverse consequence. With the use of the new technology, the success for approach to hard-to-access areas such as central nervous system [12] and intraarticular area [46] is possible. The feasibility of approach of those mentioned site is usually due to the lipid composition of the newly designed biodegradable nanomaterials [12, 46]. The technology can help target and manage of difficult-to-treat medical diseases such as malignancies [47] and intracellular infections (e.g., toxoplasmosis [48]).

7 Presently Available Degradable Nanodrugs in Clinical Medicine

As already mentioned, the development of biodegradable nanomaterials as nanodrugs is useful in clinical medicine. Several attempts on clinical trials have been done for a few years, and there are already some success and available nanodrugs.

(a) Liposomal Amphotericin B

Liposomal amphotericin B is presently available antiparasitic drug that is indicated for treatment of leishmaniasis [49]. This drug is based on liposomal nanoformulation, which is a well-known technique for construction of biodegradable nanomaterials [50]. Carrillo-Muñoz et al. performed a comparative study and found that the liposomal amphotericin B was more effective than classical drug and increased the success in drug targeting [51].

(b) Liposomal Doxorubicin

Liposomal doxorubicin is presently available anticancerous drug that was recently developed based on liposomal technology [52]. Similar to liposomal amphotericin B, liposomal doxorubicin is based on liposomal nanoformulation, which is a well-known technique for construction of biodegradable nanomaterials [52]. This drug is presently available and commercially known as Caelyx(®)/Doxil(®). In clinical trial, it was proven and found that the liposomal doxorubicin was more effective than classical drug and increased the success in drug targeting [53].

(c) Liposomal Bupivacaine

Liposomal bupivacaine is presently available drug for anesthesiology purpose that was recently developed based on liposomal technology [54]. Similar to liposomal amphotericin B and liposomal doxorubicin, liposomal bupivacaine is based on liposomal nanoformulation, which is a well-known technique for construction of biodegradable nanomaterials. This drug is presently available and commercially known as Exparel(®). In clinical trial, it was proven that

found that the liposomal doxorubicin was more effective than classical drug and increased the success in drug targeting [54, 55]. However, the clinical trial showed an important adverse effect, cardiac side effect, of the new liposomal bupivacaine [55]. Viscusi et al. found that the side effect was observable in less than 1% and usually mild [55]. Nevertheless, this finding indicates the need for further improvement of the presently available bupivacaine drug.

8 Conclusion

The biodegradable nanomaterials can be used as nanodrugs for management of several medical problems. The design of biodegradable nanomaterials has to be carefully done, and there must be the concern on the desired product and possible toxicity. The effectivity of the nanoformulation starts from the nanodrug design thorough the nanodrug administration, nanodrug delivery, targeting, and pharmacological acting. The nanodrug becomes the hope for management of incurable disease (such as cancers) and effective approach to hard-to-access pathological sites (such as the brain, intraocular space, intraarticular space). The good biodegradable nanomaterials should be stable extracellular before reaching the final target and easily biologically degraded intracellularly at the target site. Several nanoformulations can be used for preparation of biodegradable nanomaterials. Different compositions of the new nanopolymer complexes are developed and proven differences in advantages. Some newly developed biodegradable nanomaterials are already registered as new commercially available drug for use in clinical practice [56]. Although there are many reports on the development and use of biodegradable nanomaterials in clinical medicine, further researches and developments in this area are still required. Finally, the diagram presenting biodegradable nanomaterials as nanodrugs for management of medical disorder in clinical medicine is shown in Fig. 9.1.

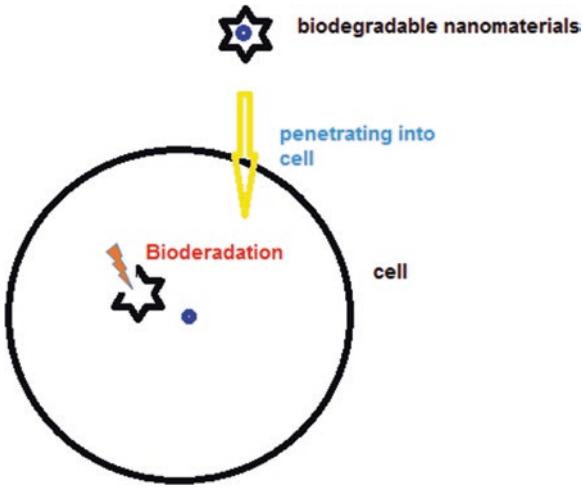


Fig. 9.1 The diagram presenting biodegradable nanomaterials as nanodrugs for management of medical disorder in clinical medicine (graphic drawing by Wiwanitkit V, 2018)

Conflict of Interest None.

References

1. Wiwanitkit, V., Sereemaspun, A., & Rojanathanes, R. (2009). Effect of gold nanoparticle on the microscopic morphology of white blood cell. *Cytopathology, 20*(2), 109–110.
2. Wiwanitkit, V., Sereemaspun, A., & Rojanathanes, R. (2009). Identification of gold nanoparticle in lymphocytes: A confirmation of direct intracellular penetration effect. *Turkish Journal of Haematology, 26*(1), 29–30.
3. Sereemaspun, A., Rojanathanes, R., & Wiwanitkit, V. (2008). Effect of gold nanoparticle on renal cell: An implication for exposure risk. *Renal Failure, 30*(3), 323–325.
4. Wan, W. K., Yang, L., & Padavan, D. T. (2007). Use of degradable and nondegradable nanomaterials for controlled release. *Nanomedicine (London, England), 2*(4), 483–509.
5. Wang, C., Wang, J., Chen, T., Luo, Z., Yang, X., Pan, X., et al. (2012). Absorption, pharmacokinetics and disposition of biodegradable nanoscale preparations. *Current Drug Metabolism, 13*(4), 429–439.
6. Lin, Y., & Qian, Z. (2012). Absorption, pharmacokinetics and disposition of biodegradable nanoscale preparations. *Current Drug Metabolism, 13*(4), 337.
7. Lu, Y., Qi, J., & Wu, W. (2012). Absorption, disposition and pharmacokinetics of nanoemulsions. *Current Drug Metabolism, 13*(4), 396–417.
8. Qi, J., Lu, Y., & Wu, W. (2012). Absorption, disposition and pharmacokinetics of solid lipid nanoparticles. *Current Drug Metabolism, 13*(4), 418–428.
9. Jian, F., Zhang, Y., Wang, J., Ba, K., Mao, R., Lai, W., et al. (2012). Toxicity of biodegradable nanoscale preparations. *Current Drug Metabolism, 13*(4), 440–446.
10. Barani, H., & Montazer, M. (2008). A review on applications of liposomes in textile processing. *Journal of Liposome Research, 18*(3), 249–262.
11. Dhiman, B., Divtrannum, D. A., & Saini, S. (2017). An appraisal on various methods of nano particulate formulations. *Pharmaceutical Nanotechnology, 5*(4), 255–262.
12. Shankar, R., Joshi, M., & Pathak, K. (2018, June 10). Lipid nanoparticles: A novel approach for brain targeting. *Pharmaceutical Nanotechnology, 6*(2), 81–93. https://doi.org/10.2174/2211738506666180611100416. [Epub ahead of print].
13. Cerqueira, B. B. S., Lasham, A., Shelling, A. N., & Al-Kassas, R. (2017). Development of biodegradable PLGA nanoparticles surface engineered with hyaluronic acid for targeted delivery of paclitaxel to triple negative breast cancer cells. *Materials Science and Engineering. C, Materials for Biological Applications, 76*, 593–600.
14. Zhang, X., Dong, Y., Zeng, X., Liang, X., Li, X., Tao, W., et al. (2014). The effect of autophagy inhibitors on drug delivery using biodegradable polymer nanoparticles in cancer treatment. *Biomaterials, 35*(6), 1932–1943.
15. Kapoor, S., Gupta, D., Kumar, M., Sharma, S., Gupta, A. K., Misro, M. M., et al. (2016). Intracellular delivery of peptide cargos using polyhydroxybutyrate based biodegradable nanoparticles: Studies on antitumor efficacy of BCL-2 converting peptide, NuBCP-9. *International Journal of Pharmaceutics, 511*(2), 876–889.
16. Aluri, R., & Jayakannan, M. (2017). Development of l-tyrosine-based enzyme-responsive amphiphilic poly(ester-urethane) nanocarriers for multiple drug delivery to cancer cells. *Biomacromolecules, 18*(1), 189–200.
17. Zhao, K., Li, D., Shi, C., Ma, X., Rong, G., Kang, H., et al. (2016). Biodegradable polymeric nanoparticles as the delivery carrier for drug. *Current Drug Delivery, 13*(4), 494–499.
18. Bisht, R., Jaiswal, J. K., & Rupenthal, I. D. (2017). Nanoparticle-loaded biodegradable light-responsive in situ forming injectable implants for effective peptide delivery to the posterior segment of the eye. *Medical Hypotheses, 103*, 5–9.

19. Salama, H. A., Ghorab, M., Mahmoud, A. A., & Abdel Hady, M. (2017). PLGA nanoparticles as subconjunctival injection for management of glaucoma. *AAPS PharmSciTech, 18*(7), 2517–2528.
20. Prakash, M., & Dhesingh, R. S. (2017). Nanoparticle modified drug loaded biodegradable polymeric contact lenses for sustainable ocular drug delivery. *Current Drug Delivery, 14*(4), 555–565.
21. Salehi, S., Czugala, M., Stafiej, P., Fathi, M., Bahners, T., Gutmann, J. S., et al. (2017). Poly (glycerol sebacate)-poly (ε-caprolactone) blend nanofibrous scaffold as intrinsic bio- and immunocompatible system for corneal repair. *Acta Biomaterialia, 50*, 370–380.
22. Mastorakos, P., Zhang, C., Song, E., Kim, Y. E., Park, H. W., Berry, S., et al. (2017). Biodegradable brain-penetrating DNA nanocomplexes and their use to treat malignant brain tumors. *Journal of Controlled Release, 262*, 37–46.
23. Ruan, S., He, Q., & Gao, H. (2015). Matrix metalloproteinase triggered size-shrinkable gelatin-gold fabricated nanoparticles for tumor microenvironment sensitive penetration and diagnosis of glioma. *Nanoscale, 7*(21), 9487–9496.
24. Bi, C., Wang, A., Chu, Y., Liu, S., Mu, H., Liu, W., et al. (2016). Intranasal delivery of rotigotine to the brain with lactoferrin-modified PEG-PLGA nanoparticles for Parkinson's disease treatment. *International Journal of Nanomedicine, 11*, 6547–6559.
25. Hu, K., Shi, Y., Jiang, W., Han, J., Huang, S., & Jiang, X. (2011). Lactoferrin conjugated PEG-PLGA nanoparticles for brain delivery: Preparation, characterization and efficacy in Parkinson's disease. *International Journal of Pharmaceutics, 415*(1–2), 273–283.
26. Sánchez-López, E., Ettcheto, M., Egea, M. A., Espina, M., Calpena, A. C., Folch, J., et al. (2017). New potential strategies for Alzheimer's disease prevention: Pegylated biodegradable dexibuprofen nanospheres administration to APPswe/PS1dE9. *Nanomedicine, 13*(3), 1171–1182.
27. Herran, E., Perez-Gonzalez, R., Igartua, M., Pedraz, J. L., Carro, E., & Hernandez, R. M. (2015). Enhanced hippocampal neurogenesis in APP/Ps1 mouse model of Alzheimer's disease after implantation of VEGF-loaded PLGA nanospheres. *Current Alzheimer Research, 12*(10), 932–940.
28. Sun, D., Li, N., Zhang, W., Zhao, Z., Mou, Z., Huang, D., et al. (2016). Design of PLGA-functionalized quercetin nanoparticles for potential use in Alzheimer's disease. *Colloids and Surfaces. B, Biointerfaces, 148*, 116–129.
29. Bangde, P., Atale, S., Dey, A., Pandit, A., Dandekar, P., & Jain, R. (2017). Potential gene therapy towards treating neurodegenerative diseases employing polymeric nanosystems. *Current Gene Therapy, 17*(2), 170–183.
30. Huang, R., Ma, H., Guo, Y., Liu, S., Kuang, Y., Shao, K., et al. (2013). Angiopep-conjugated nanoparticles for targeted long-term gene therapy of Parkinson's disease. *Pharmaceutical Research, 30*(10), 2549–2559.
31. Mohideen, M., Quijano, E., Song, E., Deng, Y., Panse, G., Zhang, W., et al. (2017). Degradable bioadhesive nanoparticles for prolonged intravaginal delivery and retention of elvitegravir. *Biomaterials, 144*, 144–154.
32. Caizhen, G., Yan, G., Ronron, C., Lirong, Y., Panpan, C., Xuemei, H., et al. (2015). Zirconium phosphatidylcholine-based nanocapsules as an in vivo degradable drug delivery system of MAP 30, a momordica anti-HIV protein. *International Journal of Pharmaceutics, 483*(1–2), 188–199.
33. Luo, L., Du, T., Zhang, J., Zhao, W., Cheng, H., Yang, Y., et al. (2016). Efficient inhibition of ovarian cancer by degradable nanoparticle-delivered survivin T34A gene. *International Journal of Nanomedicine, 11*, 501–512.
34. Zhang, F., Smolen, J. A., Zhang, S., Li, R., Shah, P. N., Cho, S., et al. (2015). Degradable polyphosphoester-based silver-loaded nanoparticles as therapeutics for bacterial lung infections. *Nanoscale, 7*(6), 2265–2270.
35. Wang, Y. F., Jin, A. M., Wei, K., Wang, X. D., Tang, S. H., & Min, S. X. (2006). Development of an anti-infection nano-hydroxyapatite drug delivery microsphere and its drug-release in vitro. *Nan Fang Yi Ke Da Xue Xue Bao, 26*(6), 754–756.

36. Martínez Rivas, C. J., Tarhini, M., Badri, W., Miladi, K., Greige-Gerges, H., Nazari, Q. A., et al. (2017). Nanoprecipitation process: From encapsulation to drug delivery. *International Journal of Pharmaceutics, 532*(1), 66–81.
37. Ho, B. N., Pfeffer, C. M., & Singh, A. T. K. (2017). Update on nanotechnology-based drug delivery systems in cancer treatment. *Anticancer Research, 37*(11), 5975–5981.
38. Grossen, P., Witzigmann, D., Sieber, S., & Huwyler, J. (2017). PEG-PCL-based nanomedicines: A biodegradable drug delivery system and its application. *Journal of Controlled Release, 260*, 46–60.
39. Gou, M., Zheng, X., Men, K., Zhang, J., Zheng, L., Wang, X., et al. (2009). Poly(epsilon-caprolactone)/poly(ethylene glycol)/poly(epsilon-caprolactone) nanoparticles: Preparation, characterization, and application in doxorubicin delivery. *The Journal of Physical Chemistry. B, 113*(39), 12928–12933.
40. Zhang, J., Men, K., Gu, Y., Wang, X., Gou, M., Guo, G., et al. (2011). Preparation of core cross-linked PCL-PEG-PCL micelles for doxorubicin delivery in vitro. *Journal of Nanoscience and Nanotechnology, 11*(6), 5054–5061.
41. Gou, M., Zheng, L., Peng, X., Men, K., Zheng, X., Zeng, S., et al. (2009). Poly(epsilon-caprolactone)-poly(ethylene glycol)-poly(epsilon-caprolactone) (PCL-PEG-PCL) nanoparticles for honokiol delivery in vitro. *International Journal of Pharmaceutics, 375*(1–2), 170–176.
42. Cho, H., Gao, J., & Kwon, G. S. (2016). PEG-b-PLA micelles and PLGA-b-PEG-b-PLGA sol-gels for drug delivery. *Journal of Controlled Release, 240*, 191–201.
43. Cho, H., & Kwon, G. S. (2014). Thermosensitive poly-(d,l-lactide-co-glycolide)-block-poly(ethylene glycol)-block-poly-(d,l-lactide-co-glycolide) hydrogels for multi-drug delivery. *Journal of Drug Targeting, 22*(7), 669–677.
44. Vijayakumar, M. R., Muthu, M. S., & Singh, S. (2013). Copolymers of poly(lactic acid) and D-α-tocopheryl polyethylene glycol 1000 succinate-based nanomedicines: Versatile multifunctional platforms for cancer diagnosis and therapy. *Expert Opinion on Drug Delivery, 10*(4), 529–543.
45. Suksiriworapong, J., Phoca, K., Ngamsom, S., Sripha, K., Moongkarndi, P., & Junyaprasert, V. B. (2016). Comparison of poly(ε-caprolactone) chain lengths of poly(ε-caprolactone)-co-d-α-tocopheryl-poly(ethylene glycol) 1000 succinate nanoparticles for enhancement of quercetin delivery to SKBR3 breast cancer cells. *European Journal of Pharmaceutics and Biopharmaceutics, 101*, 15–24.
46. Chuang, S. Y., Lin, C. H., Huang, T. H., & Fang, J. Y. (2018). Lipid-based nanoparticles as a potential delivery approach in the treatment of rheumatoid arthritis. *Nanomaterials (Basel), 8*(1), E42.
47. Wang, Y., Li, P., Truong-Dinh Tran, T., Zhang, J., & Kong, L. (2016). Manufacturing techniques and surface engineering of polymer based nanoparticles for targeted drug delivery to cancer. *Nanomaterials (Basel), 6*(2), E26.
48. Aw, M. S., & Paniwnyk, L. (2017). Overcoming *T. gondii* infection and intracellular protein nanocapsules as biomaterials for ultrasonically controlled drug release. *Biomaterials Science, 5*(10), 1944–1961.
49. Wiwanitkit, V. (2012). Interest in paromomycin for the treatment of visceral leishmaniasis (kala-azar). *Therapeutics and Clinical Risk Management, 8*, 323–328.
50. Bricaire, F. (1998). Liposomes: Promising perspectives. *Presse Médicale, 27*(Suppl 5), 7–8.
51. Carrillo-Muñoz, A. J., Quindós, G., Tur, C., Ruesga, M., Alonso, R., del Valle, O., et al. (2000). Comparative in vitro antifungal activity of amphotericin B lipid complex, amphotericin B and fluconazole. *Chemotherapy, 46*(4), 235–244.
52. Pedrini, I., Gazzano, E., Chegaev, K., Rolando, B., Marengo, A., Kopecka, J., et al. (2014). Liposomal nitrooxy-doxorubicin: One step over caelyx in drug-resistant human cancer cells. *Molecular Pharmaceutics, 11*(9), 3068–3079.
53. Rom, J., Bechstein, S., Domschke, C., Golatta, M., Mayer, C., Heil, J., et al. (2014). Efficacy and toxicity profile of pegylated liposomal doxorubicin (Caelyx) in patients with advanced breast cancer. *Anti-Cancer Drugs, 25*(2), 219–224.

54. Tong, Y. C., Kaye, A. D., & Urman, R. D. (2014). Liposomal bupivacaine and clinical outcomes. *Best Practice and Research. Clinical Anaesthesiology, 28*(1), 15–27.
55. Viscusi, E. R., Sinatra, R., Onel, E., & Ramamoorthy, S. L. (2014). The safety of liposome bupivacaine, a novel local analgesic formulation. *The Clinical Journal of Pain, 30*(2), 102–110.
56. Gnacadja, G. (2017, December 6). An invitation to pharmacostatics. *Bulletin of Mathematical Biology*. https://doi.org/10.1007/s11538-017-0369-z. [Epub ahead of print].

Chapter 10
Functionalized Antibacterial Nanoparticles for Controlling Biofilm and Intracellular Infections

Aparna Viswanathan, Jayakumar Rangasamy, and Raja Biswas

Abstract The intracellular and biofilm associated bacterial infections are difficult to treat due to the resistance potential of the organisms toward commonly used antimicrobial agents. Nanoparticles have promising future in the development of new therapeutics because of their easy functionalization and unique mode of action. This book chapter consists of three parts. The first two parts explain the limitations in the treatment of intracellular and biofilm infections in the current scenario. The last part describes how nanoparticles can be employed to tackle the problems of drug resistance and the latest research studies conducted regarding the same.

Keywords Nanoparticles · Functionalization · Biofilm · Intracellular · Antibiotics · Drug delivery

1 Introduction

The discovery of penicillin by Alexander Fleming in 1928 is the greatest advancement in the medical field, which reduced mortality due to bacterial diseases all over the world. Soon after the discovery of penicillin, other antibiotics such as bacitracin, cephalosporin, chloramphenicol, streptomycin, and clindamycin provided further therapeutic options. However, extensive use of antibiotics to treat ailments and their unchecked release into the environment resulted in the development of resistance in microbial population. The timeline in Fig. 10.1 depicts the discovery of antibiotics and the development of resistance toward these antibiotics. Large numbers of infections are now difficult to cure using single antibiotic and require combination therapies of different antibiotics. The treatment is more challenging when the pathogen adopts an intracellular lifestyle or resides within a biofilm. Treatment of these types of infections requires high dose administration of antibacterial drugs, which is often difficult due to drug toxicity, and this impedes effective treatment.

A. Viswanathan · J. Rangasamy · R. Biswas (✉)
Centre for Nanosciences and Molecular Medicine, Amrita Institute of Medical Sciences and Research Centre, Amrita Vishwa Vidyapeetham, Kochi, Kerala, India
e-mail: rjayakumar@aims.amrita.edu; rajabiswas@aims.amrita.edu

Y. V Pathak (ed.), *Surface Modification of Nanoparticles for Targeted Drug Delivery*, https://doi.org/10.1007/978-3-030-06115-9_10

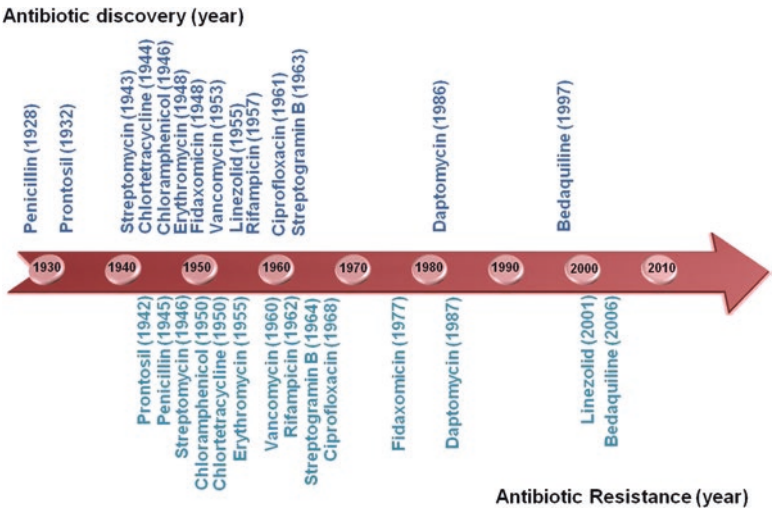


Fig. 10.1 Timeline showing the discovery of different antibiotics and the resistance reported

Intracellular bacteria evade both from the host defense system and from antibiotics. The classical examples of intracellular bacteria are *Mycobacterium tuberculosis*, *Legionella pneumophila, Chlamydia trachomatis*, *Salmonella enterica* and *Listeria monocytogenes*. Similar to intracellular infections, bacterial catheters or prosthetic joints and wound associated biofilm infections (e.g., *Staphylococccus aureus, Pseudomonas aeruginosa*) are also resistant to antibiotic treatment due to multiple phenotypic tolerance mechanisms. This calls for the need of smart systems that can deliver the drugs to the microbial niche, that is, inside the infected host cell (mainly macrophages) and into the biofilms. Intracellular and biofilm infections have significantly contributed to the death toll and the morbidity. Nanodrug delivery has got attention in the recent years due to its efficacy in targeting drug to specific cells and microenvironments [1–3].

This article summarizes the information regarding intracellular and biofilm infections, their treatment challenges and recent advances in nanoparticles mediated drug delivery systems to tackle intracellular and biofilm infections.

2 Intracellular Infections and Difficulties Associated with Their Treatment

2.1 Intracellular Bacterial Infections

Some species of bacteria are capable of residing/localizing inside the intracellular compartment of the host cells, where they multiply and grow by modulating the host immune defense mechanism. These groups of bacteria are known as

intracellular bacteria. The classical examples of intracellular bacteria are *Mycobacterium tuberculosis, Listeria monocytogenes, Salmonella enterica,* and *Chlamydia trachomatis*. These bacteria manipulate the intracellular environment of macrophages to survive. Once inside the macrophages, these intracellular bacteria use a number of strategies to evade host immune response which include interfering with phagosomal maturation, hindering of phagolysosomal fusion, neutralization of the phagosomal pH and evasion into the cytoplasm from the phagosome. Examples of intracellular bacteria which escape from the phagosomal compartment to the cytoplasm are *Listeria monocytogenes, Shigella flexneri, Francisella tularensis*, and *Burkholderia pseudomallei* [4–7]. Bacteria those maneuver and survive inside vacuolar compartments within macrophages are *Salmonella enterica* serovar Typhimurium [8], *Mycobacterium tuberculosis* [9], and *Coxiella burnetii* [10]. The obligate intracellular bacteria, *Chlamydia trachomatis,* resides inside the vacuolar compartments of epithelial cells during infection [11]. Additionally, some of the classical extracellular bacteria, such as *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* also has the ability to invade and survive inside specific host cells. For example, uropathogenic *Escherichia coli*, which causes urinary tract infections, invades and survives inside urothelial cells [12]. *P. aeruginosa* can survive inside epithelial cells [13, 14]; and *S. aureus* can survive in epithelial cells, endothelial cells and keratinocytes [15–17]. Table 10.1 shows different intracellular pathogens, associated diseases and their surviving cells.

2.2 Limitations in Intracellular Bacterial Infections Treatment Methods

Current treatment of intracellular bacterial infections requires antibiotic treatment for an extended period, as short term treatments are not effective. Under in vitro conditions, antibiotic exposure can effectively eradicate *M. tuberculosis* in the first 2 weeks. However, tuberculosis treatment requires combination antibiotic therapy for 6 months (rifampicin, pyrazinamide, isoniazid, and ethambutol for the first 2 months; and isoniazid and rifampicin for additional 4 months). Infections caused by drug resistant tuberculosis are even more difficult to treat and often requires longer duration of antibiotics treatment. Intracellular bacteria can alter their surface morphology and become more resistant to antibiotics. Ellington et al. demonstrated that once *S. aureus* is established intracellularly for 12 h within osteoblast, this bacteria alters its surface morphology and become resistant to certain antibiotics [27].

Table 10.1 Intracellular pathogens and their respective host cells

Intracellular bacteria	Residing cells	Disease	References
L. monocytogenes	Macrophages	Listeriosis	[18]
S. flexneri	Macrophages, neutrophils, and dendritic cells	Shigellosis	[19]
B. pseudomallei	Polymorphonuclear leukocytes, macrophages, epithelial cells	Melioidosis	[7]
F. tularensis	Macrophages, dendritic cells, hepatocytes, endothelial cells, polymorphonuclear neutrophils, and type II alveolar lung epithelial cells	Tularemia	[20]
Rickettsia rickettsii	Endothelial cells, smooth muscle cells	Rocky Mountain spotted fever	[18, 21]
S. enterica serovar Typhimurium	Macrophages	Salmonellosis	[22]
M. tuberculosis	Macrophages	Tuberculosis	[23]
L. pneumophila	Macrophages and monocytes	Pneumonia	[24]
C. burnetii	Monocytes, macrophages, epithelial cells and endothelial cells	Q fever	[25]
C. trachomatis	Epithelial cells	Genital tract infections	[26]
E. coli	Urothelial cells	Urinary tract infections	[12]
P. aeruginosa	Epithelial cells	Urinary tract infections, respiratory system infections, soft tissue infections, dermatitis, bacteremia, joint and bone infections	[13, 14]

3 Biofilm Infections and Limitations in Current Treatment Methods

3.1 Biofilm Infections

Biofilms are structured consortia of bacterial colonies attached on biotic or abiotic surfaces where bacteria stick to each other and get surrounded by the self-produced extracellular polymeric matrix. Biofilm formation is reported to be the most dominant approach of bacterial growth and survival [28–30]. The biofilm lifestyle not only helps the pathogen to persist in the environment but also helps in its pathogenesis.

Biofilm formation is a multistep complex process which involves (1) reversible attachment of the bacteria to the surface or nearby microbe already attached to the

surface; (2) exopolysaccharide synthesis and multicellular aggregation; (3) formation of three-dimensional mature biofilm comprising extracellular matrix containing transport channels for the circulation of nutrients within the biofilm; and (4) detachment and regrowth of the colonies as they mature.

Examples of Biofilm in Clinical Scenarios

- *P. aeruginosa* form well-structured biofilms in the lower respiratory tract. The collective action of bacteria and inflammation results in tissue damage and resultant death of the infected patient [31, 32]. They are also found to form biofilm on burn wounds [33]. *P. aeruginosa* also attach and form biofilm on contact lens. Frequent contact of the eye to the infected lens can cause to ocular diseases like keratitis [34].
- The coincidence of *Burkholderia cepacia* with *P. aeruginosa* biofilm in cystic fibrosis have shown to contribute significantly toward disease progression [35]
- Biofilm formation on kidney stones by *Proteus mirabilis* sometimes can cause urinary infections [36]
- Bone necrosis during osteomyelitis is a favorable niche for biofilm development in *S. aureus*, *S. epidermidis*, *Streptococcus pyogenes*, and *P. aeruginosa* [37]. *S. aureus* and *S. epidermidis* form biofilm on prosthetic knee and hip joints [38], pacemakers (leading to endocarditis) [39], surface of cerebrospinal fluid shunts [40], palatal obturator prostheses [41], vascular prosthetic grafts [42], and catheters and chronic wounds [43].
- Studies have found biofilm formation of *Propionibacterium acnes* on breast implants [44].
- *Porphyromonas gingivalis* forms biofilm on the soft tissues surrounding the teeth and causes periodontitis [45].
- Biofilm formation by *Serratia marcescens* is responsible for keratitis and other eye infections [46].
- Pathogenic *E. coli* forms biofilms and cause site specific diseases in host. For instance, enteropathogenic and enterohemorrhagic *E. coli* are common causative agents of gastroenteritis whereas extraintestinal *E. coli* causes sepsis, neonatal meningitis and urinary tract infections [47].
- Biofilms of *P. mirabilis* were observed on polystyrene and polyvinyl chloride catheters [48].

3.2 Current Treatment Methods Against Biofilm Infections

Biofilms are impenetrable to antibiotics which make the treatment of biofilm infections difficult. The minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC) of biofilm pathogens are 10–10,000-folds higher than the non-biofilm-forming bacterial cells. The effective concentration of antibiotics for the eradication of biofilm cannot be reached in clinical scenarios because of its toxicity. Generally, in these situations, the infected catheters or biomedical

devices are replaced with new ones, followed by empirical antibiotic therapy [49], in order to prevent biofilm regrowth on the implant. Antibiotics coated catheters and antibiotic impregnated catheters are available in the markets, which can slowly release drug and prevent biofilm formation, however use of antibiotics coated catheters always will increase the chances for the development of new drug resistant bacteria.

4 Nanoparticles for the Treatment of Intracellular and Biofilm Infections

Advances in drug delivery have led to the development of various antibiotics incorporated nanoformulations such as polymeric particles, liposomes, mesoporous silica particles, metallic and particulate suspensions which can be designed to deliver drugs to specific cells especially phagocytic cells.

Better surface area, tuned biodegradability, controlled release and distribution, stability and easy Functionalization make nanoparticles a better drug delivery agent. Table 10.2 describes the advantages and disadvantages of different nanoformulations used in drug delivery. Nanomedicine can triumph over the limitations of conventional drugs by altering its pharmacokinetics without changing the drug molecular structure. The small size and structure of nanoparticles enable them to easily penetrate physiological barriers, prevents the degradation of the drugs in unfavorable environment.

The objective of antibiotic nanoformulation is to attain better drug distribution to the subcellular organelles (for intracellular bacteria) and to the pathogenic microenvironment (for biofilm).

4.1 Current Development in Nanoparticle-Mediated Intracellular Antibiotic Delivery Systems

The drug-incorporated nanoparticles are easily internalized/endocytosed, which in turn favors delivery of the drugs into specific tissues and organs of interest. Chithrani et al. showed that the uptake of nanoparticles is highly dependent on size and shape [100]. Another advantage of these nanoparticles over the free drugs is their relatively better stability and improved bioavailability. Nanoparticles can enter the cell by the mechanism of endocytosis, which can be clathrin mediated or simple phagocytosis [6]. Once entered, nanoparticles can deliver drugs into intracellular compartments (Fig. 10.2a). Several preclinical studies were carried out in the last few years, which showed that drug-entrapped nanoparticles can be used for delivering antibiotics into intracellular compartments of various cell types. Nanoparticles can be functionalized so as to improve their binding affinity to different cell receptors. For

Table 10.2 Advantages and disadvantages of different nanoparticle systems used in drug delivery

Nanoparticles	Advantages	Disadvantages	References
Liposomes	• Enhanced cellular uptake • Flexibility in formulation for controlled release • Biocompatibility • Easy fictionalisation for targeting	• Rapid clearance • Low solubility • High production cost • Poor entrapment of hydrophilic drugs	[50–57]
Polymers (e.g., Chitosan, Gelatin, PLA, PVA, PLGA)	• Low cost of production • Fine tailoring of properties • Less toxicity	• Rapid clearance • Limited solubility • Synthetic polymers are reported to induce necrosis and apoptosis	[58–63]
Carbon Nanotubes	• Large surface area • Better conductivity and penetration capacity • High loading capacities • Chemical and thermal stability	• Toxicity • Nonbiodegradability	[64–70]
Magnetic Nanoparticles	• Controlled targeting • Easy penetration • Large surface area • Employed for diagnostic purposes	• Nonbiodegradability • Cytotoxicity	[71–76]
Dendrimers	• Tunable size and shape • Easy surface modification • Enhanced bioavailability and solubility of hydrophobic drugs • Apt for nucleic acid delivery	• Limited hemolysis and toxicity	[77–82]
Quantum dots	• High quantum yield • Good chemical and photo stability • Applied in sensors and for bioimaging	• Composition related toxicity	[83–87]
Cyclodextrin	• Better solubility and bioavailability • High stability • Biocompatible	• High production cost	[88–90]
Mesoporous silica nanoparticles	• Controlled and sustained release of drug • Good thermal and chemical stability • High drug loading efficiency • Biocompatible	• Toxicity	[91–95]
Gold nanoparticles	• Large surface area • Easy surface Functionalization • Applied in sensors and bioimaging	• Size and shape dependent toxicity	[96–99]

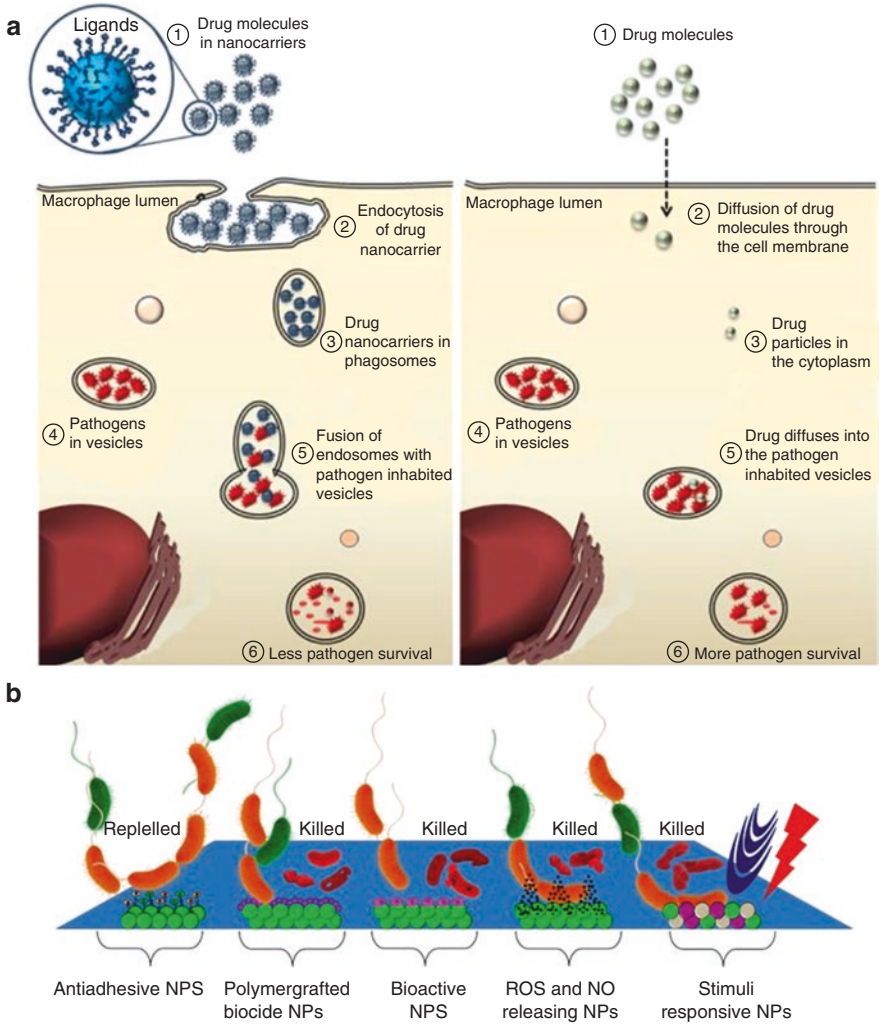


Fig. 10.2 (**a**) Schematic diagram depicting better intracellular pathogen killing when nanoparticles are used instead of bare drug. Reprinted from Biological macromolecules based targeted nanodrug delivery systems for the treatment of intracellular infections,110, V. Aparna, M. Shiva, Raja Biswas, R. Jayakumar, Biological macromolecules based targeted nanodrug delivery systems for the treatment of intracellular infections, 2–6, Copyright (2018), with permission from Elsevier [9] and (**b**) various mechanisms of biofilm formation inhibition by nanoparticles. Reprinted from 2016 Mohankandhasamy Ramasamy and Jintae Lee [101].

example, nanoparticles on conjugation with sulphated polymers like carrageenan or fucoidan showed better intracellular uptake as compared to the unconjugated ones [102, 103].

Liposomes, polymeric nanoparticles, such as poly D, L-lactide-co-glycolide (PLGA), glucan, chitosan, alginate and a mixture of alginate and chitosan; solid lipid nanoparticles (SLNs) such as fatty acids, steroids, triglycerides, partial glycerides and waxes; and micelles encapsulated antibiotics showed enhanced internalization by human macrophages, and release high concentrations of drugs into the cell which leads to efficient killing of bacteria. For example, chitosan nanoparticles loaded with ceftriaxone have shown enhanced intracellular antibacterial activity against *Salmonella* in macrophage cells [104]. Gentamicin-loaded gold nanoparticles and chloramphenicol loaded dextran sulfate nanoparticles showed better intracellular killing of intracellular *Salmonella* [2, 106]. Table 10.3 provides a list of nanoparticles, their particle size and antimicrobial action that were developed recently.

4.2 Current Development of Nanoparticles to Tackle Biofilm Infections

4.2.1 Nanoparticles for Prevention of Biofilm

Different types of nanoparticles are designed that have diverse properties to inhibit pathogens and control biofilm formation (Fig. 10.2b) which include the following:

1. *Antiadhesive nanoparticles*: Nanoparticles can be modified to make their surface superhydrophobic and smooth in order to prevent bacterial adhesion/attachment.
2. *Polymer-grafted biocide nanoparticles*: Biocides are conjugated/coated to nanoparticle surfaces which ensure pathogen death as soon as the pathogen comes in contact with the nanoparticles.
3. *Bioactive nanoparticles*: Bioactive nanoparticles are synthesized that can disrupt bacterial colonization and biofilm formation.
4. *Reactive oxygen species (ROS)- and nitric oxide (NO)-releasing nanoparticles*: Nanoparticles are developed to release NO and ROS which can lyse the exopolysaccharides of pathogens
5. *Stimuli responsive nanoparticles*: Nanoparticles can be designed to release drugs at specific conditions (pH) or a particular microniche.

Table 10.3 Nanoparticles against intracellular infections and respective pathogens

Nanoparticles	Size (nm)	Pathogen inhibited	Antimicrobial activity	References
Ceftriaxone sodium-loaded chitosan nanoparticles	210	*S. enterica* serovar Typhimurium	Improved intracellular nanoparticles uptake by *Salmonella enterica* serovar Typhimurium infected J774.2 macrophage cells	[1]
Gentamicin-loaded gold nanoparticles	180	*L. monocytogenes, S. aureus*, *E. coli*, *P. aeruginosa*, and *S. enterica* serovar Typhimurium	Enhanced killing of bacteria in infected macrophages	[2]
Enrofloxacin-loaded docosanoic acid solid lipid nanoparticles	605	*S. enterica* serovar Typhimurium	30-fold increased accumulation of nanoparticles in infected macrophages	[3]
Azithromycin and rifampin encapsulated PLGA nanoparticles	100	*Chlamydia trachomatis* and *C. pneumoniae*	Enhanced accumulation of nanoparticles in intracellular compartments and inclusion bodies	[107]
Tetracycline-loaded *O*-carboxymethyl chitosan nanoparticles	200	*S. aureus*	Sixfold enhanced killing of *S. aureus* in human embryonic kidney HEK-293 cells and in differentiated macrophage THP1cells compared to bare drug	[105]
Isoniazid, streptomycin, and rifampicin encapsulated poly-n-butylcyanoacrylate and polyisobutylcyanoacrylate nanoparticles	250	*M. tuberculosis*	Improved intracellular accumulation and three to fourfold increased antimicrobial activity of drugs against intracellular *M. tuberculosis*	[108]

(continued)

Table 10.3 (continued)

Nanoparticles	Size (nm)	Pathogen inhibited	Antimicrobial activity	References
Moxifloxacin encapsulated poly(butyl cyanoacrylate) nanoparticles	587 ± 130	*M. tuberculosis*	Tenfold enhanced antimicrobial activity against intracellular *M. tuberculosis* than free drug	[109]
rifampicin entrapped gelatin nanoparticles	264	*M. tuberculosis*	Sustained drug release for 72 h, permitting less-frequent administration to accomplish therapeutic outcome in animal models.	[110]
Rifampin-loaded mesoporous silica nanoparticle coated with polyethyleneimine	100	*M. tuberculosis*	High intracellular accumulation of nanoparticles in endosomes	[111]
Isoniazid encapsulated mesoporous silica nanoparticles with cyclodextrin-based pH-operated valves	50–100	*M. tuberculosis*	Effective intracellular pathogen killing compared to the bare drug	[112]
Penicillin G-conjugated geranyl–farnesyl nanoparticles	280 ± 17	*S. aureus*	99.9% reduced intracellular pathogen replication	[113]
Chloramphenicol-loaded dextran sulfate nanoparticles	100–200	*S. enterica* serovar Paratyphi A	Enhanced antimicrobial activity of drug encapsulated nanoparticles in ex vivo chicken intestine infection model	[106]
Rifampicin-loaded amorphous chitin nanoparticles	350 ± 50	*E. coli, S. aureus*	Five to sixfold increased delivery of rifampicin to intracellular niche	[114]

4.2.2 Nanoparticles to Treat Biofilm Infections

Inorganic nanoparticles especially silver nanoparticles and titanium dioxide nanoparticles are widely studied to prevent and eradicate biofilm in medical devices. Literature shows that silver ions released from nanoparticles interact with sulfhydryl molecules in pathogens affecting the membrane integrity, replication, enzyme kinetics, etc. and the cationic charge of the metallic nanoparticles disrupts exopolysaccharide structures [115–117].

Gold nanoparticles alone are rarely considered as effective biofilm inhibitory agent but on conjugation with active compounds and biomolecules they showed active anti-biofilm activity [118–120]. A pilot study by Lackner et al. suggested external ventricular drainage catheters impregnated with silver nanoparticles can be a used to prevent catheter-associated ventriculitis in patients [121].

Liposomes and other polymeric nanoparticles are incorporated into dental and bone cements to prevent biofilm formation in the later stages after the surgical procedures. The cationic molecules (gold, silver, etc.) in the polymer are shown to penetrate the cell membrane or produced free radicals to disrupt the biofilm. Shi et al. showed that incorporation of chitosan and its derivative nanoparticles enhanced the antibacterial and mechanical activity in poly(methyl methacrylate) bone cement [122]. Some examples of nanoparticles used for management of biofilms are given in Table 10.4.

5 Perspective

This chapter summarizes the use of nanoparticles to overcome/manage bacterial infections due to biofilms or intracellular survival. Nanoparticles can be formulated by fine tuning their properties like composition and drug release kinetics for better delivery of antibiotics to the delivery site, especially to intracellular components and biofilm niche. In case of intracellular targeting, the size of nanoformulations matters, as very small particles (<250 nm) will not get phagocytosed by the macrophages and very large particles can clog the capillaries.

Efficient delivery of a drug to a specific location by the nanoparticles enables low drug volume and low frequency of drug administration, which in turn ensures increased patient compliance and better clinical outcomes. Even with all these advantages, the effectual application of nanoparticles in human disease treatment is still limited.

More studies are needed to know the long-term toxic effects of nanoparticles. The researchers have not completely understood all the pharmacodynamics properties of the nanoformulations. The chances of nanoparticles aggregating and blocking the capillaries cannot be neglected. Investigations are to be conducted to fully recognize the interactions of the nanosystems with immune cells. Controlling the type of immune response elicited by specific nanocarriers can pave pathways for discovery of vaccines for chronic ailments [144].

Table 10.4 Nanoparticles with anti-biofilm activities

Nanoparticles	Size (nm)	Pathogen inhibited	Anti-biofilm activity	References
Nickel nanoparticles	100	*S. epidermidis*	Concentration ranging from 0.05 to 1.0 mg/mL of nickel nanoparticles inhibited the biofilm formation	[123]
Tenorite (CuO) nanoparticles	<50	*Burkholderia mallei*, *S. aureus*, *Klebsiella pneumoniae*, *E. coli*, and *P. fluorescens*	Nanoparticles with 32 μM concentration showed biofilm inhibition	[124]
Silver nanoparticles	50–60	*S. aureus*	Silver nanoparticle-coated catheters inhibited biofilm formation	[125]
Rifampicin-loaded solid lipid nanoparticles	101 ± 4.7	*S. epidermidis*	Time and concentration dependent biofilm reduction	[126]
Nitric oxide (NO) releasing silica nanoparticles	<150	*P. aeruginosa*, *E. coli*	The released NO caused effective inhibition of bacterial biofilm	[127]
Selenium nanoparticles	80–220	*S. aureus*, *P. mirabilis*, and *P. aeruginosa*	42%, 53.4%, 34.3%, biofilm inhibition of *S. aureus*, *P. mirabilis*, and *P. aeruginosa* respectively	[128]
Gentamicin-loaded gold nanoparticles	180	*L. monocytogenes*, *S. aureus*, *E. coli*, *P. aeruginosa*, and *S. enteric* serovar Typhimurium	The biofilm formation was inhibited on treatment with nanoparticles	[2]
Baicalein-decorated gold nanoparticles	26.5	*P. aeruginosa*	Around 58–76% biofilm reduction	[129]
Silver nanoparticles	50	*P. aeruginosa* and *S. epidermidis*	>95% reduction in biofilm	[130]
zinc oxide nanoparticles	20	*P. aeruginosa*	Inhibition of biofilm formation, less effective in removing preformed biofilm	[131]
Ciprofloxacin-loaded PLGA nanoparticles	200	*E. coli*	Time-dependent anti-biofilm activity on introduction of nanoparticles	[132]

(continued)

Table 10.4 (continued)

Nanoparticles	Size (nm)	Pathogen inhibited	Anti-biofilm activity	References
Cinnamaldehyde-conjugated gold nanoparticles	326 ± 48	Methicillin-resistant *S. aureus*	Distorted biofilm morphology in SEM and attenuation of bacterial virulence on treatment with nanoparticles	[133]
Levofloxacin-loaded PLGA-PVA-phosphatidyl choline nanoparticles	240 ± 50	*P. aeruginosa*	99.9% killing of pathogens and biofilm inhibition when nanoparticles are employed	[134]
Chitosan-coated iron oxide nanoparticles	15–25	*S. aureus*	500 μg/mL of nanoparticles treatment reduced biomass formation	[135]
Silver nanoparticles	20–100	*S. enteric* serovar Typhimurium and *P. aeruginosa*	25–50 ppm of nanoparticles removed bacterial biofilm	[136]
Zinc oxide nanoparticles	20	*P. aeruginosa*	Biofilm inhibition at 350 μg/ml of NPs	[137]
Rose bengal-functionalized chitosan nanoparticles	60 ± 20	*E. faecalis, Streptococcus oralis*, *Prevotella intermedia*, and *Actinomyces naeslundii*	Deeper penetration of nanoparticles into biofilm, complete disruption of biofilm	[138]
TiO_2 nanoparticle	201 ± 5	*Shewanella oneidensis*	Slower rate of biofilm development	[139]
CuO nanoparticles	40	Oral bacteria	50 μg/ml of nanoparticles inhibited extracellular polysaccharide production and biofilm formation	[140]
Zinc oxide (ZnO) nanoparticles	35	Oral bacteria	50 μg/ml of nanoparticles inhibited extracellular polysaccharide production and biofilm formation	[140]
ZnO nanoparticles	15	*S. pneumoniae*	12 μg/ml of nanoparticles attenuated biofilm	[141]
Super paramagnetic iron oxide nanoparticles	18	*S. epidermidis*	50 μg/ml of NPs prevented colony assembly	[142]

(continued)

Table 10.4 (continued)

Nanoparticles	Size (nm)	Pathogen inhibited	Anti-biofilm activity	References
Kocuran-functionalized silver nanoparticles	12	*S. aureus* and *E. coli*	Disruption of biofilms due to the induction of free radicals from nanoparticles	[143]

Most studies using nanoparticle mediated drug delivery are in vitro results and require further in vivo experimental validations. More studies are to be done to understand the mechanism by which the endocytosed drug is reaching the pathogen microniche. Also, the changes in the drug bioactivity once it reaches the microniche should also be considered.

We might still be far from the ultimate goal of the nanodrug approach to treat intracellular and biofilm infections. So far nanodrugs are approved only for clinical as nanosilver bandage/cream for wound healing and zinc oxide nanoparticles are currently under clinical trial to use for oral cavity disinfection (ClinicalTrials.gov Identifier: NCT03478150). Further research is necessary to develop suitable nano-formulations for the treatment of intracellular and biofilm infections.

Acknowledgments The authors are grateful to Centre for Nanosciences and Molecular Medicine, Amrita Institute of Medical Sciences and Research Centre, Amrita Vishwa Vidyapeetham, Kochi, 682041, India for infrastructural support and Nanomission grant (SR/NM/NT-1033/2016 G) from the Department of Science and Technology, India for financial support.

References

1. Zaki, N. M., & Hafez, M. M. (2012). Enhanced antibacterial effect of ceftriaxone sodium-loaded chitosan nanoparticles against intracellular *Salmonella* typhimurium. *AAPS PharmSciTech, 13*, 411–421. https://doi.org/10.1208/s12249-012-9758-7.
2. Mu, H., Tang, J., Liu, Q., Sun, C., Wang, T., & Duan, J. (2016). Potent antibacterial nanoparticles against biofilm and intracellular bacteria. *Scientific Reports, 6*, 18877. https://doi.org/10.1038/srep18877.
3. Xie, S., Yang, F., Tao, Y., Chen, D., Qu, W., Huang, L., Liu, Z., Pan, Y., & Yuan, Z. (2017). Enhanced intracellular delivery and antibacterial efficacy of enrofloxacin-loaded docosanoic acid solid lipid nanoparticles against intracellular *Salmonella*. *Scientific Reports, 7*, 41104. https://doi.org/10.1038/srep41104.
4. Birmingham, C. L., Canadien, V., Gouin, E., Troy, E. B., Yoshimori, T., Cossart, P., Higgins, D. E., & Brumell, J. H. (2007). *Listeria monocytogenes* evades killing by autophagy during colonization of host cells. *Autophagy, 3*, 442–451. https://doi.org/10.4161/auto.4450.
5. Ogawa, M., Yoshimori, T., Suzuki, T., Sagara, H., Mizushima, N., & Sasakawa, C. (2005). Escape of intracellular *Shigella* from autophagy. *Science, 307*, 727–731. https://doi.org/10.1126/science.1106036.
6. Butchar, J. P., Cremer, T. J., Clay, C. D., Gavrilin, M. A., Wewers, M. D., Marsh, C. B., Schlesinger, L. S., & Tridandapani, S. (2008). Microarray analysis of human monocytes infected with *Francisella tularensis* identifies new targets of host response subversion. *PLoS One, 3*, 2924. https://doi.org/10.1371/journal.pone.0002924.

7. Cullinane, M., Gong, L., Li, X., Lazar-Adler, N., Tra, T., Wolvetang, E., Prescott, M., Boyce, J. D., Devenish, R. J., & Adler, B. (2008). Stimulation of autophagy suppresses the intracellular survival of *Burkholderia pseudomallei* in mammalian cell lines. *Autophagy, 4*, 744–753. https://doi.org/10.4161/auto.6246.
8. Eswarappa, S. M., Negi, V. D., Chakraborty, S., Sagar, B. K. C., & Chakravortty, D. (2010). Division of the *Salmonella*-containing vacuole and depletion of acidic lysosomes in *Salmonella*-infected host cells are novel strategies of *Salmonella enterica* to avoid lysosomes. *Infection and Immunity, 78*, 68–79. https://doi.org/10.1128/IAI.00668-09.
9. Aparna, V., Shiva, M., Biswas, R., & Jayakumar, R. (2018). Biological macromolecules based targeted nanodrug delivery systems for the treatment of intracellular infections. *International Journal of Biological Macromolecules, 110*, 2–6. https://doi.org/10.1016/j.ijbiomac.2018.01.030.
10. Van Schaik, E. J., Chen, C., Mertens, K., Weber, M. M., & Samuel, J. E. (2013). Molecular pathogenesis of the obligate intracellular bacterium *Coxiella burnetii*. *Nature Reviews. Microbiology, 11*, 561–573. https://doi.org/10.1038/nrmicro3049.
11. Yasir, M., Pachikara, N. D., Bao, X., Pan, Z., & Fan, H. (2011). Regulation of chlamydial infection by host autophagy and vacuolar ATPase-bearing organelles. *Infection and Immunity, 79*, 4019–4028. https://doi.org/10.1128/IAI.05308-11.
12. Wang, H., Liang, F. X., & Kong, X. P. (2008). Characteristics of the phagocytic cup induced by uropathogenic *Escherichia coli*. *The Journal of Histochemistry and Cytochemistry, 56*, 597–604. https://doi.org/10.1369/jhc.2008.950923.
13. Wolska, K., Bednarz, B., & Jakubczak, A. (2003). Adherence of *Pseudomonas aeruginosa* to human buccal epithelial cells. *Acta Microbiologica Polonica, 52*, 419–423.
14. Bucior, I., Tran, C., & Engel, J. (2014). Assessing *Pseudomonas* virulence using host cells. *Methods in Molecular Biology, 1149*, 741–755. https://doi.org/10.1007/978-1-4939-0473-0_57.
15. Singh, S. K. (2017). *Staphylococcus aureus* intracellular survival: A closer look in the process. *Virulence, 8*, 1506–1507. https://doi.org/10.1080/21505594.2017.1384896.
16. Kapral, F. A., & Shayegani, M. G. (1959). Intracellular survival of Staphylococci. *The Journal of Experimental Medicine, 110*, 123–138. https://doi.org/10.1084/jem.110.1.123.
17. Gresham, H. D., Lowrance, J. H., Caver, T. E., Wilson, B. S., Cheung, A. L., & Lindberg, F. P. (2000). Survival of *Staphylococcus aureus* inside neutrophils contributes to infection. *Journal of Immunology, 164*, 3713–3722. https://doi.org/10.4049/jimmunol.164.7.3713.
18. Wilson, J. W., Schurr, M. J., LeBlanc, C. L., Ramamurthy, R., Buchanan, K. L., & Nickerson, C. A. (2002). Mechanisms of bacterial pathogenicity. *Postgraduate Medical Journal, 78*, 216–224. https://doi.org/10.1136/pmj.78.918.216.
19. Zychlinsky, A., Prevost, M. C., & Sansonetti, P. J. (1992). *Shigella flexneri* induces apoptosis in infected macrophages. *Nature, 358*, 167–169. https://doi.org/10.1038/358167a0.
20. Celli, J. (2008). Intracellular localization of *Brucella abortus* and *Francisella tularensis* in primary murine macrophages. *Methods in Molecular Biology, 431*, 133–145. https://doi.org/10.1007/978-1-60327-032-8_11.
21. Silverman, D. J., & Bond, S. B. (1984). Infection of human vascular endothelial cells by *Rickettsia rickettsii*. *The Journal of Infectious Diseases, 149*, 201–206.
22. Alpuche-Aranda, C. M., Racoosin, E. L., Swanson, J. A., & Miller, S. I. (1994). *Salmonella* stimulate macrophage macropinocytosis and persist within spacious phagosomes. *The Journal of Experimental Medicine, 179*, 601–608. https://doi.org/10.1084/jem.179.2.601.
23. Awuh, J. A., & Flo, T. H. (2017). Molecular basis of mycobacterial survival in macrophages. *Cellular and Molecular Life Sciences, 74*, 1625–1648. https://doi.org/10.1007/s00018-016-2422-8.
24. Dermine, J. F., & Desjardins, M. (1999). Survival of intracellular pathogens within macrophages. *Protoplasma, 210*, 11–24. https://doi.org/10.1007/BF01314950.
25. Ghigo, E., Colombo, M. I., & Heinzen, R. A. (2012). The *Coxiella burnetii* parasitophorous vacuole. *Advances in Experimental Medicine and Biology, 984*, 141–169. https://doi.org/10.1007/978-94-007-4315-1_8.

26. Heuer, D., Lipinski, A. R., Machuy, N., Karlas, A., Wehrens, A., Siedler, F., Brinkmann, V., & Meyer, T. F. (2009). *Chlamydia* causes fragmentation of the golgi compartment to ensure reproduction. *Nature, 457*, 731–735. https://doi.org/10.1038/nature07578.
27. Ellington, J. K., Reilly, S. S., Ramp, W. K., Smeltzer, M. S., Kellam, J. F., & Hudson, M. C. (1999). Mechanisms of *Staphylococcus aureus* invasion of cultured osteoblasts. *Microbial Pathogenesis, 26*, 317–323. https://doi.org/10.1006/mpat.1999.0272.
28. Lindsay, D., & von Holy, A. (2006). Bacterial biofilms within the clinical setting: What healthcare professionals should know. *The Journal of Hospital Infection, 64*, 313–325. https://doi.org/10.1016/j.jhin.2006.06.028.
29. Giaouris, E., Heir, E., Hébraud, M., Chorianopoulos, N., Langsrud, S., Møretrø, T., Habimana, O., Desvaux, M., Renier, S., & Nychas, G. J. (2014). Attachment and biofilm formation by foodborne bacteria in meat processing environments: Causes, implications, role of bacterial interactions and control by alternative novel methods. *Meat Science, 97*, 289–309. https://doi.org/10.1016/j.meatsci.2013.05.023.
30. Dufour, D., Leung, V., & Lévesque, C. M. (2010). Bacterial biofilm: Structure, function, and antimicrobial resistance. *Endodontic Topics, 22*, 2–16. https://doi.org/10.1111/j.1601-1546.2012.00277.x.
31. Høiby, N., Ciofu, O., & Bjarnsholt, T. (2010). *Pseudomonas aeruginosa* biofilms in cystic fibrosis. *Future Microbiology, 5*, 1663–1674. https://doi.org/10.2217/fmb.10.125.
32. Gómez, M. I., & Prince, A. (2007). Opportunistic infections in lung disease: *Pseudomonas* infections in cystic fibrosis. *Current Opinion in Pharmacology, 7*, 244–251. https://doi.org/10.1016/j.coph.2006.12.005.
33. Nickel, J. C., & Costerton, J. W. (1993). Bacterial localization in antibiotic-refractory chronic bacterial prostatitis. *The Prostate, 23*, 107–114. https://doi.org/10.1002/pros.2990230204.
34. Abidi, S. H., Sherwani, S. K., Siddiqui, T. R., Bashir, A., & Kazmi, S. U. (2013). Drug resistance profile and biofilm forming potential of *Pseudomonas aeruginosa* isolated from contact lenses in Karachi-Pakistan. *BMC Ophthalmology, 13*, 57. https://doi.org/10.1186/1471-2415-13-57.
35. Bragonzi, A., Farulla, I., Paroni, M., Twomey, K. B., Pirone, L., Lorè, N. I., Bianconi, I., Dalmastri, C., Ryan, R. P., & Bevivino, A. (2012). Modelling co-infection of the cystic fibrosis lung by *Pseudomonas aeruginosa* and *Burkholderia cenocepacia* reveals influences on biofilm formation and host response. *PLoS One, 7*, 52330. https://doi.org/10.1371/journal.pone.0052330.
36. Hellström, J. (1938). The significance of staphylococci in the development and treatment of renal and ureteral stones. *British Journal of Urology, 10*, 348–372. https://doi.org/10.1111/j.1464-410X.1938.tb10342.x.
37. Cierny, G., III, & Mader, J. T. (1984). Adult chronic osteomyelitis. *Orthopedics, 7*, 1557–1564.
38. Rohde, H., Burandt, E. C., Siemssen, N., Frommelt, L., Burdelski, C., Wurster, S., Scherpe, S., Davies, A. P., Harris, L. G., Horstkotte, M. A., Knobloch, J. K. M., Ragunath, C., Kaplan, J. B., & Mack, D. (2007). Polysaccharide intercellular adhesin or protein factors in biofilm accumulation of *Staphylococcus epidermidis* and *Staphylococcus aureus* isolated from prosthetic hip and knee joint infections. *Biomaterials, 28*, 1711–1720. https://doi.org/10.1016/j.biomaterials.2006.11.046.
39. Santos, A. P. A., Watanabe, E., & de Andrade, D. (2011). Biofilm on artificial pacemaker: Fiction or reality? *Arquivos Brasileiros de Cardiologia, 97*, 113–120. https://doi.org/10.1590/S0066-782X2011001400018.
40. Fux, C. A., Quigley, M., Worel, A. M., Post, C., Zimmerli, S., Ehrlich, G., & Veeh, R. H. (2006). Biofilm-related infections of cerebrospinal fluid shunts. *Clinical Microbiology and Infection, 12*, 331–337. https://doi.org/10.1111/j.1469-0691.2006.01361.x.
41. Murakami, M., Nishi, Y., Seto, K., Kamashita, Y., & Nagaoka, E. (2015). Dry mouth and denture plaque microflora in complete denture and palatal obturator prosthesis wearers. *Gerodontology, 32*, 188–194. https://doi.org/10.1111/ger.12073.

42. Tollefson, D. F., Bandyk, D. F., Kaebnick, H. W., Seabrook, G. R., & Towne, J. B. (1987). Surface biofilm disruption: Enhanced recovery of microorganisms from vascular prostheses. *Archives of Surgery, 122*, 38–43. https://doi.org/10.1001/archsurg.1987.01400130044006.
43. Higashi, J., & Sullam, P. (2006). *Staphylococcus aureus* biofilms. In J. L. Pace, M. E. Rupp, & R. G. Finch (Eds.), *Biofilms, infection, and antimicrobial therapy* (pp. 81–100). Boca Raton: Taylor & Francis.
44. Rieger, U. M., Mesina, J., Kalbermatten, D. F., Haug, M., Frey, H. P., Pico, R., Frei, R., Pierer, G., Luscher, N. J., & Trampuz, A. (2013). Bacterial biofilms and capsular contracture in patients with breast implants. *The British Journal of Surgery, 100*, 768–774. https://doi.org/10.1002/bjs.9084.
45. Lamont, R. J., & Jenkinson, H. F. (1998). Life below the gum line: Pathogenic mechanisms of *Porphyromonas gingivalis*. *Microbiology and Molecular Biology Reviews, 62*, 1244–1263.
46. El-Ganiny, A. M., Shaker, G. H., Aboelazm, A. A., & El-Dash, H. A. (2017). Prevention of bacterial biofilm formation on soft contact lenses using natural compounds. *Journal of Ophthalmic Inflammation and Infection, 7*, 11. https://doi.org/10.1186/s12348-017-0129-0.
47. DePas, W. H., Syed, A. K., Sifuentes, M., Lee, J. S., Warshaw, D., Saggar, V., Csankovszki, G., Boles, B. R., & Chapman, M. R. (2014). Biofilm formation protects *Escherichia coli* against killing by *Caenorhabditis elegans* and *Myxococcus xanthus*. *Applied and Environmental Microbiology, 80*, 7079–7087. https://doi.org/10.1128/AEM.02464-14.
48. Kwiecinska-Piróg, J., Bogiel, T., Skowron, K., Wieckowska, E., & Gospodarek, E. (2014). *Proteus mirabilis* biofilm—Qualitative and quantitative colorimetric methods-based evaluation. *Brazilian Journal of Microbiology, 45*, 1415–1421. https://doi.org/10.1590/S1517-83822014000400037.
49. Wu, H., Moser, C., Wang, H. Z., Høiby, N., & Song, Z. J. (2015). Strategies for combating bacterial biofilm infections. *International Journal of Oral Science, 7*, 1–7. https://doi.org/10.1038/ijos.2014.65.
50. Massing, U., Ingebrigtsen, S. G., Škalko-Basnet, N., & Holsæter, A. M. (2017). Dual centrifugation—A novel "in-vial" liposome processing technique. In *Liposomes*. doi:https://doi.org/10.5772/intechopen.68523.
51. Bozzuto, G., & Molinari, A. (2015). Liposomes as nanomedical devices. *International Journal of Nanomedicine, 10*, 975–999. https://doi.org/10.2147/IJN.S68861.
52. Salem, I. I., Flasher, D. L., & Düzgüneş, N. (2005). Liposome-encapsulated antibiotics. *Methods in Enzymology, 391*, 261–291. https://doi.org/10.1016/S0076-6879(05)91015-X.
53. Gulati, M., Grover, M., Singh, S., & Singh, M. (1998). Lipophilic drug derivatives in liposomes. *International Journal of Pharmaceutics, 165*, 129–168. https://doi.org/10.1016/S0378-5173(98)00006-4.
54. Laouini, A., Jaafar-Maalej, C., Limayem-Blouza, I., Sfar, S., Charcosset, C., & Fessi, H. (2012). Preparation, characterization and applications of liposomes: State of the art. *Journal of Colloid Science and Biotechnology, 1*, 147–168. https://doi.org/10.1166/jcsb.2012.1020.
55. Hatakeyama, H., Akita, H., Ito, E., Hayashi, Y., Oishi, M., Nagasaki, Y., Danev, R., Nagayama, K., Kaji, N., Kikuchi, H., Baba, Y., & Harashima, H. (2011). Systemic delivery of siRNA to tumors using a lipid nanoparticle containing a tumor-specific cleavable PEG-lipid. *Biomaterials, 32*, 4306–4316. https://doi.org/10.1016/j.biomaterials.2011.02.045.
56. Schroeder, A., Levins, C. G., Cortez, C., Langer, R., & Anderson, D. G. (2010). Lipid-based nanotherapeutics for siRNA delivery. *Journal of Internal Medicine, 267*, 9–21. https://doi.org/10.1111/j.1365-2796.2009.02189.x.
57. Lv, H., Zhang, S., Wang, B., Cui, S., & Yan, J. (2006). Toxicity of cationic lipids and cationic polymers in gene delivery. *Journal of Controlled Release, 114*, 100–109. https://doi.org/10.1016/j.jconrel.2006.04.014.
58. Quan, J.-S., Jiang, H.-L., Yu, J.-H., Guo, D.-D., Arote, R., Choi, Y.-J., & Cho, C.-S. (2008). Polymeric nanoparticles for oral delivery of protein drugs. *Nanoparticles New Research*, 373–386.

59. Kumari, A., Yadav, S. K., & Yadav, S. C. (2010). Biodegradable polymeric nanoparticles based drug delivery systems. *Colloids and Surfaces B: Biointerfaces, 75*, 1–18. https://doi.org/10.1016/j.colsurfb.2009.09.001.
60. Soppimath, K. S., Aminabhavi, T. M., Kulkarni, A. R., & Rudzinski, W. E. (2001). Biodegradable polymeric nanoparticles as drug delivery devices. *Journal of Controlled Release, 70*, 1–20. https://doi.org/10.1016/S0168-3659(00)00339-4.
61. Bhatia, S. (2016). *Natural polymer drug delivery systems: Nanoparticles, plants, and algae*. Cham: Springer. https://doi.org/10.1007/978-3-319-41129-3.
62. Amiji, M. M. (2007). Polymeric nanoparticles. *Nanotechnology in Cancer Therapy*, 215–230.
63. Alexis, F., Pridgen, E., Molnar, L. K., & Farokhzad, O. C. (2008). Factors affecting the clearance and biodistribution of polymeric nanoparticles. *Molecular Pharmaceutics*, 505–515. https://doi.org/10.1021/mp800051m.
64. Smart, S. K., Cassady, A. I., Lu, G. Q., & Martin, D. J. (2006). The biocompatibility of carbon nanotubes. *Carbon N. Y., 44*, 1034–1047. https://doi.org/10.1016/j.carbon.2005.10.011.
65. Yakobson, B. I., & Avouris, P. (2001). Mechanical properties of carbon nanotubes. *Carbon Nanotubes, 327*, 287–327. https://doi.org/10.1007/3-540-39947-X.
66. Endo, M., Strano, M., & Ajayan, P. (2008). Potential applications of carbon nanotubes. *Carbon Nanotubes, 62*, 13–61. https://doi.org/10.1007/978-3-540-72865-8_2.
67. Szfki, M. T. A., Nanotube, C., & Seminar, L. (2005). Purification of carbon nanotubes What do we have to purify? *Carbon N. Y., 46*, 2003–2025. https://doi.org/10.1016/j.carbon.2008.09.009.
68. McEuen, P. L. (2000). Single-wall carbon nanotubes. *Physics World, 13*, 31–36. https://doi.org/10.1088/2058-7058/13/6/26.
69. Ong, Y. T., Ahmad, A. L., Hussein, S., Zein, S., & Tan, S. H. (2010). A review on carbon nanotubes in an environmental protection and green engineering perspective. *Carbon Nanotubes, 27*, 227–242.
70. Hou, P. X., Liu, C., & Cheng, H. M. (2008). Purification of carbon nanotubes. *Carbon N. Y., 46*, 2003–2025. https://doi.org/10.1016/j.carbon.2008.09.009.
71. Bradley, J. S., Schmid, G., Talapin, D. V., Shevchenko, E. V., & Weller, H. (2003). Syntheses and characterizations: 3.2 synthesis of metal nanoparticles. *Nanoparticles*, 185–238. https://doi.org/10.1002/3527602399.ch3b.
72. Koksharov, Y. A. (2009). Magnetism of nanoparticles: Effects of size, shape, and interactions. *Magnetic Nanoparticles*, 197–254. https://doi.org/10.1002/9783527627561.ch6.
73. Nikiforov, V. N., & Filinova, E. Y. (2009). Biomedical applications of magnetic nanoparticles. *Magnetic Nanoparticles*, 393–455. https://doi.org/10.1002/9783527627561.ch10.
74. Bolden, N. W., Rangari, V. K., Jeelani, S., Boyoglu, S., & Singh, S. R. (2013). Synthesis and evaluation of magnetic nanoparticles for biomedical applications. *Journal of Nanoparticles, 2013*, 1–9. https://doi.org/10.1155/2013/370812.
75. Schrand, A. M., Rahman, M. F., Hussain, S. M., Schlager, J. J., Smith, D. A., & Syed, A. F. (2010). Metal-based nanoparticles and their toxicity assessment. *Wiley interdisciplinary Reviews: Nanomedicineand Nanobiotechnology, 2*, 544–568. https://doi.org/10.1002/wnan.103.
76. Parvathi, V. D., Rajagopal, K., Pandya, J., Lincoln, B., & Sumitha, R. (2015). Synthesis, characterisation and in vitro toxicity assessment of nano iron. *International Journal of Nanoparticles, 8*, 302. https://doi.org/10.1504/IJNP.2015.073733.
77. Van den Boorn, J. G., Daßler, J., Coch, C., Schlee, M., & Hartmann, G. (2013). Exosomes as nucleic acid nanocarriers. *Advanced Drug Delivery Reviews, 65*, 331–335. https://doi.org/10.1016/j.addr.2012.06.011.
78. Mendes, L. P., Pan, J., & Torchilin, V. P. (2017). Dendrimers as nanocarriers for nucleic acid and drug delivery in cancer therapy. *Molecules, 22*, 1401. https://doi.org/10.3390/molecules22091401.

79. Oberoi, H. S., Nukolova, N. V., Kabanov, A. V., & Bronich, T. K. (2013). Nanocarriers for delivery of platinum anticancer drugs. *Advanced Drug Delivery Reviews, 65*, 1667–1685. https://doi.org/10.1016/j.addr.2013.09.014.
80. Dufès, C., Uchegbu, I. F., & Schätzlein, A. G. (2005). Dendrimers in gene delivery. *Advanced Drug Delivery Reviews, 57*, 2177–2202. https://doi.org/10.1016/j.addr.2005.09.017.
81. Biswas, S., & Torchilin, V. P. (2013). Dendrimers for siRNA delivery. *Pharmaceuticals, 6*, 161–183. https://doi.org/10.3390/ph6020161.
82. Liu, X., Rocchi, P., & Peng, L. (2012). Dendrimers as non-viral vectors for siRNA delivery. *New Journal of Chemistry, 36*, 256–263. https://doi.org/10.1039/C1NJ20408D.
83. Probst, C. E., Zrazhevskiy, P., Bagalkot, V., & Gao, X. (2013). Quantum dots as a platform for nanoparticle drug delivery vehicle design. *Advanced Drug Delivery Reviews, 65*, 703–718. https://doi.org/10.1016/j.addr.2012.09.036.
84. Qi, L., & Gao, X. (2008). Emerging application of quantum dots for drug delivery and therapy. *Expert Opinion on Drug Delivery, 5*, 263–267. https://doi.org/10.1517/17425247.5.3.263.
85. Mattoussi, H., Palui, G., & Bin Na, H. (2012). Luminescent quantum dots as platforms for probing in vitro and in vivo biological processes. *Advanced Drug Delivery Reviews, 64*, 138–166. https://doi.org/10.1016/j.addr.2011.09.011.
86. Smith, A. M., Duan, H., Mohs, A. M., & Nie, S. (2008). Bioconjugated quantum dots for in vivo molecular and cellular imaging. *Advanced Drug Delivery Reviews, 60*, 1226–1240. https://doi.org/10.1016/j.addr.2008.03.015.
87. Zrazhevskiy, P., Sena, M., & Gao, X. (2010). Designing multifunctional quantum dots for bioimaging, detection, and drug delivery. *Chemical Society Reviews, 39*, 4326. https://doi.org/10.1039/b915139g.
88. Tiwari, G., Tiwari, R., & Rai, A. K. (2010). Cyclodextrins in delivery systems: Applications. *Journal of Pharmacy & Bioallied Sciences, 2*, 72–79. https://doi.org/10.4103/0975-7406.67003.
89. Uekama, K. (1999). Cyclodextrins in drug delivery system. *Advanced Drug Delivery Reviews, 36*, 1–2. https://doi.org/10.1016/S0169-409X(98)00051-9.
90. Challa, R., Ahuja, A., Ali, J., & Khar, R. K. (2005). Cyclodextrins in drug delivery: An updated review. *AAPS PharmSciTech, 6*, 329–357. https://doi.org/10.1208/pt060243.
91. Mamaeva, V., Sahlgren, C., & Lindén, M. (2013). Mesoporous silica nanoparticles in medicine-Recent advances. *Advanced Drug Delivery Reviews, 65*, 689–702. https://doi.org/10.1016/j.addr.2012.07.018.
92. Slowing, I. I., Vivero-Escoto, J. L., Wu, C.-W., & Lin, V. S.-Y. (2008). Mesoporous silica nanoparticles as controlled release drug delivery and gene transfection carriers. *Advanced Drug Delivery Reviews, 60*, 1278–1288. https://doi.org/10.1016/j.addr.2008.03.012.
93. Bharti, C., Gulati, N., Nagaich, U., & Pal, A. (2015). Mesoporous silica nanoparticles in target drug delivery system: A review. *International Journal of Pharmaceutical Investigation, 5*, 124. https://doi.org/10.4103/2230-973X.160844.
94. Tang, F., Li, L., & Chen, D. (2012). Mesoporous silica nanoparticles: Synthesis, biocompatibility and drug delivery. *Advanced Materials, 24*, 1504–1534. https://doi.org/10.1002/adma.201104763.
95. Slowing, I. I., Trewyn, B. G., Giri, S., & Lin, V. S.-Y. (2007). Mesoporous silica nanoparticles for drug delivery and biosensing applications. *Advanced Functional Materials, 17*, 1225–1236. https://doi.org/10.1002/adfm.200601191.
96. Tréguer-Delapierre, M., Majimel, J., Mornet, S., Duguet, E., & Ravaine, S. (2008). Synthesis of non-spherical gold nanoparticles. *Gold Bulletin, 41*, 195–207. https://doi.org/10.1007/BF03216597.
97. Li, Y., Schluesener, H. J., & Xu, S. (2010). Gold nanoparticle-based biosensors. *Gold Bulletin, 43*, 29–41. https://doi.org/10.1007/BF03214964.
98. Online, V. A., Fragouli, D., Ruffilli, R., & Athanassiou, A. (2014). Localised synthesis of gold nanoparticles in anisotropic alginate. *RSC Advances, 4*, 20449–20453. https://doi.org/10.1039/b000000x.

99. Manson, J., Kumar, D., Meenan, B. J., & Dixon, D. (2011). Polyethylene glycol functionalized gold nanoparticles: The influence of capping density on stability in various media. *Gold Bulletin, 44*, 99–105. https://doi.org/10.1007/s13404-011-0015-8.
100. Chithrani, B. D., Ghazani, A. A., & Chan, W. C. W. (2006). Determining the size and shape dependence of gold nanoparticle uptake into mammalian cells. *Nano Letters, 6*, 662–668. https://doi.org/10.1021/nl052396o.
101. Ramasamy, M., & Lee, J. (2016). Recent nanotechnology approaches for prevention and treatment of biofilm-associated infections on medical devices. *BioMed Research International, 2016*. https://doi.org/10.1155/2016/1851242.
102. Aparna, V., Melge, A. R., Rajan, V. K., Biswas, R., Jayakumar, R., & Gopi Mohan, C. (2018). Carboxymethylated ι-carrageenan conjugated amphotericin B loaded gelatin nanoparticles for treating intracellular *Candida glabrata* infections. *International Journal of Biological Macromolecules, 110*, 140–149. https://doi.org/10.1016/j.ijbiomac.2017.11.126.
103. Sandhya, M., Aparna, V., Maneesha, S. K., Raja, B., Jayakumar, R., & Sathianarayanan, S. (2018). Amphotericin B loaded sulfonated chitosan nanoparticles for targeting macrophages to treat intracellular *Candida glabrata* infections. *International Journal of Biological Macromolecules, 110*, 133–139. https://doi.org/10.1016/j.ijbiomac.2018.01.028.
104. Elbi, S., Nimal, T. R., Rajan, V. K., Baranwal, G., Biswas, R., Jayakumar, R., & Sathianarayanan, S. (2017). Fucoidan coated ciprofloxacin loaded chitosan nanoparticles for the treatment of intracellular and biofilm infections of *Salmonella*. *Colloids and Surfaces B: Biointerfaces, 160*, 40–47. https://doi.org/10.1016/j.colsurfb.2017.09.003.
105. Maya, S., Indulekha, S., Sukhithasri, V., Smitha, K. T., Nair, S. V., Jayakumar, R., & Biswas, R. (2012). Efficacy of tetracycline encapsulated *O*-carboxymethyl chitosan nanoparticles against intracellular infections of *Staphylococcus aureus*. *International Journal of Biological Macromolecules, 51*, 392–399. https://doi.org/10.1016/j.ijbiomac.2012.06.009.
106. Kiruthika, V., Maya, S., Suresh, M. K., Anil Kumar, V., Jayakumar, R., & Biswas, R. (2015). Comparative efficacy of chloramphenicol loaded chondroitin sulfate and dextran sulfate nanoparticles to treat intracellular *Salmonella* infections. *Colloids and Surfaces B: Biointerfaces, 127*, 33–40. https://doi.org/10.1016/j.colsurfb.2015.01.012.
107. Toti, U. S., Guru, B. R., Hali, M., McPharlin, C. M., Wykes, S. M., Panyam, J., & Whittum-Hudson, J. A. (2011). Targeted delivery of antibiotics to intracellular chlamydial infections using PLGA nanoparticles. *Biomaterials, 32*, 6606–6613. https://doi.org/10.1016/j.biomaterials.2011.05.038.
108. Anisimova, Y. V., Gelperina, S. I., a Peloquin, C., & Heifets, L. B. (2000). Nanoparticles as antituberculosis drugs carriers: Effect on activity against *Mycobacterium tuberculosis* in human monocyte-derived macrophages. *Yao Hsueh Hsueh Pao [Acta Pharmaceutica Sinica], 2*, 165–171. https://doi.org/10.1023/a:1010061013365.
109. Kisich, K. O., Gelperina, S., Higgins, M. P., Wilson, S., Shipulo, E., Oganesyan, E., & Heifets, L. (2007). Encapsulation of moxifloxacin within poly(butyl cyanoacrylate) nanoparticles enhances efficacy against intracellular *Mycobacterium tuberculosis*. *International Journal of Pharmaceutics, 345*, 154–162. https://doi.org/10.1016/j.ijpharm.2007.05.062.
110. Saraogi, G. K., Gupta, P., Gupta, U. D., Jain, N. K., & Agrawal, G. P. (2010). Gelatin nanocarriers as potential vectors for effective management of tuberculosis. *International Journal of Pharmaceutics, 385*, 143–149. https://doi.org/10.1016/j.ijpharm.2009.10.004.
111. Clemens, D. L., Lee, B. Y., Xue, M., Thomas, C. R., Meng, H., Ferris, D., Nel, A. E., Zink, J. I., & Horwitz, M. A. (2012). Targeted intracellular delivery of antituberculosis drugs to *Mycobacterium tuberculosis* infected macrophages via functionalized mesoporous silica nanoparticles. *Antimicrobial Agents and Chemotherapy, 56*, 2535–2545. https://doi.org/10.1128/AAC.06049-11.
112. Hwang, A. A., Lee, B. Y., Clemens, D. L., Dillon, B. J., Zink, J. I., & Horwitz, M. A. (2015). Tuberculosis: pH responsive isoniazid loaded nanoparticles markedly improve tuberculosis treatment in mice (Small 38/2015). *Small, 11*, 5065. https://doi.org/10.1002/smll.201570235.

113. Abed, N., Saïd-Hassane, F., Zouhiri, F., Mougin, J., Nicolas, V., Desmaële, D., Gref, R., & Couvreur, P. (2015). An efficient system for intracellular delivery of β-lactam antibiotics to overcome bacterial resistance. *Scientific Reports, 5*, 13500. https://doi.org/10.1038/srep13500.
114. Smitha, K. T., Nisha, N., Maya, S., Biswas, R., & Jayakumar, R. (2015). Delivery of rifampicin-chitin nanoparticles into the intracellular compartment of polymorphonuclear leukocytes. *International Journal of Biological Macromolecules, 74*, 36–43. https://doi.org/10.1016/j.ijbiomac.2014.11.006.
115. Monteiro, D. R., Gorup, L. F., Takamiya, A. S., Ruvollo, A. C., Camargo, E. R., & Barbosa, D. B. (2009). The growing importance of materials that prevent microbial adhesion: antimicrobial effect of medical devices containing silver. *International Journal of Antimicrobial Agents, 34*, 103–110. https://doi.org/10.1016/j.ijantimicag.2009.01.017.
116. Oldenburg, S. J. (2017). *Silver nanoparticles: Properties and applications*. Sigma-Aldrich, Sigma-Aldrick.
117. Wagener, M. (2006). Antimicrobial coatings: Nanocomposite coatings can reduce infections within a medical environment. *Polymers Paint Colour Journal, 610*. http://dialog.proquest.com/professional/docview/773702398?accountid=156179.
118. Yu, Q., Li, J., Zhang, Y., Wang, Y., Liu, L., & Li, M. (2016). Inhibition of gold nanoparticles (AuNPs) on pathogenic biofilm formation and invasion to host cells. *Scientific Reports, 6*, 26667. https://doi.org/10.1038/srep26667.
119. Chamundeeswari, M., Sobhana, S. S. L., Jacob, J. P., Kumar, M. G., Devi, M. P., Sastry, T. P., & Mandal, A. B. (2010). Preparation, characterization and evaluation of a biopolymeric gold nanocomposite with antimicrobial activity. *Biotechnology and Applied Biochemistry, 55*, 29–35. https://doi.org/10.1042/BA20090198.
120. Brown, A. N., Smith, K., Samuels, T. A., Lu, J., Obare, S. O., & Scott, M. E. (2012). Nanoparticles functionalized with ampicillin destroy multiple-antibiotic-resistant isolates of *Pseudomonas aeruginosa* and *Enterobacter aerogenes* and methicillin-resistant *Staphylococcus aureus*. *Applied and Environmental Microbiology, 78*, 2768–2774. https://doi.org/10.1128/AEM.06513-11.
121. Lackner, P., Beer, R., Broessner, G., Helbok, R., Galiano, K., Pleifer, C., Pfausler, B., Brenneis, C., Huck, C., Engelhardt, K., Obwegeser, A. A., & Schmutzhard, E. (2008). Efficacy of silver nanoparticles-impregnated external ventricular drain catheters in patients with acute occlusive hydrocephalus. *Neurocritical Care, 8*, 360–365. https://doi.org/10.1007/s12028-008-9071-1.
122. Shi, Z., Neoh, K. G., Kang, E. T., & Wang, W. (2006). Antibacterial and mechanical properties of bone cement impregnated with chitosan nanoparticles. *Biomaterials, 27*, 2440–2449. https://doi.org/10.1016/j.biomaterials.2005.11.036.
123. Vahedi, M., Hosseini-Jazani, N., Yousefi, S., & Ghahremani, M. (2017). Evaluation of antibacterial effects of nickel nanoparticles on biofilm production by *Staphylococcus epidermidis*. *Iranian Journal of Microbiology, 9*, 160–168.
124. Mubarak Ali, D., Arunkumar, J., Pooja, P., Subramanian, G., Thajuddin, N., & Alharbi, N. S. (2015). Synthesis and characterization of biocompatibility of tenorite nanoparticles and potential property against biofilm formation. *Saudi Pharmaceutical Society, 23*, 421–428. https://doi.org/10.1016/j.jsps.2014.11.007.
125. Namasivayam, S. K. R., Christo, B. B., Arasu, S. M. K., Kumar, K. A. M., & Deepak, K. (2013). Anti biofilm effect of biogenic silver nanoparticles coated medical devices against biofilm of clinical isolate of *Staphylococcus aureus*. *Global Journal of Medical Research, 13*, 25–30.
126. Fazly Bazzaz, B. S., Khameneh, B., Zarei, H., & Golmohammadzadeh, S. (2016). Antibacterial efficacy of rifampin loaded solid lipid nanoparticles against *Staphylococcus epidermidis* biofilm. *Microbial Pathogenesis, 93*, 137–144. https://doi.org/10.1016/j.micpath.2015.11.031.
127. Hetrick, E. M., Shin, J. H., Paul, H. S., & Schoenfisch, M. H. (2009). Anti-biofilm efficacy of nitric oxide-releasing silica nanoparticles. *Biomaterials, 30*, 2782–2789. https://doi.org/10.1016/j.biomaterials.2009.01.052.

128. Shakibaie, M., Forootanfar, H., Golkari, Y., Mohammadi-Khorsand, T., & Shakibaie, M. R. (2015). Anti-biofilm activity of biogenic selenium nanoparticles and selenium dioxide against clinical isolates of *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Proteus mirabilis*. *Journal of Trace Elements in Medicine and Biology, 29*, 235–241. https://doi.org/10.1016/j.jtemb.2014.07.020.
129. Rajkumari, J., Busi, S., Vasu, A. C., & Reddy, P. (2017). Facile green synthesis of baicalein fabricated gold nanoparticles and their antibiofilm activity against *Pseudomonas aeruginosa* PAO1. *Microbial Pathogenesis, 107*, 261–269. https://doi.org/10.1016/j.micpath.2017.03.044.
130. Kalishwaralal, K., BarathManiKanth, S., Pandian, S. R., Deepak, V., & Gurunathan, S. (2010). Silver nanoparticles impede the biofilm formation by *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*. *Colloids and Surfaces B Biointerfaces, 79*, 340–344. https://doi.org/10.1016/j.colsurfb.2010.04.014\rS0927-7765(10)00217-1.
131. Sangani, M. H., Moghaddam, M. N., & Forghanifard, M. M. (2015). Inhibitory effect of zinc oxide nanoparticles on *Pseudomonas aeruginosa* biofilm formation. *Nanomedicine Journal, 2*, 121–128. https://doi.org/10.7508/nmj.2015.02.004.
132. Cheow, W. S., Chang, M. W., & Hadinoto, K. (2010). Antibacterial efficacy of inhalable antibiotic-encapsulated biodegradable polymeric nanoparticles against *E. coli* biofilm cells. *Journal of Biomedical Nanotechnology, 6*, 391–403. https://doi.org/10.1166/jbn.2010.1116.
133. Mohankandhasamy, R., Lee, J. H., & Lee, J. (2017). Development of gold nanoparticles coated with silica containing the antibiofilm drug cinnamaldehyde and their effects on pathogenic bacteria. *International Journal of Nanomedicine, 12*, 2813–2828. https://doi.org/10.2147/IJN.S132784.
134. Cheow, W. S., Chang, M. W., & Hadinoto, K. (2011). The roles of lipid in anti-biofilm efficacy of lipid-polymer hybrid nanoparticles encapsulating antibiotics. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 389*, 158–165. https://doi.org/10.1016/j.colsurfa.2011.08.035.
135. Shi, S., Jia, J., Xiao-kui, G., Zhao, Y., Chen, D., Guo, Y., & Zhang, X. (2016). Reduced *Staphylococcus aureus* biofilm formation in the presence of chitosan-coated iron oxide nanoparticles. *International Journal of Nanomedicine, 11*, 6499–6506. https://doi.org/10.2147/IJN.S41371.
136. Kyaw, K., Harada, A., Ichimaru, H., Kawagoe, T., Yahiro, K., Morimura, S., Ono, K., Tsutsuki, H., Sawa, T., & Niidome, T. (2017). Silver nanoparticles as potential antibiofilm agents against human pathogenic bacteria. *Chemistry Letters, 46*, 594–596. https://doi.org/10.1246/cl.161198.
137. Sangani, M. H., Moghaddam, M. N., & Forghanifard, M. M. (2015). Inhibitory effect of zinc oxide nanoparticles on *Pseudomonas aeruginosa* biofilm formation Inhibition of biofilm formation by zinc oxide nanoparticles. *Nanomedicine Journal, 2*, 121–128. https://doi.org/10.7508/nmj.2015.02.004.
138. Shrestha, A., & Kishen, A. (2014). Antibiofilm efficacy of photosensitizer-functionalized bioactive nanoparticles on multispecies biofilm. *Journal of Endodontia, 40*, 1604–1610. https://doi.org/10.1016/j.joen.2014.03.009.
139. Maurer-Jones, M. A., Gunsolus, I. L., Meyer, B. M., Christenson, C. J., & Haynes, C. L. (2013). Impact of TiO_2 nanoparticles on growth, biofilm formation, and flavin secretion in *Shewanella oneidensis*. *Analytical Chemistry, 85*, 5810–5818. https://doi.org/10.1021/ac400486u.
140. Tabrez Khan, S., Ahamed, M., Al-Khedhairy, A., & Musarrat, J. (2013). Biocidal effect of copper and zinc oxide nanoparticles on human oral microbiome and biofilm formation. *Materials Letters, 97*, 67–70. https://doi.org/10.1016/j.matlet.2013.01.085.
141. Bhattacharyya, P., Agarwal, B., Goswami, M., Maiti, D., Baruah, S., & Tribedi, P. (2018). Zinc oxide nanoparticle inhibits the biofilm formation of *Streptococcus pneumoniae*. *Antonie Van Leeuwenhoek, 111*, 89–99. https://doi.org/10.1007/s10482-017-0930-7.

142. Taylor, E. N., & Webster, T. J. (2009). The use of superparamagnetic nanoparticles for prosthetic biofilm prevention. *International Journal of Nanomedicine, 4*, 145–152. https://doi.org/10.2147/IJN.S5976.
143. Kumar, C. G., & Sujitha, P. (2014). Green synthesis of Kocuran-functionalized silver glyconanoparticles for use as antibiofilm coatings on silicone urethral catheters. *Nanotechnology, 25*, 325101. https://doi.org/10.1088/0957-4484/25/32/325101.
144. Berzofsky, J. A., Ahlers, J. D., Janik, J., Morris, J., Oh, S., Terabe, M., & Belyakov, I. M. (2004). Progress on new vaccine strategies against chronic viral infections. *The Journal of Clinical Investigation, 114*, 450–462. https://doi.org/10.1172/JCI22674.

Chapter 11
Surface Modifications of Liposomes for Drug Targeting

Doniya Milani, Umi Athiyah, Dewi Melani Hariyadi, and Yashwant V Pathak

Abstract Medical treatment through the use of pharmaceuticals is dependent on the ability of therapeutic agents to reach their intended targets while evading unintended interactions, endosomal sequestration, and degradation. By developing targeted therapies, our treatments of different diseases can be tremendously improved in ways that not only enhance the functionality of relevant drugs, but also improve the patients' experiences. Liposomes are nanocarriers that encapsulate their payloads, protecting active ingredients from biological environments and degradation. Their use in nanomedicine has the ability to reshape drug administration, from improved specificity and prolonged circulation to decreased cytotoxicity and fewer negative side effects. The efficacy and functionality of liposomes can be further refined and enhanced through surface modification. By conjugating liposomes with various moieties, drug delivery can become a much more targeted process.

Keywords Surface modification · Nanocarriers · Nanotechnology · Cell targeting · Drug targeting · Drug delivery · Liposomes · Stealth · Targeted therapy · PEG

1 Introduction

Nanocarriers have introduced a unique and promising method of drug delivery. One significant challenge of drug administration is ensuring that the drug not only reaches the correct destination but also does so without damaging or affecting the

D. Milani · U. Athiyah
Faculty of Pharmacy, Universitas Airlangga, Surabaya, Indonesia

Y. V Pathak (✉)
College of Pharmacy, University of South Florida, Tampa, FL, USA

Adjunct professor at Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia
e-mail: ypathak1@health.usf.edu

D. M. Hariyadi
College of Pharmacy, University of South Florida, Tampa, FL, USA

Faculty of Public Health, Airlangga University, Surabaya, Indonesia

Y. V Pathak (ed.), *Surface Modification of Nanoparticles for Targeted Drug Delivery*, https://doi.org/10.1007/978-3-030-06115-9_11

surrounding organs, cells, and/or tissues. Nanocarriers can maneuver around the problems typically associated with conventional drug delivery. One of their most exciting capabilities is specifically targeting diseased organs or cells while circumventing healthy, nondiseased organs and cells in the body [1]. Drug delivery through nanoparticles and nanocarriers allows higher efficacy with fewer side effects [1]. Nanocarriers, which range from 1 to 1000 nm, offer several advantageous properties, including high surface to volume ratio, increased tissue and membrane penetration, targeted and controlled drug release, and biological mobility [2].

Liposomes are nanocarriers that protect the active ingredient they are carrying from degradation [3]. They are currently considered to be the most successful drug-carrier system [4]. Liposomes' lipid compositions determine their chemical properties [3]. The efficacy of liposomes can be altered by tweaking their physiochemical properties, such as their size, surface charge, and lipid organization [4]. Much of the research on nanocarriers now focuses on surface modifications that can improve the efficacy of drug targeting. Surface modifications and functionalization with moieties, which alter the range of stimuli recognized, can further fine-tune the liposomes are nanocarriers [1]. Different surface modifications present different benefits. For example, polyethylene glycol (PEG) modification of liposomes can potentially improve blood circulation and reduce nonspecific interactions while avoiding the reticuloendothelial system (RES) that usually poses a significant challenge in intravenous administration [5].

Surface modifications and functionalization of liposomes can tremendously improve the treatment of solid tumors and cancer by presenting ways to overcome the current physiological and biological barriers [6]. Altering these liposomes opens the door for finding new and efficient methods of delivery for anticancer agents [6]. Treating malignant tumors through conventional treatment approaches is a particular obstacle due to their unique physiology. Uncontrollable growth of mutated cells that initiate within an organism's own cells contributes to the difficulty of selective treatment of cancer [6]. However, with the use of nanocarriers and nanoparticles, site-specific delivery of anticancer drugs is a possibility. Considering the tremendous number of deaths per year that cancer is responsible for, improving anticancer drug delivery is vital. This review will explore the current barriers in selective drug targeting, examples of surface modifications of liposomes, the limitations of functionalized and modified liposomes, and how these alterations aid in overcoming the present barriers.

2 Liposomes

Liposomes are spherical artificial vesicles that contain at least one phospholipid bilayer between aqueous phases [7]. These favorable drug delivery systems vary considerably in terms of properties and compositions. Liposomes have contributed significantly to the advancement of drug delivery due to their biocompatibility,

ability to encapsulate hydrophilic and hydrophobic compounds, low toxicity, size, surface charge, improved penetrability, and site-specific targeting [7]. However, liposomes must overcome certain limitations and barriers as well, such as their short half-life, low solubility, and their tendency to occasionally undergo oxidation and hydrolysis-like reactions [7]. By overcoming these barriers, advantages such as biocompatibility and site-specific drug delivery to tumors can be utilized [8].

2.1 Liposome Circulation Time Obstacles

Typical liposomes are taken up by the reticuloendothelial system (RES), which contributes to their shorter half-life [5]. The RES is a crucial defense mechanism of the body [9]. Upon intravenous administration, a liposome is absorbed by Opsonin, a serum protein which recognizes the liposome as a foreign substance [5, 9]. Once a liposome is opsonized, it is demolished by phagocytes that are a part of the RES [9]. The interaction of the RES and liposomes affects organs such as the liver, spleen, and bone marrow [10]. Another challenge that typically faces liposomes is their tendency to release their contents during circulation. This problem can be circumvented through surface modification; as an effort to increase the circulation time of a liposome, it can be modified with a hydrophilic polymer, polyethyleneglycol (PEG) [5, 9]. These liposomes are referred to as PEGylated or stealth liposomes [9]. Once a liposome's surface is PEGylated, it becomes more stable and evasive, hence the name "stealth" liposome.

3 PEGylated Liposomes

3.1 Advantages of PEGylated Liposomes

PEGylated liposomes have several advantages. These modified nanocarriers have increased circulation time in systemic circulation and a decreased uptake by the phagocytes in the RES [11]. As opposed to non-PEGylated liposomes, stealth liposomes have a much greater ease of entry due to the enhanced permeability and retention (EPR) effect, and improved riddance of tumors due to the increased accumulation in the tumor tissue [11]. PEGylated molecules have a heavier weight, which lessens its clearance through glomerular filtration [12]. This in turn augments the drug efficacy [12]. Additionally, PEGylation modifies and disguises the protein surface, which minimizes the body's immune response [12].

Following the discovery of stealth liposomes, site-specific liposomes came into existence. By conjugating the open ends of PEG polymers with different moieties, such as antibodies and peptides, the liposomes can engage in more specific targeting [13]. This surface functionalization allows the liposomes to interact

with particular overexpressed receptors that are located on the cell surface [13]. Neutral PEGylated liposomes can be easily modified and have multifunctional proteins on their surface [14].

In addition to their stability and increased circulation time, PEGylated liposomes can also avoid enzymatic degradation [12]. The PEG-liposomes are eliminated from the body in a different way that is related to molecular mass, which is heavier than a normal liposome due to its increased water solubility [12]. The increased weight also allows for reduction in clearance via glomerular filtration [15, 16]. PEGylated liposomes with a weight below 20 kDa are eradicated through renal filtration, whereas the heavier ones are primarily eliminated by the liver [16].

3.2 Targeted Therapy Through PEGylation

Because of the EPR effect that PEGylation brings about, stealth liposomes have the ability to specifically target tumors [17]. In a study done by Matsumura and coworkers, the EPR effect was first introduced. The study characterized tumors as having leaky vasculature and poor lymphatic systems [18]. While fenestrated endothelium gaps normally range in size from 200 to 900 nm, the openings within tumor vasculature are even larger [19]. This allows nanoparticles to favorably accumulate in tumors [19]. This preferential accumulation improves the efficacy of drug delivery by allowing more targeted delivery.

The creation of nanoparticles that can target virtually anything in the body is an exciting feat for pharmaceuticals. However, this poses its own challenges as well, including the issue of systemic toxicity [17]. During circulation, nanoparticles may encounter erythrocytes. Their interactions can lead to erythrocyte aggregation and/or hemolysis [17]. This is of particular significance with cationic nanoparticles that are attracted to negatively charged cell surfaces due to electrostatic interactions between the two [17]. However, PEGylation can decrease hemolysis and erythrocyte aggregation through conjugation [20]. When conjugated to PEG, polymers such as polylysine (PLL) and poly(ethyleneimine) (PEI) showed decreased cytotoxicity [21].

3.3 PEGylation and Surface Modification as a Method of Penetrating Mucus

PEGylation is also useful for increasing penetration efficacy through mucus [22]. Mucus is a barrier that protects the human body from a plethora of foreign invaders, including viruses and bacteria [22]. Its mesh-like structure and intricate composition make mucus a challenging barrier for pharmaceutical advancement [22]. For issues such as mucus permeation, several alternative polymers (in addition to PEG) have been researched. Polymers such as poly(2-alkyl-2-oxazolines), polysarcosine,

poly(vinyl alcohol), zwitterionic polymers, and mucolytic enzymes are some of the alternatives that have been considered as methods of improving and enabling mucus permeation of nanoparticles [22]. Certain polymers are able to adhere to the surfaces of mucosal membranes, thus improving the drug's bioavailability [23]. This is of particular relevance when dealing with drug delivery to the airways; overcoming the dilemma of efficient drug delivery through mucus in the airways could open the door to new treatments for serious conditions such as cystic fibrosis [24]. In a series of studies conducted by Hanes et al., a method of enhancing mucosal penetration of nanoparticles was discovered [25, 26]. Nanoparticles with poor diffusion abilities in mucus were functionalized using PEG and the result was superior penetration and diffusion [25, 26]. This improvement is due to the reduction of mucoadhesion, preventing hydrophobic or electrostatic interactions, thus allowing the nanoparticles to permeate the mucus [22].

4 The Effects of Surface Functionalization on Liposomes

4.1 Interactions in the Body

A disadvantage of a nanoparticle is its interactions with biological fluids, which have high ionic strengths [27]. Due to the nature of nanoparticles and the interactions in biological fluids, colloidal stability may be weakened, leading to aggregation of nanoparticles [27]. Colloidal stability is especially weakened in the presence of aggregation. The two primary approaches used when addressing colloidal stabilization are stabilization through electrostatic repulsion and steric stabilization [27]. Functionalization is achieved by attaching polymers to the surface of the liposome. Attaching polymers such as PEG or poly(vinyl pyrrolidone) (PVP) can increase the stability of nanoparticles in biological fluid [28].

4.2 Liposomal Surface Chemistry

Surface functionalization of a liposome also requires considering surface chemistry. Incorporation of a given ligand onto the surface of a nanoparticle requires considering the nanoparticle composition and surface affinity [27]. There are three general classes of chemical groups when dealing with surface functionalization. The first class is noble metals, such as Au and Ag, that are typically functionalized with thiols, amines, and cyanides [27]. The second class is oxides, which are primarily used with magnetic nanoparticles and can be coated with acidic or hydroxyl groups using oxygen bonding [27]. Lastly, there are binary compounds (elements from Groups 12–16 on the Periodic Table), which have high affinities toward thiols and hydroxyl groups, but also may use amino groups [27].

PEG head groups can be modified in a variety of ways, making these molecules extremely versatile and advantageous. This presents several opportunities and possibilities for further bio-functionalization of liposomal surfaces [29]. Examples of terminal functional moieties that are used to modify PEG head groups include carboxylic (–COOH) and amine ($–NH_2$) due to the fact that they can be introduced into PEG molecules without compromising the PEGylated nanoparticle's colloidal stability in blood and plasma [29, 30]. The ease of PEG head group modification combined with the hydrophilic nature of PEG that enables steric stabilization is why PEG is the preferred, and most widely used, ligand [31].

4.3 PEG Coating

PEG coating is done in different ways, depending on the class of the nanoparticle. As mentioned, the first class is plasmonic nanoparticles (the noble metals). Plasmonic nanoparticles' surfaces are commonly coated with thiol-terminated derivatives (PEG-SH) through covalent bonding [27]. For citrate-stabilized nanoparticles, this is simply accomplished by adding a solution of PEG-SH to the nanoparticles, which eventually leads to ligand exchange with PEG-SH, creating solutions that are extremely stable in solutions with high ionic strengths and also in biological fluids [27, 32].

4.4 Alternative Ligands to PEG

Although PEG is the most widely used ligand, alternative ligands are also used to increase the stability of nanoparticles in biological fluids. Zwitterionic ligands, ligands that have mixed charge functional groups that contribute to a net charge of zero, have also been used with nanoparticles. In fact, nanoparticles with zwitterionic ligands show even lower levels of opsonization than PEGylated liposomes [33]. In a study conducted by Bawendi et al., the importance of surface charge arrangement was considered [34]. The study demonstrated that zwitterionic nanoparticles with positive charges on their outermost surface displayed nonspecific accumulation and absorption, whereas zwitterionic nanoparticles with negative surface charges showed minimal nonspecific absorptions and fewer protein interactions [34]. There were also differences between the in vivo behavior of zwitterionic nanoparticles and nonionic nanoparticles. The study elucidated that exposed charged groups enhanced the interactions between zwitterionic nanoparticles and the environment [34]. This study highlighted the importance of considering spatial arrangement of charge when creating nanoparticles to be used in vivo. In order to minimize nonspecific interactions, specific attention to the spatial arrangement of positive charges needs to be considered [34].

5 Variables Affecting PEG Liposomes

5.1 PEG Molecular Weight

The circulation time of PEGylated liposomes is dependent on several different variables, such as PEG molecular weight, content, conformation, and surface density [17]. The molecular weight of a PEG chain is proportional to the length of its polymer chain, thus making the molecular weight a pertinent element of surface shielding ability [17]. In a study performed by Duvall et al. using 50D mixed micelles administered intravenously in vivo, results indicated that increasing the PEG molecular weight in the corona significantly increased its half-life for blood circulation [35]. In a different study, PEGylated liposomes were coated with 750 kDa PEG and compared to non-PEGylated liposomes [36]. Although the two were initially comparable, increasing the PEG molecular weight to 5 kDa resulted in extended circulation time and decreased uptake by the mononuclear phagocyte system [36]. Since opsonin proteins in the blood serum easily bind to non-PEGylated nanoparticles, they can be quickly recognized and eliminated by the mononuclear phagocytic system, preventing the nanoparticles to effectively do their jobs [37, 38]. By modifying the surface of a nanoparticle with the addition of a PEG polymer, a hydrophilic layer is formed around the nanoparticle that creates steric repulsion forces, protecting the nanoparticle from opsonin proteins [38]. Generally, the minimum PEG molecular weight required to effectively shield nanoparticle surfaces from opsonin proteins and opsonization by the mononuclear phagocyte system is 2 kDa or greater [38]. However, this number is subject to change in certain circumstances. For example, according to a different study, human monocytic leukemia cell line-derived macrophages (THP-1) require a PEG molecular weight of at least 10 kDa in order to achieve effective shielding of nanoparticles [39]. However, PEG molecular weight may alter the molecule's density, thus creating a possible confounding factor that can influence results.

5.2 PEG Surface Density and Conformation

In addition to PEG molecular weight, PEG surface density and conformation are important factors influencing circulation time and varying interactions with components of the blood. In a study conducted by Yang et al., it was discovered that a "dense brush" conformation was necessary in order to avoid uptake by human monocytic THP-1 cells in vitro [40]. Brush conformations are assumed by the PEG chains at higher grafting densities when the Flory radius (R_F) of the PEG coils divided by the grafting distance (D) equals greater than 1 [40]. The "dense brush" conformation required for evasion of THP-1 cells is adopted when the PEG layer thickness is greater than the Flory radius by at least twofold ($R_F/D > 2.8$) [40]. The authors of the study also discovered that increases in PEG surface density were necessary for increased blood circulation time in vivo [40]. Yang and coworkers concluded that

nanoparticle interactions with the mononuclear phagocyte system cells significantly depend on PEG chain conformations and that a highly dense brush conformation is critical for increasing circulation time [40].

6 Liposome Drug Entrapment

Another challenge with liposomes is drug entrapment efficiency. Liposomes entrap lipophilic drugs in the lipid film and hydrophilic drugs in the aqueous part [4]. While entrapment of lipophilic drugs is typically easily accomplished, hydrophilic drugs exhibit encapsulation efficiency with liposomes [41]. A promising method of accomplishing entrapment of hydrophilic drugs into liposomes is the microencapsulation vesicle (MCV) method, which uses "water-in-oil-in-water" emulsion [42]. In a study conducted by Kaimoto and coworkers, the MCV method was used to prepare surface-modified liposomes with PEG and a site-directed ligand [42]. In the last phase of the experiment, a peptide ligand with an affinity to adipose tissue vasculature was utilized [42]. After injecting mice with the fluorescent-labeled liposomes, there was considerable liposomal accumulation in the adipose tissue vessels, indicating that the MCV method with solvent optimization may be a very useful technique for achieving high drug entrapment efficiency and targeting delivery in surface-modified liposomes [42].

7 Disadvantages of Surface Modification of Liposomes

7.1 The Effects of PEGylation on Liposomal Stability

Although surface modification via PEGylation is advantageous and offers several improvements in nanoparticle drug delivery systems, some drawbacks do exist. PEG chains have both hydrophobic and hydrophilic tendencies [43]. Because of the hydrophobic characteristics PEG exhibits, the hydration of membrane phospholipid head groups cannot occur [43, 44]. In a study performed by Tirosh et al., it was concluded that hydration in part contributes to the thermodynamic stability of PEG liposomes [44]. Thus, these hydrophobic restraints can lead to destabilization of the liposomes and problems regarding drug entrapment and loading [43, 44]. Following a study that evaluated the effect cholesterol has on preventing the PEG-induced phase separation of lipid 1-palmitoyl-2[6-(pyren-1-yl)]decanoyl-sn-glycero-3-phosphocholine (PPDPC), it was suggested that excess cholesterol be used in PEGylated liposomes due to its ability to lessen PEG chain–chain interactions [45]. PEGylation can also negatively affect the stability of liposome preparation in relation to storage conditions [43]. However, long-term stability is possible through freezing and freeze-drying in conjunction with the use of a cryoprotective agent [46]. Cryoprotective agents aid in the prevention of liposomal fusion or degradation during the freezing process [46].

7.2 Immunogenic Responses of PEGylated Liposomes

In addition to these drawbacks of using PEGylated liposomes, it has been shown that they can also prompt adverse immunogenic responses, such as acute immune toxicity, that arise not through IgE like immediate type I hypersensitivity reactions, but rather the activation of the complement system [47, 48]. These responses present themselves through hypersensitivity reactions [47]. These infusion reactions that may be induced by PEGylated liposomes are referred to as complement activation-related pseudoallergy (CARPA) [47, 48]. There are a multitude of factors that play a role in a PEG-liposome's ability to activate the complement system, including hydrophobicity, the inclusion of cholesterol, and particle size [47–49]. In addition to these, the presence of preexisting PEG antibodies in people have been considered and implicated in the manifestation of infusion reactions [48, 50]. The presence of these anti-PEG antibodies can affect the targeting, efficacy, and clearance rate of a liposome-encapsulated drug [51], thus making it an important area for research. The consideration of the possible limitations, disadvantages, and consequences of PEG liposomes has increased interest in the discovery and utilization of different lipopolymers as PEG substitutes.

7.3 In Vivo Effects of Surface-Modified Liposomes

The goal of surface modification of liposomes is to improve their overall functionality by overcoming some of the properties of regular liposomes while avoiding or minimizing the effects to the clearance mechanisms of the body [43]. However, this has not been entirely perfected yet. Although PEG liposomes exhibit higher circulation times, the PEG-induced stealth properties of liposomes have finite extents [52]. As the stealth properties of these polymer coatings diminish, these surface-modified liposomes are eventually recognized and cleared by the mononuclear phagocyte system [52], thus limiting the in vivo stabilization effects of PEGylation.

Another in vivo consequence of extended system circulation is an increase in the possibility of drug interaction and exposure of the other nontargeted tissues, potentially leading to toxicity [53]. In some cases, this potential for toxicity challenges the very use of PEGylated liposomes in certain treatments [54].

To improve the in vivo efficacy of PEGylated liposomes, targeting strategies can be utilized. For example, the addition of a ligand to a PEG liposome can improve its activity in comparison to a PEG liposome that is without a ligand. This has been demonstrated in a variety of ways, using a variety of ligands. One example is a study conducted by Yamada et al. in which in vivo antitumor efficacy was assessed through PEGylation and targeting [55]. As a result of PEGylated-induced steric hindrance, the association between a liposome-bound ligand with its receptor can be hampered [55]. This was tested by Yamada and group using three types of folate-linked PEGylated liposomal doxorubicin: untargeted PEGylated doxorubicin-loaded lipo-

somes, non-PEGylated liposomes concealing folate, and PEGylated liposomes with surface exposure to folate [55]. Their research found that the PEGylated, folate-linked, doxorubicin-loaded liposomes exhibited the greatest antitumor efficacy.

8 Conclusion

The use of liposomes in drug delivery is a developing and promising field of nanotechnology that offers a plethora of advancements. As research grows, ways of circumventing the restrictions, limitations, and disadvantages of traditional liposomes appear. A major method of fine-tuning these nanocarriers is surface modification. The most commonly seen routine for this is the addition of a PEG molecule to a liposome. PEGylated liposomes are generally seen as safe, effective, and extremely useful. With their proper usage, drug delivery systems can be redefined to include enhancements such as site-specific delivery of drugs, improved biocompatibility, greater drug efficacy, increased permeation, decreased contact with nontargeted tissues and organs, and decreased toxicity. PEGylated liposomes are currently being used to treat a variety of diseases and ailments. PEG liposomes are being more intensely studied, leading to the discovery of the benefits of ligand attachment. Surface modification of liposomes has facilitated further refinement of these small drug delivery vesicles. The introduction of ligands to PEG liposomes has further expanded the uses and advantages of liposomes, leading to new discoveries in methods of drug delivery and therapeutic treatments.

Although liposomes are generally effective vesicles for drug delivery on a nanoscale, PEG liposomes have some drawbacks that must be considered and continuously researched as an effort to improve the safety and efficacy of surface-modified liposomes as drug delivery systems. However, the benefits of stealth liposomes cannot and should not be ignored. For improving issues such as circulation time, PEGylation is considered to be the gold standard [43]. Despite this, the increase in research on PEG substitutes has led to the discovery of several alternatives that provide a whole gamut of refinements and improvements to surface-modified liposomes.

9 Future Trends

Surface-modified liposomes have proven to be effective and useful vesicles for systemic delivery of drugs. By altering traditional liposomes via PEGylation, these nanoparticles have managed to overcome several of the limitations and barriers that liposomes have faced. PEGylation is one of the primary methods of modification being studied. PEG coatings on liposomes have increased overall function, safety, and efficacy of drug agents. These nanocarriers are certainly going to continue being applied in clinical settings.

The expansion of research on ligand additions to PEGylated liposomes will lead to the discovery of safer, more specific, and more efficacious systems of drug delivery. Surface-modified liposomes offer several advantages over traditional liposomes, such as prolonged circulation, preferential accumulation, subcellular targeting, decreased toxicity, enhanced cellular uptake, and new treatment strategies for countless diseases, including cancer. This may fundamentally change the outcomes and overall experiences of afflicted individuals. In addition, more efficacious vaccines can be delivered, improving general public health, leading to a decrease in healthcare costs. Lower concentrations of therapeutic agents required to achieve intended effects, new methods of disease prevention, and early detection and diagnostic abilities can significantly lower the costs of medical treatment for patients, practitioners, and pharmaceutical companies. However, more research is required before nanocarriers become widespread. In the future, there will most likely be an increase in the versatility of and demand for surface-modified nanocarriers and particles in medical, agricultural, electronic, and environmental fields. Nanocarriers, albeit small, offer huge incentives and possibilities that may reshape our approach to life entirely. Thus, it is in the interest of both economics and innovation to continue researching and improving nanocarriers.

References

1. Fakhar ud, D., et al. (2017). Effective use of nanocarriers as drug delivery systems for the treatment of selected tumors. *International Journal of Nanomedicine, 12*, 7291–7309. PMC. Web: August 28, 2018, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5634382/
2. Siafaka, P. I., Okur, N. Ü., Karavas, E., & Bikiaris, D. N. (2016). Surface modified multifunctional and stimuli responsive nanoparticles for drug targeting: Current status and uses. *International Journal of Molecular Sciences, 17*(9), 1440. MDPI. Accessed August 28, 2018, from http://www.mdpi.com/1422-0067/17/9/1440/htm
3. Kothalawala, N., Mudalige, T. K., Sisco, P., & Linder, S. W. (2018). Novel analytical methods to assess the chemical and physical properties of liposomes. *Journal of Chromatography B, 1091*, 14–20.
4. Bozzuto, G., & Molinari, A. (2015). Liposomes as nanomedical devices. *International Journal of Nanomedicine*, 975–999. https://doi.org/10.2147/ijn.s68861.
5. Riaz, M., et al. (2018). Surface Functionalization and targeting strategies of liposomes in solid tumor therapy: A review. *International Journal of Molecular Sciences, 19*, 195.
6. Sriraman, S. K., Aryasomayajula, B., & Torchilin, V. P. (2014). Barriers to drug delivery in solid tumors. *Tissue Barriers, 2*, e29528. https://doi.org/10.4161/tisb.29528.
7. Akbarzadeh, A., et al. (2013). Liposome: Classification, preparation, and applications. *Nanoscale Research Letters, 8*(1), 102. https://doi.org/10.1186/1556-276X-8-102. PMC. Web: August 22, 2018.
8. Hofheinz, R. D., Gnad-Vogt, S. U., Beyer, U., & Hochhaus, A. (2005). Liposomal encapsulated anti-cancer drugs. *Anti-Cancer Drugs, 16*, 691–707. https://doi.org/10.1097/01.cad.0000167902.53039.5a.
9. Hatakeyama, H., Akita, H., & Harashima, H. (2013). The polyethyleneglycol dilemma: Advantage and disadvantage of PEGylation of liposomes for systemic genes and nucleic acids delivery to tumors. *Biological and Pharmaceutical Bulletin, 36*, 892–899.

10. Immordino, M. L., Dosio, F., & Cattel, L. (2006). Stealth liposomes: Review of the basic science, rationale, and clinical applications, existing and potential. *International Journal of Nanomedicine, 1*(3), 297–315. Print.
11. Liu, X., Peng, H., & Wang, Q. (2014). Surface engineering of liposomal formulations for targeted drug delivery. *Chemical Engineering and Process Techniques, 2*(1), 1024.
12. Milla, P., Dosio, F., & Cattel, L. (2012). PEGylation of proteins and liposomes: A powerful and flexible strategy to improve the drug delivery. *Current Drug Metabolism, 13*, 105. https://doi.org/10.2174/138920012798356934.
13. Shen, Z., Ye, H., Kröger, M., & Li, Y. (2018). Aggregation of polyethylene glycol polymers suppresses receptor-mediated endocytosis of PEGylated liposomes. *Nanoscale, 10*, 4545–4560.
14. Fisher, R. K., et al. (2017). Improving the efficacy of liposome-mediated vascular gene therapy via lipid surface modifications. *Journal of Surgical Research, 219*, 136–144.
15. Harris, J. M., Martin, N. E., & Modi, M. (2001). Pegylation: a novel process for modifying pharmacokinetics. *Clinical Pharmacokinetics, 40*(7), 539–551.
16. Roberts, M. J., Bentley, M. D., & Harris, J. M. (2002). Chemistry for peptide and protein PEGylation. *Advanced Drug Delivery Reviews, 54*(4), 459–476.
17. Suk, J. S., et al. (2016). PEGylation as a strategy for improving nanoparticle-based drug and gene delivery. *Advanced Drug Delivery Reviews, 99*(Pt A), 28–51. PMC. Web: August 23, 2018 from https://www.ncbi.nlm.nih.gov/pubmed/26456916
18. Matsumura, Y., & Maeda, H. (1986). A new concept for macromolecular therapeutics in cancer chemotherapy: Mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. *Cancer Research, 46*, 6387–6392.
19. Hobbs, S. K., et al. (1998). Regulation of transport pathways in tumor vessels: Role of tumor type and microenvironment. *Proceedings of the National Academy of Sciences of the United States of America, 95*(8), 4607–4612. Print.
20. Qi, R., Gao, Y., Tang, Y., He, R. R., Liu, T. L., He, Y., Sun, S., Li, B. Y., Li, Y. B., & Liu, G. (2009). PEG-conjugated PAMAM dendrimers mediate efficient intramuscular gene expression. *The AAPS Journal, 11*, 395–405.
21. Jevprasesphant, R., Penny, J., Jalal, R., Attwood, D., McKeown, N. B., & D'Emanuele, A. (2003). The influence of surface modification on the cytotoxicity of PAMAM dendrimers. *International Journal of Pharmaceutics, 252*, 263–266. https://doi.org/10.1016/S0378-5173(02)00623-3.
22. Khutoryanskiy, V. V. (2018). Beyond PEGylation: Alternative surface-modification of nanoparticles with mucus-inert biomaterials. *Advanced Drug Delivery Reviews, 124*, 140–149.
23. Sosnik, A., das Neves, J., & Sarmento, B. (2014). Mucoadhesive polymers in the design of nano-drug delivery systems for administration by non-parenteral routes: A review. *Progress in Polymer Science, 39*, 2030–2075.
24. Schneider, C. S., et al. (2017). Nanoparticles that do not adhere to mucus provide uniform and long-lasting drug delivery to airways following inhalation. *Science Advances, 3*, e1601556.
25. Mert, O., et al. (2012). A poly(ethylene glycol)-based surfactant for formulation of drug-loaded mucus penetrating particles. *Journal of Controlled Release, 157*, 455–460.
26. Xu, Q. G., Boylan, N. J., Cai, S. T., Miao, B., Patel, H., & Hanes, J. (2013). Scalable method to produce biodegradable nanoparticles that rapidly penetrate human mucus. *Journal of Controlled Release, 170*, 279–286.
27. Guerrini, L., Alvarez-Puebla, R. A., & Pazos-Perez, N. (2018). Surface modifications of nanoparticles for stability in biological fluids. *Materials, 11*, 1154.
28. Gref, R., Lück, M., Quellec, P., Marchand, M., Dellacherie, E., Harnisch, S., Blunk, T., & Müller, R. H. (2000). 'Stealth' corona-core nanoparticles surface modified by polyethylene glycol (PEG): Influences of the corona (PEG chain length and surface density) and of the core composition on phagocytic uptake and plasma protein adsorption. *Colloids and Surfaces B: Biointerfaces, 18*, 301–313.
29. Thanh, N. T. K., & Green, L. A. W. (2010). Functionalization of nanoparticles for biomedical applications. *Nano Today, 5*, 213–230.

30. Carril, M., Padro, D., Del Pino, P., Carrillo-Carrion, C., Gallego, M., & Parak, W. J. (2017). In situ detection of the protein corona in complex environments. *Nature Communications, 8*, 1542.
31. Zhang, G., Yang, Z., Lu, W., Zhang, R., Huang, Q., Tian, M., Li, L., Liang, D., & Li, C. (2009). Influence of anchoring ligands and particle size on the colloidal stability and in vivo biodistribution of polyethylene glycol-coated gold nanoparticles in tumor-xenografted mice. *Biomaterials, 30*, 1928–1936.
32. Rahme, K., Nolan, M. T., Doody, T., McGlacken, G. P., Morris, M. A., O'Driscoll, C., & Holmes, J. D. (2013). Highly stable pegylated gold nanoparticles in water: Applications in biology and catalysis. *RSC Advances, 3*, 21016–21024.
33. Longmire, M., Choyke, P. L., & Kobayashi, H. (2008). Clearance properties of nano-sized particles and molecules as imaging agents: Considerations and caveats. *Nanomedicine, 3*, 703–717.
34. Han, H.-S., et al. (2013). Spatial charge configuration regulates nanoparticle transport and binding behavior *in vivo*. *Angewandte Chemie (International edition in English)*, *52*(5), 1414–1419. PMC. Web: August 24, 2018.
35. Miteva, M., et al. (2015). Tuning PEGylation of mixed micelles to overcome intracellular and systemic siRNA delivery barriers. *Biomaterials*, *38*, 97–107. PMC. Web: August 25, 2018.
36. Mori, A., Klibanov, A. L., Torchilin, V. P., & Huang, L. (1991). Influence of the steric barrier activity of amphipathic poly(ethyleneglycol) and ganglioside GM1on the circulation time of liposomes and on the target binding of immunoliposomes in vivo. *FEBS Letters, 284*, 263–266.
37. Gref, R., Domb, A., Quellec, P., et al. (1995). The controlled intravenous delivery of drugs using PEG-coated sterically stabilized nanospheres. *Advanced Drug Delivery Reviews, 16*, 215–233.
38. Owensiii, D., & Peppas, N. (2006). Opsonization, biodistribution, and pharmacokinetics of polymeric nanoparticles. *International Journal of Pharmaceutics, 307*, 93–102.
39. He, Q., Zhang, J., Shi, J., Zhu, Z., Zhang, L., Bu, W., Guo, L., & Chen, Y. (2010). The effect of PEGylation of mesoporous silica nanoparticles on nonspecific binding of serum proteins and cellular responses. *Biomaterials, 31*, 1085–1092.
40. Yang, Q., Jones, S. W., Parker, C. L., Zamboni, W. C., Bear, J. E., & Lai, S. K. (2014). Evading immune cell uptake and clearance requires PEG grafting at densities substantially exceeding the minimum for brush conformation. *Molecular Pharmaceutics, 11*, 1250–1258.
41. Eloy, J. O., et al. (2014). Liposomes as carriers of hydrophilic small molecule drugs: Strategies to enhance encapsulation and delivery. *Colloids and Surfaces B: Biointerfaces, 123*, 345–363.
42. Kajimoto, K., Katsumi, T., Nakamura, T., Kataoka, M., & Harashima, H. (2018). Liposome microencapsulation for the surface modification and improved entrapment of cytochrome c for targeted delivery. *Journal of the American Oil Chemists Society, 95*, 101–109.
43. Nag, O. K., & Awasthi, V. (2013). Surface engineering of liposomes for stealth behavior. *Pharmaceutics*, *5*(4), 542–569. PMC. Web: August 27, 2018.
44. Tirosh, O., et al. (1998). Hydration of polyethylene glycol-grafted liposomes. *Biophysical Journal, 74*(3), 1371–1379.
45. Lehtonen, J. Y., & Kinnunen, P. K. (1995). Poly(ethylene Glycol)-induced and temperature-dependent phase separation in fluid binary phospholipid membranes. *Biophysical Journal*, *68*(2), 525–535. PMC. Web: August 27, 2018.
46. Stark, B., Pabst, G., & Prassl, R. (2010). Long-term stability of sterically stabilized liposomes by freezing and freeze-drying: Effects of cryoprotectants on structure. *European Journal of Pharmaceutical Sciences, 41*, 546–555. https://doi.org/10.1016/j.ejps.2010.08.010.
47. Szebeni, J. (2005). Complement activation-related pseudoallergy: A new class of drug-induced acute immune toxicity. *Toxicology, 216*, 106–121. https://doi.org/10.1016/j.tox.2005.07.023.
48. Neun, B., Barenholz, Y., Szebeni, J., & Dobrovolskaia, M. (2018). Understanding the role of anti-PEG antibodies in the complement activation by doxil in vitro. *Molecules, 23*, 1700.
49. Szebeni, J., Alving, C. R., Rosivall, L., Bunger, R., Baranyi, L., Bedocs, P., Toth, M., & Barenholz, Y. (2007). Animal models of complement-mediated hypersensitivity reactions to liposomes and other lipid-based nanoparticles. *Journal of Liposome Research, 17*, 107–117.

50. Chen, B. M., Su, Y. C., Chang, C. J., Burnouf, P. A., Chuang, K. H., Chen, C. H., Cheng, T. L., Chen, Y. T., Wu, J. Y., & Roffler, S. R. (2016). Measurement of pre-existing IgG and IgM antibodies against polyethylene glycol in healthy individuals. *Analytical Chemistry, 88*, 10661–10666.
51. Yang, Q., Ma, Y., Zhao, Y., She, Z., Wang, L., Li, J., Wang, C., & Deng, Y. (2013). Accelerated drug release and clearance of pegylated epirubicin liposomes following repeated injections: A new challenge for sequential low-dose chemotherapy. *International Journal of Nanomedicine, 8*, 1257–1268.
52. Nag, O. K., Yadav, V. R., Hedrick, A., & Awasthi, V. (2013). Post-modification of preformed liposomes with novel non-phospholipid poly(ethylene glycol)-conjugated hexadecylcarbamoylmethyl hexadecanoic acid for enhanced circulation persistence *in vivo*. *International Journal of Pharmaceutics, 446*, 119–129. https://doi.org/10.1016/j.ijpharm.2013.02.026.
53. Gabizon, A., Goren, D., Horowitz, A. T., Tzemach, D., Lossos, A., & Siegal, T. (1997). Long-circulating liposomes for drug delivery in cancer therapy: A review of biodistribution studies in tumor-bearing animals. *Advanced Drug Delivery Reviews, 24*, 337–344. https://doi.org/10.1016/S0169-409X(96)00476-0.
54. Cui, J., Li, C., Guo, W., Li, Y., Wang, C., Zhang, L., Zhang, L., Hao, Y., & Wang, Y. (2007). Direct comparison of two pegylated liposomal doxorubicin formulations: Is auc predictive for toxicity and efficacy? *Journal of Controlled Release, 118*, 204–215. https://doi.org/10.1016/j.jconrel.2006.12.002.
55. Yamada, A., Taniguchi, Y., Kawano, K., Honda, T., Hattori, Y., & Maitani, Y. (2008). Design of folate-linked liposomal doxorubicin to its antitumor effect in mice. *Clinical Cancer Research, 14*(24), 8161–8168.

Chapter 12
Surface Modification of Nanoparticles to Oppose Uptake by the Mononuclear Phagocyte System

Komal Parmar and Jayvadan K. Patel

Abstract Drug delivery has become an important aspect of medicine field with invention of specific potent molecules. New possibilities by understanding the disease pathways are emerging for its treatment and prevention at early basis. This provides development of customized systems that are designed to achieve specific control. This chapter provides an overview of recent advances in surface modification of nanoparticles to oppose uptake by mononuclear phagocytic system in order to achieve targeted drug delivery.

Keywords Targeted nanotechnology · Surface modification · MPS

1 Introduction

A successful drug delivery depends on the release of an optimal dose of the drug at the required site over a given time period without any side effects. Over the years researchers have investigated novel drug delivery approaches in order to develop ideal drug delivery systems. Genetic mutations and intracellular infections are major challenges of intracellular diseases. Targeted drug delivery provides accumulation of drug concentration into specific regions of interest in the body after successful delivery. It is also referred to as smart drug delivery sometimes, with an ability to bind specifically to the desired site of action. However, the task of targeting a desirable site in vivo is challenging. Here, the challenge is on three fronts: first to find the target for the disease; second to find the appropriate drug molecule to bind to that target and treat the disease; and thirdly to find an appropriate means to carry the drug to the specific site in a stable form.

K. Parmar (✉)
ROFEL, Shri G.M. Bilakhia College of Pharmacy, Vapi, Gujarat, India

J. K. Patel
Nootan Pharmacy College, Faculty of Pharmacy, Sankalchand Patel University, Visnagar, Gujarat, India

Y. V Pathak (ed.), *Surface Modification of Nanoparticles for Targeted Drug Delivery*, https://doi.org/10.1007/978-3-030-06115-9_12

Efficient treatment of intracellular disease relies on the development of small drug molecules or development of nanoparticulate drug delivery system which can diffuse through the intracellular compartment via cell membrane. Limitations associated with development of new small drug molecules persists which increases the gap between understanding of disease mechanism and development of new drug molecules. Nanoparticles with size in nano range have attended much attraction in various fields of medicine [1]. These nanoparticles with specialized functions have opened up the doors for development of more advanced technologies. Nanoparticles offer advantages such as good colloidal stability, effective encapsulation, protection of drug molecules against enzymes and hydrolysis, and ease of preparation method [2]. Thus, nanomedicine (application of nanotechnology in medicine) industry is flourishing day after day where in the medicine works at nanoscale in cellular structures of body. Nanoparticles with their functional chemistry can overcome biological barriers and target even single cell entities for treatment. However, one needs to investigate and understand the clinical interaction of nanoparticles with body system for better efficacy.

Nanocarriers as targeted drug delivery were firstly proposed by Paul Ehrlich in the nineteenth century. In 1960s, firstly nanoparticles were investigated for vaccination processes and then till date various nanotechnology based pharmaceutical products have flourished the market and still many are under investigation [3]. Over the years, many versatile nanocarriers has been investigated successfully with various active molecules for targeted drug delivery including liposomes [4], solid lipid nanoparticles [5], gold nanoparticles [6], silica nanoparticles [7], carbon nanotubes [8], micelles [9, 10], dendrimers [11], nanogels [12], nanoemulsion [13], and nanocrystals [14]. Figure 12.1 demonstrates a schematic representation of various nanoparticles with efficiency to target various organs.

Recent advancement in nanotechnology has influenced diagnosis and treatment procedures of complex diseases like cancer and HIV to a great extent [15–19]. Along with such complex diseases, promising efforts are made in development of novel therapies for the treatment of cardiac and other diseases intended for site-specific administration of drug molecules with minimal side effects [20–24].

Clearance kinetics and biodistribution of nanoparticles are governed by their surface properties and particle size. Small size nanoparticles with size less than 5 μm will be taken up by mononuclear phagocytic system (MPS) in liver and spleen. Surface modification of nanoparticles has received much attention as promising approach in recent years for efficient drug delivery [25–27]. Smart nanocarriers modified to have surfaced positive charge will interact with surface negative charge of cells rapidly and efficiently, which helps endocytosis to occur easily, thereby supporting targeted drug delivery. The choice of polymeric materials plays a major role in preparation of such specific modified nanoparticles. Unique property of nanoparticle is attributed to the polymeric properties utilized in preparation. Thus, here the nanoparticles are modified for specific objectives to be fulfilled while intended for targeted drug delivery. Surface modification of nanoparticles renders specific characteristics on the surface such that it will orient itself toward specific site in the body. Surface modification of nanoparticles is done by simply coating of core with

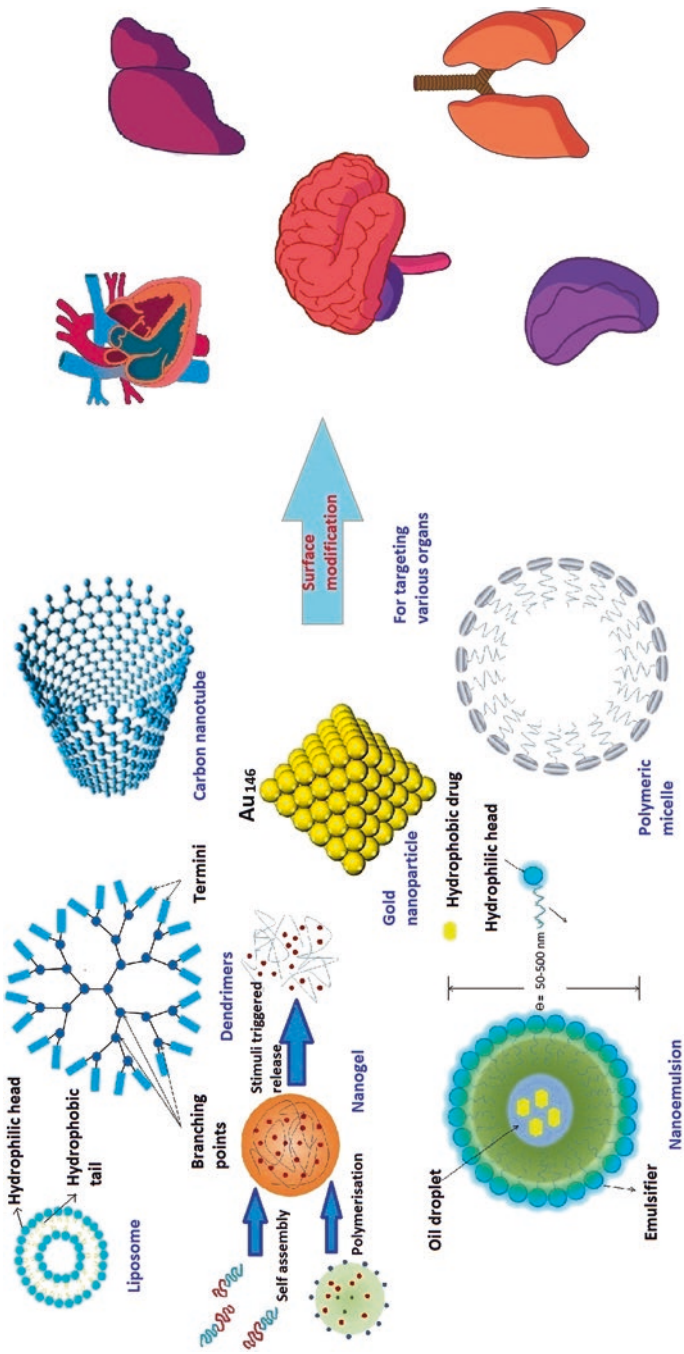


Fig. 12.1 Various nanocarriers capable to target important organs after surface modification

hydrophilic polymers intended for long circulation in body, and/or coupled with specific ligands or proteins for targeted drug delivery in specific site [28–31]. In general, smart use of nanoparticles has revolutionized formulation and delivery of drugs. This chapter focuses on the application of various nanocarriers in targeted drug delivery systems. Emphasis is given to surface modification by using functional agents which enables nanoparticles to oppose the mononuclear phagocytic system and circulate for prolonged time in blood, recognize the environmental properties of the body, communicate and respond appropriately, and also deliver the active molecule to the intended site of action.

2 Surface Modification for Functionalised Nanoparticles

Polymeric nanoparticles are widely utilized in targeted drug delivery. Most synthetic polymers used in the preparation of modified nanoparticles are hydrophobic. Our body recognizes such hydrophobic systems as a foreign material and coats them with blood components, mainly opsonins. Such opsonized modified nanoparticles are readily taken up by the mononuclear phagocytic system (MPS) or reticular endothelial system (RES), peculiarly in the liver [32]. However, when phagocytic system is not targeted, the goal of surface modification turns to protection from MPS/RES. To overcome this problem, new strategies are worked out directing the nanoparticles with targeting capabilities. Mirshafiee et al. (2016) investigated impact of precoating of protein on nanoparticles. They utilized precoating of gamma-globulin which impeded the binding of opsonins on their target cell surface receptors of macrophages, thereby making the nanoparticles available for site-specific delivery [33].

2.1 Prolonged Circulation of Nanoparticles

Long circulating nanoparticles can be obtained by coating the surface with hydrophilic polymers. Such coating prevents opsonization of nanoparticles and thereby protect from MPS/RES uptake [34–36]. Coating with polyethylene glycols (PEGs) is well known for preparing stealth nanoparticles. Such PEG coating provides a protective layer on the surface of nanoparticles which has ability to repel the absorption of opsonin proteins. Steric forces play an important role in such repulsion phenomena which leads to steric stabilization, reducing surface–surface interaction and thereby blocking the initiation of opsonization process [37]. Surface charge density, chain length, and shape of polymers are found to influence the macrophage uptake and surface hydrophilicity of nanoparticles thereby leading to their long circulation in body [38]. Figure 12.2 demonstrates effect of surface charge density of hydrophilic polymer on opsonization. Gref et al. (2000) described advantages of PEGylation of nanoparticles. Nanoparticles without surface modification showed presence of

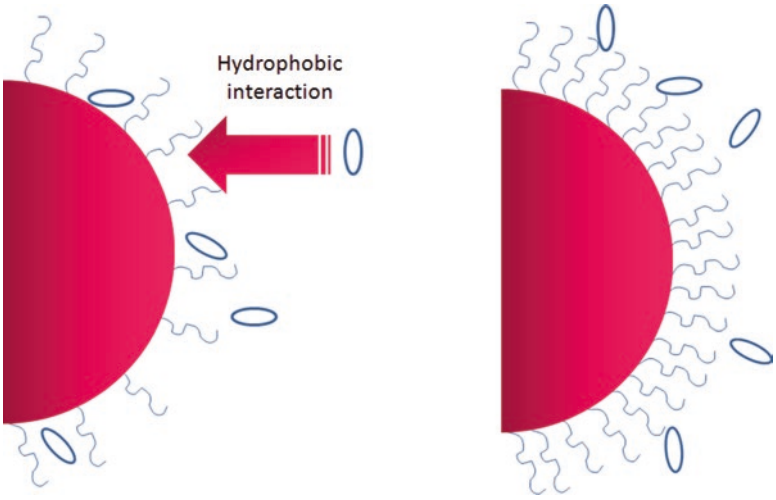


Fig. 12.2 Opsonization prevented due to surface charge density of hydrophilic polymer

apolipoproteins. Further, they reported effect of chain length of PEG on opsonization process on nanoparticles. The results suggested an optimal range of molecular mass of polymer between 2 and 5 kDa which reduced plasma protein adsorption [39]. Dos Santos et al. (2007) reported PEG-Lipid conjugates with prolonged circulation. Further effect of various molecular weights was analyzed on circulation lifetimes to protein binding. The results demonstrated that as little as 0.5 mol% of 1,2-distearoyl-sn-glycero-3-phosphatidylethanolamine (DSPE) modified with PEG having a mean molecular weight of 2000 (DSPE-PEG2000) substantially increased plasma circulation of liposomes [40]. Particle size of nanoparticles also determines the protein corona and thereby influences phagocytic uptake [41, 42]. Biopolymers especially proteins forms a protein corona that is colligated with the nanoparticles. Larger nanoparticles incline to adsorb more protein as compared to lower sizes and thus were found to be readily taken up by the phagocytic system [43, 44].

Poloxamers are another class of polymers which have gained much attention in surface modification and preparation of nanoparticles. These triblock copolymers composed of hydrophilic polyethylene oxide (PEO) chains linked to hydrophobic backbone of polypropylene oxide (PPO), that is, PEO-PPO-PEO when used for the stabilization of nanoparticles, the PEO segment forms an entangled structure which helps the nanoparticles to remain masked from the phagocytic system [45]. Here, PEO shows affinity toward the particle surface, whereas PPO remains on the outer side forming a polymeric star like conformation. At bulk polymer concentrations the polymer concentration reaches to a plateau. The thickness of the adsorption of polymer depends on the hydrophobicity of the particle surface and hydrophilic–lipophilic balance of the poloxamer type. For instance, poloxamer 188 forms a 20-nm thick layer on PLGA (poly (lactic-co-glycolic acid)) nanoparticles. At concentrations equal or more than CMC, growth in thickness of the layer is observed

which is attributed to hemimicelle adsorption [46]. Stolnik et al. (2001) demonstrated the effect of surface coverage of poloxamer 407 on biological fate of nanoparticles. Increase in the surface coverage resulted in increase in volume fraction of PEO chains in the adsorbed layer. This in turn ensued into reduced protein interaction with the nanoparticle surface leading to prolonged in vivo circulation [47]. Poloxamine-coated nanoparticles are reported to have increase in vivo circulation and reduction in uptake by liver [48].

Thus coating or surface modification via hydrophilic polymers effectively prevent uptake of nanoparticles by preventing opsonization and thereby increasing the circulation time. However, control of physiological processes of the body is difficult which in turn limits these applications.

2.2 Localization of Nanoparticles

Surface modification of nanoparticles for site-specific drug delivery can be divided into two groups, passive targeting and active targeting. In passive targeting the drug entry is based on enhanced permeation rate in the tumor tissue. It is widely known that because of several abnormalities resulting into healthy tissue, the resulting tumor tissue comprises a leaky blood vessel network. The tumor blood vessels lack pericytes and comprise highly multiplying endothelial cells. Enhanced permeation in the tumor tissue facilitates an opportunity for tumor targeted drug delivery [49]. Enhanced permeation rate can be mediated by several mediators including bradykinins, nitric oxide, vascular endothelial growth factor, cytokines, prostaglandins, and matrix metalloproteinases [50]. From various studies it has found that tumor possess pore size ranging between 380 and 780 nm [51–53]. Greish and coresearchers reported high tumor targeting efficiency of pirarubicin micelles made up of copoly(styrene-maleic acid) with little toxicity. The conjugate showed higher accumulation in tumor tissue by enhanced permeation rate effect [54]. Yang et al. (2011) reported antitumor efficacy of PEG-liposomal oxaliplatin in xenograft tumor bearing mouse model for colorectal cancer. The results demonstrated that following intravenous administration liposomal conjugate was found to accumulate in the tumor via leaky tumor vasculature [55].

In context with other organs, after intravenous administration nanoparticles are rapidly cleared by MPS/RES and then accumulated in liver and spleen [56]. This natural process can be utilized as site-specific drug delivery for both organs/a single organ. Tammam et al. (2012) reported tacrolimus biodegradable nanoparticles for liver and spleen targeting. The results of the study concluded that poly(lactic) acid (PLA) nanoparticles (NP) of tacrolimus was successfully targeted to liver and spleen via RES which proved beneficial in graft survival with reduced side effects. Release pattern of tacrolimus from PLA-NP determined by the dialysis bag method demonstrated 77 ± 45.72% drug release within 4 days [57]. But when MPS/RES is to be avoided so as to target the tumor other than liver or spleen, other approaches are investigated.

Gu and coresearchers investigated PEGylated mesoporous silica nanoparticles (PEG-MSN) to target doxorubicin to liver. From the study it was observed that uptake of PEG-MSN of doxorubicin was significantly higher than that of MSN of doxorubicin benefited from the galactose receptor-mediated endocytosis phenomenon [58]. The amphiphilic property of PEGs with good solubility is responsible for better biocompatibility for cell membranes. Therefore, PEG-coated nanoparticles show higher efficiency to penetrate compared to unmodified nanoparticles [59].

Drug delivery by passive targeting undergoes non selective uptake by organs which may lead to unnecessary accumulation of drugs resulting into severe adverse effects. Therefore, active targeting becomes indispensable for delivery drug to right cells. Active targeting is based on positive interactions between antibody/ligand and antigen/receptor molecules. Thus, the drug delivery system is manipulated to improve its distribution pattern and target to the specific biosite. The attachment of specific ligand on nanoparticles facilitates site-specific drug delivery. Ligands are conjugated on the surface of nanoparticles with chemical strategies which can find the tumor cells as a target at the same time excluding the healthy cells, leading to minimal adverse effects of chemotherapy [60].

Many researchers have demonstrated that attachment of folate groups as ligands on the surface of nanoparticles results into enhancement cellular uptake by tumor tissues, as a function of surface density balance against PEG steric resistance [61, 62]. Quintana et al. 2002 developed a therapeutic nanodevice intended to target tumor cells through the folate receptor. Folic acid and methotrexate were covalently linked to the surface of ethylenediamine core polyamidoamine dendrimer. The results demonstrated improved targeting to 100-fold by successful surface modification using ligand based approach [63]. Quadir et al. (2017) reported folate-targeted nanoparticles loaded with doxorubicin that target the folate receptor-over-expressing tumor cells. The system comprised pH-responsive polymeric part which drives the nanocarrier and ligand conjugated PEG unit which targets the folate receptor. The results demonstrated suppression of tumor growth due to successive accumulation of drug in tumor cells [64].

Drug delivery to brain is a challenging task for researchers due to complex structure of blood–brain barrier (BBB). Poly (lactic-co-glycolic acid) (PLGA)/polylactic acid (PLA) is widely utilized to prepare nanoparticles to target brain. However from the studies it is demonstrated that modified nanoparticles show enhanced brain uptake as compared to unmodified PLGA/PLA nanoparticles [65, 66]. Song and coworkers demonstrated brain drug delivery system by attaching lactoferrin (a multifunctional protein) on silica nanoparticles. Nanoparticles were further modified with PEG to reduce protein absorption. The results suggested enhanced transport efficacy of the nanoparticles across BBB. Maximum efficacy was found with nanoparticles less than 25 nm in diameter [67]. Wang et al. (2010) reported trimethylated chitosan (TMC) surface-modified PLGA nanoparticles for brain delivery. TMC was covalently linked to the surface of nanoparticles via carbodiimide mediated linkage. Average diameter of nanoparticles were of 150 nm and were found to accumulate in the cortex, paracoel, third ventricle, and choroid plexus epithelium, while no brain uptake was observed with unmodified PLGA nanoparticles [68].

2.3 Some Other Examples of Nanocarriers for Targeted Drug Delivery by Surface Modification

The types of nanocarriers mentioned here are the most challenging and frequently used surface-modified nanopharmaceuticals for targeted drug delivery. Table 12.1 enlists marketed surface-modified targeted nanopharmaceuticals.

Carbon based nanoparticles such as carbon nanotubes have exhibited a prominent application in site-specific drug delivery. Carbon nanotubes are low dimensional carbon nanoparticles having unique physical and chemical properties. Lu et al. (2012) developed conjugates of multiwalled carbon nanotubes and iron oxide magnetic nanoparticles as dual targeting nanocarrier of doxorubicin. Further, the nanoparticles were functionalized with poly(acrylic acid) through free radical polymerization conjugated with folic acid ligand. Site-specific drug delivery was achieved under the guidance of magnetic field and through ligand receptor interactions. The results showed enhanced cytotoxicity toward U87 human glioblastoma cells as compared to free doxorubicin [78]. Hou et al. (2016) reported graphene oxide loaded with mitoxantrone with aim to reduce drug resistance in cancer. The nanoparticles were functionalized using hyaluronic acid and pluronics. The results suggested enhanced uptake of nanosheets by MCF-7/ADR cells via receptor mediated endocytosis [79].

Gold nanoparticles with size ranging between 1 and 100 nm are extensively studied for drug and gene delivery. In a recent study, PEGylated doxorubicin gold nanoparticles were prepared to target glioma cells. Ligand-based functionalization was carried out to mediate the system to penetrate blood–brain barrier. Angiopep-2, low density lipoprotein receptor related protein-1 enabled the system to target to the glioma cells in brain [80]. Another research utilized peptide TAT modified gold nanoparticle of an anticancer molecule in order to assess multi drug resistance and thereby its antiproliferative activity [81]. Locatelli et al. (2014) reported multifunctional polymeric nanocomposites containing two cytotoxic agents, alisertib and silver nanoparticles. Further the nanocarrier was conjugated with chlorotoxin, an active targeting 36-amino acid-long peptide that specifically binds to MMP-2, a receptor overexpressed by brain cancer cells. The results suggested reduction in the tumor area when studied using cell line U87MG [82]. Figure 12.3 describes surface-modified gold nanoparticle formation for targeted drug delivery.

In contrast to conventional nanoparticles, *mesoporous nanoparticles* are porous in interior region. They are nontoxic in nature, easily modified, have large loading capacity and are biocompatible. Polydopamine-based surface modification method was employed to prepare doxorubicin loaded mesoporous silica nanoparticles. Peptide CSNRDARRC conjugation was carried out to enhance the therapeutic effects on bladder cancer. The results suggested recognition of human bladder cancer cell line HT-1376 by the modified nanoparticles and thereby highest cellular uptake due to receptor ligand interaction [83]. Figure 12.4 describes schematic diagram of surface modification of mesoporous nanoparticles.

Table 12.1 demonstrates few examples of marketed/under research surface-modified targeted nanopharmaceuticals

Product	Drug	Formulation	Mechanism	Application	References
Doxil®	Doxorubicin	PEGylated nanoliposomes	Passive target to tumors by EPR effect	Ovarian cancer, Kaposi's sarcoma, multiple myeloma	[69]
Abraxane®	Paclitaxel	Albumin-bound paclitaxel nanoparticles	Albumin receptor (gp60)-mediated transcytosis across endothelial cells	Various cancers like breast cancer, pancreatic cancer, and lung cancer	[70]
Myocet®	Doxorubicin	Liposome encapsulated	Passive target to tumors by EPR effect	Breast cancer	[71]
DaunoXome®	Daunorubicin	Liposome encapsulated	Passive target to tumors by EPR effect	HIV-related Kaposi's sarcoma	[72]
EndoTAG-I	Paclitaxel	Cationic liposome	Targets activated tumor endothelial cells with negative charge	Breast cancer/ pancreatic cancer	[73]
Aurimmune (CYT-6091)	TNF-α (Tissue necrosis factor)	TNF-α and PEG bound to colloidal gold nanoparticles	TNF-α plus EPR	Advanced cancer	[74]
CRLX101	Camptothecin	Polymeric nanoparticles made up of cyclodextrin and PEG	Linkage hydrolysis	Various cancers	[75]
BIND-014	Docetaxel	Polymeric nanoparticles of PLA coated with PEG attached with ligands targeted to PSMA (prostrate-specific membrane antigen)	Ligand mediated	Various solid malignancies	[76]
Genexol®	Paclitaxel	Micelles composed of block copolymer poly(ethylene glycol)-poly(D,L-lactide)	Passive targeting via EPR effect	Metastatic breast cancer, lung cancer	[77]

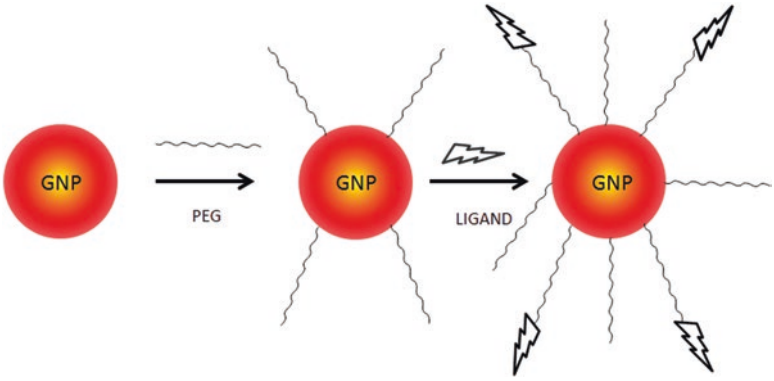
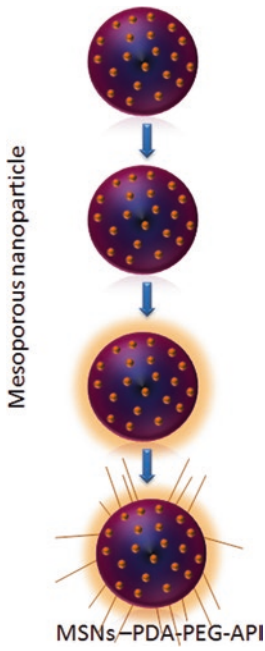


Fig. 12.3 Gold nanoparticles, surface modification by PEG and ligand attachment for site-specific drug delivery

Fig. 12.4 Mesoporous nanoparticle for site-specific drug delivery after surface functionalization



Lipid nanoparticles have emerged as new possible carrier systems to deliver drugs. Choice of lipids can alter the biopharmaceutical characteristics of the drug molecule taken and thus changes its circulation. Paclitaxel loaded solid lipid nanoparticles were prepared to target lung cancer. Surface functionalization of nanocarriers was carried out using lectin conjugation. Nanoconjugates were found to be rapidly taken up by A549 cells through receptor-mediated endocytosis [84]. Neves et al. (2016) reported loaded solid lipid nanoparticles of resveratrol, a neuroprotective compound. Further the nanocomposites were functionalized by

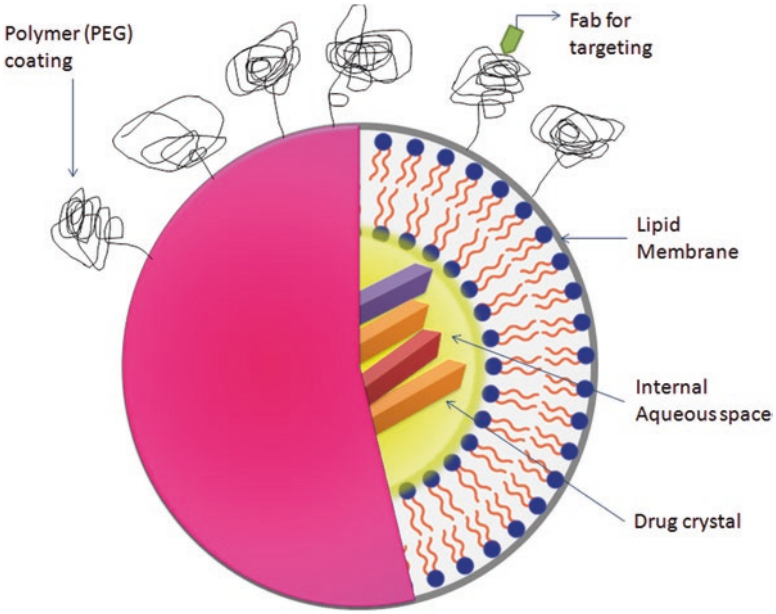


Fig. 12.5 Diagrammatic representation of surface-modified liposome. Statement: The authors hereby declare that all the figures in the chapter are not taken from any source and are self-drawn or modified

apolipoprotein E which are recognized by LDL receptors over expressed on the blood–brain barrier. The results demonstrated permeability of resveratrol-loaded solid lipid nanoparticles functionalized with apolipoprotein E through hCMEC/D3 monolayers with a significant increase (1.8-fold higher) [85].

Dendritic molecules have played an emerging role in targeted drug delivery strategies. With capacity of the peripheral molecules to under surface modification with antibody, or proteins, dendrimers are capable to host several molecules. Zong et al. (2012) reported multifunctional generation 5 polyamidoamine dendrimers of methotrexate. Folic acid conjugation was done for the complex to get bind selectively to the over expressed folate receptor on tumor cells [86].

Liposomal drug delivery system has been implied as another promising nanocarrier system for site-specific drug delivery. The aqueous core and the lipidic shell enable the system to encapsulate both hydrophilic and hydrophobic molecules. Antibody-modified liposomes were evaluated for in vivo antitumor activity of timosaponin AIII. The results suggested higher selectivity of CD44 liposomes toward CD44 tumor positive cells and thereby exhibited stronger tumor inhibition [87]. Figure 12.5 describes a diagrammatic sketch of the liposomal targeted drug delivery.

Novel nanocarriers like *micelles* have emerged as an important class of targeted drug delivery systems for delivery of various chemotherapeutics. One such work reported comprises a polymeric micelle system of paclitaxel to tumor targeting

delivery. Micellar formulation consist of sodium cholate and monomethoxy poly (ethylene glycol)-block-poly (D,L-lactide). Significant antitumor efficacy of paclitaxel micellar formulation was observed in mice bearing BEL-7402 hepatocellular carcinoma and A549 lung carcinoma [88].

3 Future Perspectives

Over the past few years, nanomedicine has emerged as a versatile tool for the targeted drug delivery system. Various nanocarriers have been investigated for sustained, controlled, and targeted effects. However, surface functionalization of nanocarriers has provided an extra merit in the targeted delivery system approaches. Such modification enables the unit to direct toward the specific site by avoiding MPS/RES uptake (to the level of receptors in the cells). Thus, target to specific cells is now possible approach via surface modification of nanocarriers. Looking forward on this, surface modification of nanocarriers represents the future of nanotechnology in the area of targeted drug delivery systems.

References

1. De Jong, W. H., & Borm, P. J. A. (2008). Drug delivery and nanoparticles: Applications and hazards. *International Journal of Nanomedicine, 3*(2), 133–149.
2. Siafaka, P., Betsiou, M., Tsolou, A., et al. (2015). Synthesis of folate-pegylated polyester nanoparticles encapsulating ixabepilone for targeting folate receptor over expressing breast cancer cells. *Journal of Materials Science. Materials in Medicine, 26*(12), 275.
3. Kreuter, J. (2007). Nanoparticles. A historical perspective. *International Journal of Pharmaceutics, 331*(1), 1–10.
4. Belhadj, Z., Zhan, C., Ying, M., et al. (2017). Multifunctional targeted liposomal drug delivery for efficient glioblastoma treatment. *Oncotarget, 8*(40), 66889–66900.
5. Rehman, M., Ihsan, A., Madni, A., et al. (2017). Solid lipid nanoparticles for thermoresponsive targeting.evidence from spectrophotometry, electrochemical, and cytotoxicity studies. *International Journal of Nanomedicine, 12*, 8325–8336.
6. Du, Y., Xia, L., Jo, A., et al. (2018). Synthesis and evaluation of doxorubicin-loaded gold nanoparticles for tumor-targeted drug delivery. *Bioconjugate Chemistry, 29*(2), 420–430.
7. Lin, Y. Q., Zhang, J., Liu, S. J., & Ye, H. (2018). Doxorubicin loaded silica nanoparticles with dual modification as a tumor-targeted drug delivery system for colon cancer therapy. *Journal of Nanoscience and Nanotechnology, 18*(4), 2330–2336.
8. Singh, N., Sachdev, A., & Gopinath, P. (2018). Polysaccharide functionalized single walled carbon nanotubes as nanocarriers for delivery of curcumin in lung cancer cells. *Journal of Nanoscience and Nanotechnology, 18*(3), 1534–1541.
9. Zhong, P., Qiu, M., Zhang, J., et al. (2017). cRGD-installed docetaxel-loaded mertansine prodrug micelles: Redox-triggered ratio metric dual drug release and targeted synergistic treatment of B16F10 melanoma. *Nanotechnology, 28*(29), 295103.
10. Wang, L., Zhang, H., Qin, A., Jin, Q., Tang, B. Z., & Ji, J. (2016). Theranostic hyaluronic acid prodrug micelles with aggregation-induced emission characteristics for targeted drug delivery. *Science China Chemistry, 59*(12), 1609–1615.

11. Nabavizadeh, F., Fanaei, H., Imani, A., et al. (2016). Evaluation of nanocarrier targeted drug delivery of capecitabine-pamam dendrimer complex in a mice colorectal cancer model. *Acta Medica Iranica, 54*(8), 485–493.
12. Moon, S. G., Thambi, T., Phan, V. H. G., Kim, S. H., & Lee, D. S. (2017). Injectable hydrogel-incorporated cancer cell-specific cisplatin releasing nanogels for targeted drug delivery. *Journal of Materials Chemistry B, 5*, 7140–7152.
13. Boche, M., & Pokharkar, V. (2017). Quetiapine nanoemulsion for intranasal drug delivery: Evaluation of brain-targeting efficiency. *AAPS PharmSciTech, 18*(3), 686–696.
14. Kraft, J. C., McConnachie, L. A., Koehn, J., et al. (2017). Long-acting combination anti-HIV drug suspension enhances and sustains higher drug levels in lymph node cells than in blood cells and plasma. *AIDS, 31*(6), 765–770.
15. Parboosing, R., Maguire, G. E. M., Govender, P., & Kruger, H. G. (2012). Nanotechnology and the treatment of HIV infection. *Viruses, 4*(4), 488–520.
16. Kim, P. S., & Read, S. W. (2010). Nanotechnology and HIV: Potential applications for treatment and prevention. *Wiley Interdisciplinary Reviews. Nanomedicine and Nanobiotechnology, 2*(6), 693–702.
17. Kumar, L., Verma, S., Prasad, D. N., Bhardwaj, A., Vaidya, B., & Jain, A. K. (2015). Nanotechnology. A magic bullet for HIV AIDS treatment. *Artificial Cells Nanomedicine and Biotechnology, 43*(2), 71–86.
18. Sanna, V., Pala, N., & Sechi, M. (2014). Targeted therapy using nanotechnology: Focus on cancer. *International Journal of Nanomedicine, 9*, 467–483.
19. Kim, G. J., & Nie, S. (2005). Targeted cancer nanotherapy. *Materials Today, 8*(8), 28–33.
20. Liu, M., Li, M., Wang, G., et al. (2014). Heart-targeted nanoscale drug delivery systems. *Journal of Biomedical Nanotechnology, 10*(9), 2038–2062.
21. Colzi, I., Troyan, A. N., Perito, B., et al. (2015). Antibiotic delivery by liposomes from prokaryotic microorganisms: Similia cum similis works better. *European Journal of Pharmaceutics and Biopharmaceutics, 94*, 411–418.
22. Drulis-Kawa, Z., & Dorotkiewicz-Jach, A. (2010). Liposomes as delivery systems for antibiotics. *International Journal of Pharmaceutics, 387*(1–2), 187–198.
23. Zhang, X., Wu, Y., Zhang, M., et al. (2017). Sodium cholate-enhanced polymeric micelle system for tumor-targeting delivery of paclitaxel. *International Journal of Nanomedicine, 12*, 8779–8799.
24. Clares, B., Ruiz, M. A., Gallardo, V., & Arias, J. L. (2012). Drug delivery to inflammation based on nanoparticles surface decorated with biomolecules. *Current Medicinal Chemistry, 19*(19), 3203–3211.
25. Yang, Z., Ma, H., Jin, Z., et al. (2017). BSA-coated fluorescent organic–inorganic hybrid silica nanoparticles preparation and drug delivery. *New Journal of Chemistry, 41*, 1637–1644.
26. Bi, D., Zhao, L., Yu, R., et al. (2018). Surface modification of doxorubicin-loaded nanoparticles based on polydopamine with pH-sensitive property for tumor targeting therapy. *Drug Delivery, 25*(1), 564–575.
27. Parodi, A., Haddix, S. G., Taghipour, N., et al. (2014). Bromelain surface modification increases the diffusion of silica nanoparticles in the tumor extracellular matrix. *ACS Nano, 8*(10), 9874–9883.
28. Gupta, A. K., & Curtis, A. S. (2004). Surface modified superparamagnetic nanoparticles for drug delivery. interaction studies with human fibroblasts in culture. *Journal of Materials Science: Materials in Medicine, 15*(4), 493–496.
29. Venkatasubbu, G. D., Ramasamy, S., Avadhani, G. S., Ramakrishnan, V., & Kumar, J. (2013). Surface modification and paclitaxel drug delivery of folic acid modified polyethylene glycol functionalized hydroxyapatite nanoparticles. *Powder Technology, 235*, 437–442.
30. Storm, G., Belliot, S. O., Daeman, T., & Lasic, D. D. (1995). Surface modification of nanoparticles to oppose uptake by the mononuclear phagocyte system. *Advanced Drug Delivery Reviews, 17*(1), 31–48.

31. Wang, X., Sun, X., Lao, J., et al. (2014). Multifunctional grapheme quantum dots for simultaneous targeted cellular imaging and drug delivery. *Colloids and Surfaces B: Biointerfaces, 122*, 638–644.
32. Aggarwal, P., Hall, J. B., McLeland, C. B., Dobrovolskaia, M. A., & McNeil, S. E. (2009). Nanoparticle interaction with plasma proteins as it relates to particle biodistribution, biocompatibility and therapeutic efficacy. *Advanced Drug Delivery Reviews, 61*(6), 428–437.
33. Mirshafiee, V., Kim, R., Park, S., Mahmoudi, M., & Kraft, M. L. (2016). Impact of protein pre-coating on the protein corona composition and nanoparticle cellular uptake. *Biomaterials, 75*, 295–304.
34. Amoozgar, Z., & Yeo, Y. (2012). Recent advances in stealth coating of nanoparticle drug delivery systems. *Wiley Interdisciplinary Reviews. Nanomedicine and Nanobiotechnology, 4*(2), 219–233.
35. Gustafson, H. H., Holt-Casper, D., Grainger, D. W., & Ghandehari, H. (2015). Nanoparticle uptake: The phagocyte problem. *Nano Today, 10*(4), 487–510.
36. Wei, W., Zhang, X., Chen, X., Zhou, M., Xu, R., & Zhang, X. (2016). Smart surface coating of drug nanoparticles with cross-linkable polyethylene glycol for bio-responsive and highly efficient drug delivery. *Nanoscale, 8*(15), 8118–8125.
37. Owens, D. E., III, & Peppas, N. A. (2006). Opsonization, biodistribution, and pharmacokinetics of polymeric nanoparticles. *International Journal of Pharmaceutics, 307*(1), 93–102.
38. Alexis, F., Pridgen, E., Molnar, L. K., & Farokhzad, O. C. (2008). Factors affecting the clearance and biodistribution of polymeric nanoparticles. *Molecular Pharmaceutics, 5*(4), 505–515.
39. Gref, R., Luck, M., Quellec, P., et al. (2000). 'Stealth' corona-core nanoparticles surface modified by polyethylene glycol (PEG). Influences of the corona (PEG chain length and surface density) and of the core composition on phagocytic uptake and plasma protein adsorption. *Colloids and Surfaces B: Biointerfaces, 18*(3–4), 301–313.
40. Dos Santos, N., Allen, C., Doppen, A. M., et al. (2007). Influence of poly(ethylene glycol) grafting density and polymer length on liposomes: Relating plasma circulation lifetimes to protein binding. *Biochimica et Biophysica Acta, 1768*(6), 1367–1377.
41. Lundqvist, M., Stigler, J., Elia, G., Lynch, I., Cedervall, T., & Dawson, K. A. (2008). Nanoparticle size and surface properties determine the protein corona with possible implications for biological impacts. *Proceedings of the National Academy of Sciences of the United States of America, 105*(38), 14265–14270.
42. Shi, B., Fang, C., & Pei, Y. (2006). Stealth PEG-PHDCA niosomes: Effects of chain length of PEG and particle size on niosomes surface properties, *in vitro* drug release, phagocytic uptake, *in vivo* pharmacokinetics and antitumor activity. *Journal of Pharmaceutical Sciences, 95*(9), 1873–1887.
43. Nagayama, S., Ogawara, K., Fukuoka, Y., Higaki, K., & Kimura, T. (2007). Time-dependent changes in opsonin amount associated on nanoparticles alter their hepatic uptake characteristics. *International Journal of Pharmaceutics, 342*(1–2), 215–221.
44. Fang, C., Shi, B., Pei, Y. Y., Hong, M. H., Wu, J., & Chen, H. Z. (2006). *In vivo* tumor targeting of tumor necrosis factor-alpha-loaded stealth nanoparticles: Effect of MePEG molecular weight and particle size. *European Journal of Pharmaceutical Sciences, 27*(1), 27–36.
45. Shubhra, Q. T. H., Toth, J., Gyenis, J., & Feczko, T. (2014). Poloxamers for surface modification of hydrophobic drug carriers and their effects on drug delivery. *Polymer Reviews, 54*(1), 112–138.
46. Santander-Ortega, M. J., Jódar-Reyes, A. B., Csaba, N., Bastos-González, D., & Ortega-Vinuesa, J. L. (2006). Colloidal stability of Pluronic F68-coated PLGA nanoparticles. A variety of stabilisation mechanisms. *Journal of Colloid and Interface Science, 302*(2), 522–529.
47. Stolnik, S., Daudali, B., Arien, A., et al. (2001). The effect of surface coverage and conformation of poly(ethylene oxide) (PEO) chains of poloxamer 407 on the biological fate of model colloidal drug carriers. *Biochimica et Biophysica Acta, 1514*(2), 261–279.
48. Redhead, H. M., Davis, S. S., & Illum, L. (2001). Drug delivery in poly(lactide-co-glycolide) nanoparticles surface modified with poloxamer 407 and poloxamine 908: *In vitro* characteristics and *in vivo* evaluation. *Journal of Controlled Release, 70*(3), 353–363.

49. Azzi, S., Hebda, J. K., & Gavard, J. (2013). Vascular permeability and drug delivery in cancers. *Frontiers in Oncology, 3*, 211.
50. Iyer, A. K., Khaled, G., Fang, J., & Maeda, H. (2006). Exploiting the enhanced permeability and retention effect for tumor targeting. *Drug Discovery Today, 11*(17–18), 812–818.
51. Thakor, A. S., & Gambhir, S. S. (2013). Nanooncology. The future of cancer diagnosis and therapy. *CA: A Cancer Journal for Clinicians, 63*(6), 395–418.
52. Charrois, G. J. R., & Allen, T. M. (2003). Rate of biodistribution of STEALTH® liposomes to tumor and skin: Influence of liposome diameter and implications for toxicity and therapeutic activity. *Biochimica et Biophysica Acta, 1609*(1), 102–108.
53. Mei, K. C., Bai, J., Lorrio, S., Wang, J. T. W., & Al-Jamal, K. T. (2016). Investigating the effect of tumor vascularization on magnetic targeting *in vivo* using retrospective design of experiment. *Biomaterials, 106*, 276–285.
54. Greish, K., Nagamitsu, A., Fang, J., & Maeda, H. (2005). Copoly(styrene-maleic acid)-Pirarubicin micelles: High tumor-targeting efficiency with little toxicity. *Bioconjugate Chemistry, 16*(1), 230–236.
55. Yang, C., Liu, H. Z., Lu, W. D., & Fu, Z. X. (2011). PEG-liposomal oxaliplatin potentialization of antitumor efficiency in a nude mouse tumor-xenograft model of colorectal carcinoma. *Oncology Reports, 25*(6), 1621–1628.
56. Yang, L., Kuang, H., Zhang, W., Aguilar, Z. P., Wei, H., & Xu, H. (2017). Comparisons of the biodistribution and toxicological examinations after repeated intravenous administration of silver and gold nanoparticles in mice. *Scientific Reports, 7*, 3303. https://doi.org/10.1038/s41598-017-03015-1.
57. Tammam, S., Mathur, S., & Afifi, N. (2012). Preparation and biopharmaceutical evaluation of tacrolimus loaded biodegradable nanoparticles for liver targeting. *Journal of Biomedical Nanotechnology, 8*(3), 439–449.
58. Gu, J., Su, S., Zhu, M., et al. (2012). Targeted doxorubicin delivery to liver cancer cells by PEGylated mesoporous silica nanoparticles with a pH-dependent release profile. *Microporous and Mesoporous Materials, 161*, 160–167.
59. Liu, D., Wu, W., Ling, J., Wen, S., Gu, N., & Zhang, X. (2011). Effective PEGylation of iron oxide nanoparticles for high performance *in vivo* cancer imaging. *Advanced Functional Materials, 21*(8), 1498–1504.
60. Agarwal, A., Saraf, S., Asthana, A., Gupta, U., Gajbhiye, V., & Jain, N. K. (2008). Ligand based dendritic systems for tumor targeting. *International Journal of Pharmaceutics, 350*(1–2), 3–13.
61. van Dongen, M. A., Silpe, J. E., Dougherty, C. A., et al. (2014). Avidity mechanism of dendrimer-folic acid conjugates. *Molecular Pharmaceutics, 11*(5), 1696–1706.
62. Poon, Z., Chen, S., Engler, A. C., et al. (2010). Ligand-clustered "patchy" nanoparticles for modulated cellular uptake and in vivo tumor targeting. *Angewandte Chemie (International Edition in English), 49*(40), 7266–7270.
63. Quintana, A., Raczka, E., Piehler, L., et al. (2002). Design and function of a dendrimer-based therapeutic nanodevice targeted to tumor cells through the folate receptor. *Pharmaceutical Research, 19*(9), 1310–1316.
64. Quadir, M. A., Morton, S. W., Mensah, L. B., et al. (2017). Ligand-decorated click polypeptide derived nanoparticles for targeted drug delivery applications. *Nanomedicine, 13*(5), 1797–1808.
65. Mathew, A., Fukuda, T., Nagaoka, Y., et al. (2012). Curcumin loaded-PLGA nanoparticles conjugated with Tet-1 peptide for potential use in Alzheimer's disease. *PLoS One, 7*(3), e32616.
66. Li, J., & Sabliov, C. (2013). PLA/PLGA nanoparticles for delivery of drugs across the blood-brain barrier. *Nanotechnology Reviews, 2*(3), 241–257.
67. Song, Y., Du, D., Li, L., Xu, J., Dutta, P., & Lin, Y. (2017). In vitro study of receptor-mediated silica nanoparticles delivery across blood–brain barrier. *ACS Applied Materials and Interfaces, 9*(24), 20410–20416.
68. Wang, Z. H., Wang, Z. Y., Sun, C. S., Wang, C. Y., Jiang, T. Y., & Wang, S. L. (2010). Trimethylated chitosan-conjugated PLGA nanoparticles for the delivery of drugs to the brain. *Biomaterials, 31*(5), 908–915.

69. Barenholz, Y. C. (2012). Doxil®-the first FDA-approved nano-drug: Lessons learned. *Journal of Controlled Release, 160*(2), 117–134.
70. Yuan, D. M., Lv, Y. L., Yao, Y. W., et al. (2012). Efficacy and safety of Abraxane in treatment of progressive and recurrent non-small cell lung cancer patients. A retrospective clinical study. *Thoracic Cancer, 3*(4), 341–347.
71. Swenson, C. E., Perkins, W. R., Roberts, P., & Janoff, A. S. (2001). Liposome technology and the development of Myocet™ (liposomal doxorubicin citrate). *The Breast, 10*(2), 1–7.
72. Rosenthal, E., Poizot-Martin, I., Saint-Marc, T., Spano, J. P., & Cacoub, P. (2002). Phase IV study of liposomal daunorubicin (DaunoXome) in AIDS-related Kaposi sarcoma. *American Journal of Clinical Oncology, 25*(1), 57–59.
73. Fasol, U., Frost, A., Büchert, M., et al. (2012). Vascular and pharmacokinetic effects of EndoTAG-1 in patients with advanced cancer and liver metastasis. *Annals of Oncology, 23*(4), 1030–1036.
74. Pedro, R. N., Thekke-Adiyat, T., Goel, R., et al. (2010). Use of tumor necrosis factor-alpha-coated gold nanoparticles to enhance radiofrequency ablation in a translational model of renal tumors. *Urology, 76*(2), 494–498.
75. Gaur, S., Wang, Y., Kretzner, L., Chen, L., Yen, T., et al. (2014). Pharmacodynamic and pharmacogenomic study of the nanoparticle conjugate of camptothecin CRLX101 for the treatment of cancer. *Nanomedicine, 10*(7), 1477–1486.
76. Von Hoff, D. D., Mita, M. M., Ramanathan, R. K., et al. (2016). Phase I study of PSMA-targeted docetaxel-containing nanoparticle BIND-014 in patients with advanced solid tumors. *Clinical Cancer Research, 22*(13), 3157–3163.
77. Werner, M. E., Cummings, N. D., Sethi, M., et al. (2013). Preclinical evaluation of Genexol-PM, a nanoparticle formulation of paclitaxel, as a novel radiosensitizer for the treatment of non-small cell lung cancer. *International Journal of Radiation Oncology, Biology, Physics, 86*(3), 463–468.
78. Lu, Y. J., Wei, K. C., Ma, C. C., Yang, S. Y., & Chen, J. P. (2012). Dual targeted delivery of doxorubicin to cancer cells using folate-conjugated magnetic multi-walled carbon nanotubes. *Colloids and Surfaces B: Biointerfaces, 89*, 1–9.
79. Hou, L., Feng, Q., Wang, Y., et al. (2016). Multifunctional hyaluronic acid modified graphene oxide loaded with mitoxantrone for overcoming drug resistance in cancer. *Nanotechnology, 27*(1), 015701.
80. Ruan, S., Yuan, M., Zhang, L., et al. (2015). Tumor microenvironment sensitive doxorubicin delivery and release to glioma using angiopep-2 decorated gold nanoparticles. *Biomaterials, 37*, 425–435.
81. Wang, R. H., Bai, J., Deng, J., Fang, C. J., & Chen, X. (2017). TAT-modified gold nanoparticle carrier with enhanced anticancer activity and size effect on overcoming multidrug resistance. *ACS Applied Materials and Interfaces, 9*(7), 5828–5837.
82. Locatelli, E., Naddaka, M., Uboldi, C., et al. (2014). Targeted delivery of silver nanoparticles and alisertib: *In vitro* and *in vivo* synergistic effect against glioblastoma. *Nanomedicine (London, England), 9*(6), 839–849.
83. Wei, Y., Gao, L., Wang, L., et al. (2017). Polydopamine and peptide decorated doxorubicin-loaded mesoporous silica nanoparticles as a targeted drug delivery system for bladder cancer therapy. *Drug Delivery, 24*(1), 681–691.
84. Pooja, D., Kulhari, H., Kuncha, M., et al. (2016). Improving efficacy, oral bioavailability, and delivery of paclitaxel using protein-grafted solid lipid nanoparticles. *Molecular Pharmaceutics, 13*(11), 3903–3912.
85. Neves, A. R., Queiroz, J. F., & Reis, S. (2016). Brain-targeted delivery of resveratrol using solid lipid nanoparticles functionalized with apolipoprotein E. *Journal of Nanbiotechnology, 14*, 27. https://doi.org/10.1186/s12951-016-0177-x.
86. Zong, H., Thomas, T. P., Lee, K. H., et al. (2012). Bifunctional PAMAM dendrimer conjugates of folic acid and methotrexate with defined ratio. *Biomacromolecules, 13*(4), 982–991.
87. Lu, L., Ding, Y., Zhang, Y., et al. (2018). Antibody-modified liposomes for tumor-targeting delivery of timosaponin AIII. *International Journal of Nanomedicine, 13*, 1927–1944.
88. Zhang, S., Langer, R., & Traverso, G. (2017). Nanoparticulate drug delivery systems targeting inflammation for treatment of inflammatory bowel disease. *NanoToday, 16*, 82–96.

Chapter 13
Surface Modification of Metallic Nanoparticles

Vignesh Nagarajan, Tafadzwa Justin Chiome, and Sanjay Sudan

Abstract Nanomaterials are particles with various characteristic shapes, dimensions ranging from zero-dimensional to three-dimensional structures, and sizes ranging between 1 nm and 100 nm. The properties of nanomaterials are due to this small size, which thereby increases their surface area and reactivity. This nanoscale size of particles results in different properties than those of the corresponding bulk material, such as mechanical strength, optical properties, magnetic susceptibility, and electrical conductivity. The specific surface area of a spherical nanoparticle increases by 1/10; modest-size nanoparticles have a high surface-to-mass ratio and hence a high interfacial energy. The high reactive surface area of the nanomaterial can be modified by the choice of application for a certain industry. The most widely used nanomaterials in various industries are metals and metal oxides due to their unique changes in properties and characteristic features compared with their respective bulk materials. Because of their high reactivity and high available surface area, nanoparticles usually tend to be unstable. To avoid aggregation, it is often recommended to stabilize or functionalize such nanoparticles to improve the shelf life of a nanomaterial. Thus, modification of the surface of nanoparticles is an important chemical step that adds value to the final product. This chapter discusses various methods of surface modification, how metals and metal nanoparticles can be protected by using surfactants, and how the biocompatibility of these ligands can be used to introduce novel functionalities that broaden the range of application.

Keywords Induction coupled plasma · Surface charge · Thiol group · Polymer nanoparticles · Gold nanoparticles · TiO_2 · SiO_2 · Functionalized nanocomposites ·Synthesis · Silver · Stabilized · Nanofillers · Polymers

V. Nagarajan (✉) · T. J. Chiome · S. Sudan
Matrix Nano (A Saveer Group Company), Greater Noida, India
e-mail: vignesh@saveer.com

Y. V Pathak (ed.), *Surface Modification of Nanoparticles for Targeted Drug Delivery*, https://doi.org/10.1007/978-3-030-06115-9_13

1 Introduction to Surface Modification

Surface modification is the scientific technique of depositing a well-controlled coating or material on the surface of a nanomaterial to broaden the scope of the nanomaterial's applications based on its physical and chemical properties. Surface modification is a rapidly growing area of focus in the fields of nanoscience and technology, along with the design and development of nanomaterials. Surface modification is necessary to stabilize a nanoparticle and prevent agglomeration. Agglomeration occurs when a bulk material is made into a nanomaterial. However, the increased surface area also increases its reactivity, making it more unstable at its desired state. Therefore, the surface is usually stabilized at its desired size using suitable organic groups. This practice is also carried out to increase the shelf life of the material.

Another reason to modify the surface of a nanomaterial is to alter its compatibility in various phases, as compatibility is the key factor in the choice of applications for nanomaterials [1–3]. A nanomaterial may exhibit a desired property but could be incompatible because of its existing surface morphology, ionic properties, and phobicity. Therefore, the surface is designed in such a way to be compatible with the phase of application without changing its actual properties. The addition of an extra layer allows the nanomaterial to remain intact at the core as desired. Another interesting example of modification for phase compatibility is the use of biocompatible modified inorganic nanomaterials (usually metals and metal oxides) as (nano)fillers between organic polymer chains; this is known as functionalization of the polymer chain.

The purpose of modification is to create homogeneity and avoid compatibility problems between two phases, thus improving the availability and utility of the properties of the material in the desired application. The recent interest in surface modification is to allow nanomaterials to stabilize and self-organize within a chemical reaction, forming an equilibrium in the structural process [2–4]. Typically, simple organic groups are adequate to prevent the agglomeration of nanoparticles. However, the use of complex functional organic groups as capping agents on the particle surface may increase the interaction of the nanoparticles with various surfaces and materials in a phase of application [5, 6]. The most common surface-modified nanomaterials are metals and metal oxides due to their wide range of applications in various industries. This chapter discusses the surface modification of some commonly used nanomaterials in a top-down approach using the most advanced and purest method of nanomaterial synthesis of induction-coupled plasma, which results in an average particle size ranging from 20 to 100 nm [7, 8].

2 Modification of Metal Nanoparticles

The literature on metal nanoparticles is already well established in the application of noble metals such as silver and gold. Through the process of evolution in nanotechnology, various applications for these nanomaterials have been discovered, with key roles as catalysts and in chemical sensing, bio-labelling, and photonics in

various bio-chemical processes. The bio-applications of gold and silver nanoparticles have broadened because of their anti-oxidant properties, ease of synthesis by both by chemical and green methods, and optical properties. Gold and silver nanoparticles that are surface modified with suitable ligands can be biocompatible. Gold nanoparticles can act as biotransporters for tracers, which has recently expanded to the use of magnetic iron nanoparticles. Silver nanoparticles are known to have efficient absorbance [4, 9, 10] and light scattering when a certain wavelength of light is used on it. The surface modification of noble metals is typically carried out by adherence, mainly with a thiol group, disulphide ligands, amines, nitriles, carboxylic acids, and phosphines. The following sections provide examples.

2.1 Thiols and Disulphides

The chemical feasibility of forming strong covalent bonds with various noble metals, such as Ag, Cu, Pt, Hg, and Fe, has led to the development and use of organo-sulphur in the surface modification of noble metals. The key factor that facilitates the strong coordinate bond between the metal surface organo-sulphur is the higher affinity of sulphur to metal surfaces, thus making the organo-sulphur compounds readily absorbable. The strong coordinate bond between the metal surface and sulphur anchors and immobilizes the thiol group from the metal surface of the metal nanoparticles. The oxidation of the thiol functional group to form sulphate or sulfionate reduces the affinity of the interaction with the metal surface.

Thiol- or disulphide-capped nanoparticles can be prepared by two approaches. First, the pre-synthesized noble metal nanoparticle can be used graft sulphur compounds on the surface by replacing the pre-existing capping agent with the new Sulphur-containing ligands. In this case of synthesis of a noble metal nanoparticle by induction-coupled plasma, the metal nanoparticle is a capping agent that is free synthesized in an inert environment with a residual surface charge, allowing for easy grafting of Sulphur-containing ligands, The alternate approach available is the synthesis of an organo-sulphur capped nanoparticle in a one-step process by wet chemistry, in which the metal precursor nucleates and is capped by a protective ligand simultaneously in a single-step reaction [10, 11].

3 Surface Modification of Oxides

Nanoparticles are widely used in a variety of potential applications, primarily in energy storage, conversion, and biomedicine catalysis. Oxide nanoparticles are often used due to their diverse properties and wide variety of possible structures [10–12]. Frequently used oxide nanoparticles include silica (SiO_2), titanium dioxide (TiO_2), and iron oxide.

3.1 Silicon Dioxide and Silica Nanoparticles

Improvements in mechanical strength and increased chemical stability have been the keys to the success of silica nanoparticles. In addition, they are commercially cheaper, which makes them common in a variety of fields based on the properties of application, such as in fabrics, paper, electronics, coatings, and cements [2, 3, 5]. They are often used as chemical/catalytic bridges to control rheology and improve the mechanical properties of material composites, such as in rubber and paint-based industries.

SiO_2 nanoparticles can be broadly synthesized into two forms based on the application: free-flowing powders and colloidal solutions. The widely preferred forms for industrial and commercial applications are the powders, which are mostly synthesized by precipitation, vapour deposition, or flame processes. Currently, the highly pure uniform powders use induction-coupled plasma. Induction-coupled plasma is a high-temperature method with increased time of flight in a vacuum-inert environment, which can synthesize a consistent powder with an average particle size of 50–100 nm.

Silicon dioxide (SiO_2) nanoparticles are hydrophobic by nature. To make them biocompatible and hydrophilic, they can be surface-modified by binding the surface with a water-soluble polymer, such as nonionic poly(oxyethylene methacrylate) or ionic poly(styrene sulfonic acid), via a three-step synthetic approach:

1. Surface activation of the silanol groups (–OH) in SiO_2 nanoparticles.
2. Surface modification by grafting a chlorine (–Cl) group.
3. Grafting polymers onto the nanoparticle surface using atom transfer radical polymerization [3, 9, 11].

4 Surface Modification of Polymer Nanocomposites

Nanostructures have properties that are defined by the same factors as traditional composites but more complicated structures. They are multiphase materials with repeated distances between the phases, which results in a complex material having at least one dimension that is less than 100 nm. With the high compatibility of polymers to perform certain tasks, their performance is increased by dispersing nanomaterials into an inorganic polymer matrix to form a material known as a polymer nanocomposite.

As the new generation of composites, polymer nanocomposites provide a better alternative with excellent properties including modulus, strength, barriers, solvent and heat resistance, and lower flammability. Under optimum conditions, polymer chains can grow and separate the nanoparticle, intertwining within the interlayer space to create a polymer nanocomposite. The surface modification of alumina-coated silica nanoparticles can be performed using phosphonic acids while dispersed in water, thereby creating a variation in the dispersion of the aqueous solution by altering the modification parameters.

4.1 Nanocomposites

Nanocomposites are characterized by phases that have different physical and chemical properties, with the capability of being separated by an interface that is distinct. The matrix is the material found in greater quantity; the smaller embedded amount is known as the reinforcement, which is there to improve the mechanical properties of the matrix. The presence of a reinforcement in the matrix results in distinct anisotropic properties in the hybrid material. Based on this upgrade, the new material has several advantages compared with conventional composites, including the following:

1. Improved matrix properties due to the presence of a reinforcement material.
2. Reduced weight of the composite due to the addition of a nanofiller.
3. Enhanced electrical, mechanical, thermal, magnetic, and optical properties of the material.

As a good alternative to conventional composites, nanocomposites are now in greater demand due to their improved and more efficient properties that have applications in both the industrial and medical sectors. The advancement of nanocomposites to include polymers has increased the demand for materials such as polymer/clay nanocomposites in academic and industrial research. This has allowed for the development of several pathways by which the nanoclay can be incorporated into the matrix through methods such as polymerization, solution mixing, in situ polymerization, and other latex methods.

4.2 Polymer Matrix Nanocomposites

To create such complex structures, the polymer has a matrix with reinforcements that can be one-dimensional (nanofibers and nanotubes), two-dimensional (stacked materials), or three-dimensional (nanospheres). This type of composite has tremendous advantages due to the fact that a small addition of filler results in great improvement of the composite's properties, giving it better properties such as barrier and wear resistance [13].

These new composites have gained popularity and attracted the attention of the aerospace industry. A thermoplastic polymer known as polyamide, which has glass and carbon as fillers, can be used to make reinforcement materials. Weak intermolecular forces create bonds between the matrix and the filler; however, these bonds can be made stronger by creating chemical bonds at an atomic level, which results in remarkable property improvements.

The modification of nanomaterials using polymers provides great advantages because polymers are lightweight with easy processing, high durability, corrosion resistance, low cost, and ductility, although they also have poor heat resistance and gas barriers. Recent work has demonstrated that a small amount of nanoclay (as little as 0.03%) in Pulseless Electrical Activity (PEA) of rubber

results in a 450% increase in epoxy, thus showing that the nano-effect has great synergistic advantages. The high surface area and high surface energy of the nanoparticles creates a much stronger matrix–nanoparticle interaction while decreasing the intermolecular distance.

4.3 Synthesis

The fabrication of polymer matrix nanocomposites can be achieved by the following methods:

1. Intercalation of a polymer from solution.
2. In-situ polymerization.
3. Direct mixing of polymer and fillers.
4. Template synthesis.
5. Melting intercalation.
6. Sol–gel process.

With several methods available for the synthesis of polymer matrix nanocomposites, a simple one that can be easily followed step by step is to thoroughly mix the matrix and the filler material while maintaining the filler material at 25%. To allow for proper mixing to occur, the polymer first should be dissolved in a polar solvent, the filler should be added (in this case, nanoclay/alumina beads), and then homogenous mixing should be performed using a magnetic stirrer for several hours. After proper mixing, the solvent is then evaporated by pouring it into a container with a large surface area, which allows for equal and quick evaporation. To ensure complete removal, the material is further dried in a vacuum at 100 °C. After this, a solution of pure polymer is added to meet the required particle volume fraction.

4.4 Properties of Polymer Matrix Nanocomposites

Regardless of the advantages exhibited by the matrix and the filler individually, the properties shown by their combination in the polymer matrix nanocomposite are based on other external control parameters, including the following:

- Method of synthesis.
- Type of nanoparticle used.
- Method and degree at which the two phases were mixed.
- Type of interaction observed in the interface.
- Filler volume used.
- Size and shape of filler.
- System morphology.

The surface modification of these composites relies on the proper dispersion and distribution of the filler material inside the matrix; otherwise, defects in the form of agglomeration occur and result in the deterioration of the composite. Modification of the material is also achieved by the nature of the interaction between the two phases: the stronger the interaction, the more profound the properties. The interfacial surface should be modified and optimized because most of the interphase properties depend on it. The high surface area of the nanoparticle determines the extent to which the phase interface will contribute to the properties of the nanocompound. Based on this principle, nanoparticles can be further modified to increase their surface area by attaching ligands and other functional groups to their surfaces.

Polymers are also used for the modification of the nanoparticles through methods such as polymer grating and saline grating. The interaction between the nanoparticle and the polymer forms sequences that have adsorbed and unadsorbed segments; the point of contact with the particles forms anchors, whereas the free segments form loops as they become entangled with polymer chains of neighboring segments. The modification of the material plays a major role because the thickness of the interphase region greatly affects factors such as stress; this outcome can be influenced by parameters such as flexibility, the extent of entanglement, and the energy of polymer adsorption. For an outstanding polymer matrix nanocomposite, this modification should create a good interaction between the polymer and the nanofiller. By meeting this criterion, the composite will have outstanding shear strength, fatigue, thermal stability, and corrosion resistance with minimum reinforcement volume.

Surface modification with strong interactions between the two phases produces a flat dense layer on the filler surface, whereas weak interactions cause the loosely attached chains to extend into the matrix as tails and entangle together. As a filler's size is reduced and approaches the nano range, recent R&D at our lab reported that the phase boundary interactions improve to a greater extent, giving the material enhanced properties. Research has demonstrated the advantages of polymer matrix nanocomposites over traditional composites, thereby increasing their demand in all industrial sectors due to their improved properties from just a small volume of reinforcement material.

5 Conclusion

Nanomaterials (1–100 nm) display rare and unusual properties that have great advantages in various industrial sectors. Due to these exotic properties, nanotechnology is on the leading edge of technological innovations. Nanoparticles provide a wide range of unique mechanical, optical, and physical properties, amongst other things. However, nanomaterials also tend to be highly reactive; furthermore, due to their inorganic nature, they tend to be rejected by the body when applied to biological entities. To make nanoparticles compatible for a desired task, surface modification can be performed to create a more efficient and reliable material. Several

modification methods may be used, including tagging the particle with materials such as ligands, polymers, and other functional groups. This allows the modified material to bind to specific sites or segments of different materials in its proximity to induce a desired effect. Surface modification allows for a predetermined reaction of the material at the target, thereby increasing the nanomaterial's efficiency.

Acknowledgement We thank Saveer Biotech for the opportunity to write a chapter with an esteemed pharmacologist, as well as Justin Chiome, Vignesh Nagarjan's parents, and Parvati Palankar for their support in carrying forward this modern field of science.

References

1. Schmitt Pauly, C., Genix, A.-C., Alauzun, J. G., Sztucki, M., Oberdisse, J., & Mutin, P. H. (2015). Surface modification of alumina-coated silica nanoparticles in aqueous sols with phosphonic acids and impact on nanoparticle interactions. *Physical Chemistry Chemical Physics, 17*(29), 19173–19182.
2. Vert, M., Doi, Y., Hellwich, K.-H., Hess, M., Hodge, P., Kubisa, P., et al. (2012). Terminology for biorelated polymers and applications (IUPAC recommendations 2012). *Pure and Applied Chemistry, 84*(2), 377–410.
3. Arzt, E. (1998). Size effects in materials due to microstructural and dimensional constraints: A comparative review. *Acta Materialia, 46*(16), 5611–5626.
4. Steinhögl, W., Schindler, G., Steinlesberger, G., & Engelhardt, M. (2002). Size-dependent resistivity of metallic wires in the mesoscopic range. *Physical Review B, 66*(7), 075414.
5. Patzke, G. R., Zhou, Y., Kontic, R., & Conrad, F. (2011). Oxide nanomaterials: Synthetic developments, mechanistic studies, and technological innovations. *Angewandte Chemie International Edition, 50*(4), 826–859.
6. Rodriguez, J. A., & Fernandez-Garcia, M. (2007). *Synthesis, properties, and applications of oxide nanomaterials*. Hoboken, NJ: Wiley.
7. van Ooij, W. J., & Chityala, A. (2000). In K. L. Mittal (Ed.), *Surface modification of powders by plasma polymerization* (p. 243). Utrecht: VSP.
8. van Ooij, W. J., Zhang, N., & Guo, S. (1999). In J. P. Blitz & C. B. Little (Eds.), *Fundamental and applied aspects of chemically modified surfaces* (p. 191). Cambridge: Royal Society of Chemistry.
9. Forrest, J., Dalnoki-Veress, K., Stevens, J., & Dutcher, J. (1996). Effect of free surfaces on the glass transition temperature of thin polymer films. *Physical Review Letters, 77*(10), 2002.
10. Pankhurst, Q. A., Connolly, J., Jones, S., & Dobson, J. (2003). Applications of magnetic nanoparticles in biomedicine. *Journal of Physics D: Applied Physics, 36*(13), R167.
11. Kelly, K. L., Coronado, E., Zhao, L. L., & Schatz, G. C. (2003). The optical properties of metal nanoparticles: The influence of size, shape, and dielectric environment. *The Journal of Physical Chemistry B, 107*(3), 668–677.
12. Schmitt Pauly, C., Genix, A.-C., Alauzun, J. G., Guerrero, G., Appavou, M.-S., Pérez, J., et al. (2015). Simultaneous phase transfer and surface modification of TiO2 nanoparticles using alkylphosphonic acids: Optimization and structure of the organosols. *Langmuir, 31*, 10966–10974.
13. Siegel, R. W., (1993). Nanostructured Materials-Mind over matter, *Nanostructured Materials, 3*, 1–18.

Chapter 14
Successful Delivery of Zidovudine-Loaded Docosanol Nanostructured Lipid Carriers (Docosanol NLCs) into Rat Brain

Tapash Chakraborty, Malay K. Das, Lopamudra Dutta, Biswajit Mukherjee, Sanjoy Das, and Anupam Sarma

Abstract The major challenges to the clinical application of zidovudine are its moderate aqueous solubility, relative short half-life, and incapability to go across BBB after systemic administration makes the brain one of the dominant HIV reservoirs. We investigated the development of zidovudine-loaded NLCs based on docosanol and oleic acid which were further surface modified with PEG4000 and HAS. The drug content and entrapment efficiencies were assessed by UV analysis. The mean diameter of the SyLN was found to be at 54.7 ± 1.4 nm with a zeta potential of −21.6 ± 0.2 mV and relatively low polydispersity. The NLCs showed excellent stability in the refrigerated condition, in blood serum and were safe for IV administration. In vitro release studies showed a sustained release profile of zidovudine in aCSF. In vivo plasma and brain pharmacokinetics investigation in a rat model showed that SyLN and SyLN-Peg NLCs rapidly reached the brain and yielded higher MRT, *C*max, and AUC. The rat brain pharmacokinetic data confirm the brain localization and accumulation of the developed NLCs delivering AZT in a sustained manner for a prolonged period of time, which is further confirmed by CLSM images of brain cryosections labeled with SyLN-C6 NLCs. Our results suggest that the developed docosanol NLCs could be a promising drug delivery system for long-term brain delivery of zidovudine in the treatment of Neuro-AIDS.

Keywords Docosanol NLCs · Zidovudine · Blood–brain barrier · Brain targeted NLCs · Sustained release NLCs · Neuro-AIDS

The original version of this chapter was revised. The correction to this chapter is available at https://doi.org/10.1007/978-3-030-06115-9_26

T. Chakraborty · M. K. Das (✉) · S. Das · A. Sarma
Drug Delivery Research Laboratory, Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh, Assam, India
e-mail: mkdps@dibru.ac.in

L. Dutta · B. Mukherjee
Department of Pharmaceutical Technology, Jadavpur University, Kolkata, West Bengal, India

Y. V Pathak (ed.), *Surface Modification of Nanoparticles for Targeted Drug Delivery*, https://doi.org/10.1007/978-3-030-06115-9_14

1 Introduction

Acquired immunodeficiency syndrome (AIDS) is one of the deadliest diseases known to humankind. According to WHO reports, AIDS has killed an estimated 39 million people until the year 2014. Today, about 37.3 million people are living with HIV, which results in 1.2 million deaths every year. Destroying the human body defense system by infecting the macrophages and CD4 T-cells are the primary hematopoietic targets of HIV. In the brain, the perivascular and parenchymal macrophages/microglia, oligodendrocytes, endothelial cells, neurons, and astrocytes are the targets of HIV. The process of neuroinvasion by HIV occurs via the HIV-infected circulating monocytes entering the brain during the course of routine immune surveillance and replacement of the perivascular macrophages. The HIV then replicates within the central nervous system (CNS), causing adverse neurological dysfunctions like the AIDS–dementia complex, which includes motor, cognitive, and behavioral impairments that culminate in morbidity at advanced stages of the infection [1, 2]. Zidovudine (AZT) was the first drug approved by the US FDA to treat HIV and AIDS in the year 1986. AZT shows its activity by inhibiting the HIV-encoded reverse transcriptase (RT) enzyme after conversion to AZT-5′-triphosphate by host cell kinases. AZT prevents the mother-to-child spread of HIV during birth or after a needle stick injury or other potential exposure. The oral and intravenous AZT induces immunologic, virologic, and neurologic improvements in HIV-1 infected patients [3]. On administering with other antiretroviral drugs, AZT reduces the blood level of the viruses below detectable ranges. While untreated AIDS patients have an average lifespan of 11 years after infections, AZT-treated patients can live a rather normal life [4]. The half-life of AZT in plasma is approximately 1 h, which necessitates frequent doses to maintain effective therapeutic AZT levels [5]. Although AZT enters cerebrospinal fluid readily, its ability to cross the blood–brain barrier (BBB) is less than optimal. The concentration ration of the CSF and plasma is about 0.06 [3, 5]. AZT is a high water-soluble drug belonging to the class III of the Biopharmaceutics Classification System (BCS). For adults, the recommended oral dose of AZT is 600 mg per day in divided doses (300 mg every 12 h or 200 mg orally every 8 h). The oral bioavailability of administered AZT is about 64 ± 10%. The IC50 and IC90 values (50% and 90% inhibitory concentrations) of AZT are 3–13 ng/mL and 30–130 ng/mL, respectively. The LD50 of AZT is 3084 mg/kg B.W [6].

Literature survey showed that AZT-loaded solid lipid nanoparticles [7–9], solidified lipid microparticles [10], liposomes [11], and polymeric nanoparticles have already been developed for effective brain delivery of AZT [12]. The major drawbacks of these formulations are larger particle size, poor brain target ability, toxicity, instability, drug loss on storage, expensive, no sustained release properties inside the brain, and lack of commercial viability.

NLCs are one of the latest drug delivery systems designed to deliver drugs to various tissues and organs. It is the next generation of the solid lipid nanoparticles (SLNs) which eliminates the drugs leakage on standing from SLN particles, the

main disadvantage of SLNs. It has several advantages over other polymeric drug delivery systems. NLC can be utilized to target a wide range of body areas like tumors, brain, blood cells, and body cavities. It can be administered through different routes like oral, intravenous, and topical. The NLC is a suitable carrier for both lipophilic and hydrophilic drugs and has higher drug loading capability over SLNs and liposomes. NLCs are less or nontoxic, easily eliminable, biocompatible, and biodegradable [13, 14]. The main advantage of NLC is that it can be very easily scaled up for industrial production. Therefore, zidovudine loaded docosanol NLCs could eliminate all disadvantages of all the previously reported novel nanocarrier systems. Until date, no brain targeted NLC formulation of zidovudine has been reported. However, human brain cell line (C6) compatible nano lipid carriers (NLCs) of azidothymidine had been reported [15] with no in vivo efficacy.

1-Docosanol, also known as behenyl alcohol, is a saturated fatty alcohol used traditionally as an emollient, emulsifier and thickener in cosmetics and nutritional supplement, either alone or with other constituents of policosanol, which is effective as a lipid-lowering agent [16–19]. Docosanol is easily metabolized by oxidation to *n*-docosanoic acid and then incorporated as an acyl group on polar lipids and internalized by cells [20, 21]. Studies have shown that docosanol is nontoxic to rats, rabbits, and dogs [22, 23]. Docosanol has been approved by the US FDA as a 10% over-the-counter cream for the treatment of recurrent orolabial herpes. NLC formulations have been developed and reported with behenyl alcohol as well as glyceryl behenate (Compritol 888 ATO) [24] for topical application. No brain targeting NLC formulation have yet been developed using docosanol [24].

Oleic acid is the most widely utilized liquid lipid in the development of NLC formulations due to its great stability, easy availability, and nontoxic nature [25–27]. Brain-targeted SLN and NLC formulations have also been developed and reported with oleic acid [28].

The reticuloendothelial system (RE system) recognized NLC formulations as xenobiotics. Hence, RE system tends to eliminate the carrier system from blood as soon as possible. The surface coating of the NLC formulation with the hydrophilic material (e.g., PEG), or with other material that is indigenous to the human body, like human serum albumin (HAS), the carrier systems can be protected from the RE system. The surface modification increases the chances of reaching the delivery systems to its target site before being eliminated by the RE system [29–31]. Moreover, the nanocarrier surface coating changes the way the particles interact with the biological membranes. An altered zeta potential or altered lipophilicity changes the affinity by which the particles bind to the membranes and moves through them.

In our current study, we investigated the development of zidovudine-loaded NLCs based on docosanol and oleic acid which were further surface-modified with PEG4000 and HAS for effective brain delivery of zidovudine. The brain targeting efficiency of the developed NLCs was investigated in rat model via intravenous administration.

2 Materials and Methods

2.1 Materials

AZT was gifted by Macleods Pharmaceuticals, Mumbai, India. Ethanol was purchased from Merck Millipore, Mumbai, India. 1-Docosanol (Behenyl alcohol) and Oleic acid were purchased from Tokyo Chemicals Industry Co., Ltd. Tokyo, Japan. Tween 80 and Polyethylene glycol 4000 (PEG4000) was purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. Human serum albumin (HAS) was purchased from Sigma-Aldrich Co., USA. Urethane, adult bovine serum, coumarin 6 (C6), and phosphate buffer saline, pH 7.4 were purchased from HiMedia, Mumbai, India. All other chemicals and reagents used were of analytical grade.

2.2 Preparation of Docosanol NLCs

The Docosanol NLC was prepared using previously reported 'modified emulsion method' with slight modifications [14, 32]. Briefly, 10 mg of docosanol, 7.5 mg of oleic acid, and 5 mg of AZT were dissolved in 2 mL of absolute ethanol in a beaker. This mixture was heated to 55 °C over a hot plate-cum-magnetic stirrer (IKA® RCT basic, IKA, Germany). In another beaker, 10 mL aqueous solution of 1% w/v Tween 80 was heated to 55 °C on a hot plate-cum-magnetic stirrer with continuous stirring at 500 rpm. The optimum amount of solid lipid, liquid lipid, drug, and surfactant was determined by trial formulations in different ratios of the excipients. The ethanol mixture was taken in a 2-mL syringe fitted with a 24-gauge needle and gradually injected into the stirring Tween 80 solution in the beaker. The mixture was stirred for 5 min at the same temperature before ultrasonication for 20 min in a bath sonicator (UCB 30, Spectra Lab, Navi Mumbai, India). The NLC formulation was then magnetically stirred until the smell of ethanol disappears indicating complete evaporation of the solvent.

To remove the unentrapped drug and excess amounts of surfactant from the formulation suspension, it was filtered through PD-10 Desalting Columns (GE Healthcare Bio-Sciences AB, Sweden) containing Sephadex™ G-25 resin. The eluent fractions containing the NLC formulations were then concentrated back to its initial volume using Vivaspin6® (Sartorius Stedim Biotech, Germany, MWCO 100). Formulations were finally kept in airtight polypropylene centrifuge tubes at 4–8 °C protected from light [4, 32].

2.3 Coating of Docosanol NLCs

Freshly prepared NLC formulation (SyLN) was separately coated with polyethylene glycol 4000 (PEG4000) and human serum albumin (HAS). The weighted amount of coating material was added to the NLC suspension to make 1% w/v

solution of the coating material. The optimum concentration of the coating material was finalized by trial-and-error method based on particle size. This mixture was then stirred for 1 h on a magnetic stirrer at room temperature. The coated formulation was stored in polypropylene centrifuge tubes at 4–8 °C for future applications [30, 33].

2.4 Particle Size and Zeta Potential

A 1:100 dilution of newly formulated SyLN, SyLN-Peg, and SyLN-HSA in double-distilled water was used for the measurements. The average particle size and polydispersity index (PDI) of the formulations were measured by particle size analyzer (NanoBrook 90Plus, Brookhaven Instruments Corporation, New York; USA). The zeta potential of the formulations was measured by Zetasizer (Nano ZS90, Malvern, Worcestershire; UK). The particle size of the NLCs was measured at a 90° scattering angle using Dynamic Light Scattering. The zeta potential of the NLCs was measured by using laser Doppler micro-electrophoresis [34].

2.5 Entrapment Efficiency (EE) and Drug Loading

The entrapment efficiency (EE) was determined using the size exclusion chromatography. The amount of entrapped drug was determined by subtracting the amount of free drug from the total drug added during the formulation process [5, 35]. The EE was calculated based on the equation below:

$$\%\text{Entrapment efficiency} = \frac{\text{Weight of AZT added} - \text{Free AZT}}{\text{Weight of AZT added}} \times 100\% \quad (14.1)$$

To determine drug loading, 1 mL of the NLC formulation was lyophilized in a lyophilizer (SS1-LYO, Southern Scientific Lab Instruments, Chennai, India). The weight of the dried sample was determined from the tare weight of the vessel and the final weight of the vessel. To the dried formulation, 10 mL of ethanol was added and mixed well to a vortex mixture (Swirlex-Vortex Shaker, Abdos Labtech Private Limited, New Delhi, India). The mixture was then centrifuged at 13,000 rpm for 15 min. The amount of AZT was determined spectrophotometrically by measuring the absorbance of the clear supernatant at λ_{max} of 265 nm. Each experiment was performed in triplicate. Placebo formulation treated like that of the sample was used as blank for UV absorbance [25, 36]. The percentage drug loading was calculated based on the equation below:

$$\%\text{Drug loading} = \frac{\text{Amount of entrapped drug}}{\text{Weight of the NLC formulation}} \times 100\% \quad (14.2)$$

2.6 Transmission Electron Microscopy (TEM)

The shape and size of the uncoated and coated NLC formulations were studied using a transmission electron microscope (JEM-2100, 200 kV, Jeol Ltd., Tokyo, Japan). Each sample was diluted 100 times with deionized water and one drop was deposited on a carbon film-covered copper grid to form a thin-film specimen. The solvent was then evaporated by heating the grids in a hot-air oven at 45 °C. The sample was then examined and photographed under the microscope [37].

2.7 Compatibility Study of NLC Formulation

To determine any incompatibility between the components of the NLC formulation, differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FT-IR) study, and powder X-ray diffraction (XRD) studies were performed. For that, NLC formulation was lyophilized with 5% mannitol in a lyophilizer. In all the studies, the spectrum of the individual components, the physical mixture of the components and the lyophilized formulation were obtained [27, 38].

For IR study, powder samples were analyzed by the FT-IR instrument (Bruker, ALPHA, Billerica, Massachusetts, USA) over a wave number region of 400–4000 cm^{-1} (wavelength of 2.5–25 μ) and spectra were recorded.

DSC study was done with a differential scanning calorimeter (PerkinElmer, DSC 4000, Waltham, Massachusetts, USA) using a closed aluminum crucible. Indium was used to calibrate the DSC apparatus. 5–10 mg of the sample powder was placed in the alumina crucible and heated from 30 to 445 °C with nitrogen flow at 50 mL/min at a heating rate of 10 °C/min and a thermogram was recorded [39].

The X-ray diffraction (XRD) patterns were analyzed using an X-ray diffractometer (D8 Focus, Bruker AXS, Germany.). Scanning for the powder samples was conducted over 2θ, ranging from 10° to 80°, at a voltage of 45 kV and a current of 40 mA [38].

2.8 Stability Study of NLC Formulation

To determine the stability of the formulated NLC in its aqueous suspension form, the uncoated, PEG4000 and HSA coated formulations were put in 1 mL Eppendorf tubes and stored at cold temperature (4–8 °C) and at room temperature for 6 months. A 100-μL sample was withdrawn at 1, 2, 3, and 6 months and analyzed for particle size and entrapment efficiency. After 6 months of study, zeta potential and in vitro drug release pattern of the samples were studied and compared with the initial data [40, 41].

2.9 In Vitro Drug Release Study

The in vitro drug release study was performed using dialysis tubes having a molecular weight cut-off 12,000 Da in artificial cerebrospinal fluid (aCSF). The aCSF was prepared by dissolving sodium chloride (8.66 g), potassium chloride (0.224 g), calcium chloride dihydrate (0.206 g), and magnesium chloride hexahydrate (0.163 g) in 500 mL of pyrogen-free, sterile water. NLC formulation containing a specific amount of encapsulated AZT was loaded into a dialysis bag and both the ends were tied with thread. The bag was then immersed in 100 mL of the dissolution medium (aCSF) kept in a beaker under continuous stirring (100 rpm) over a hot plate-cum-magnetic stirrer. The temperature of the medium was maintained at 37 °C. A 1-mL sample was withdrawn at time intervals of 1, 2, 4, 8, 24, and 48 h, which was replaced with 1 mL of fresh medium. The same study was conducted with AZT solution also. Samples were analyzed using a UV spectrophotometer at 266 nm against the blank medium [42].

The estimation of AZT in the samples collected from in vitro studies was performed by UV spectrophotometric method. An accurately weight amount of AZT was solubilized in artificial cerebrospinal fluid (aCSF) and adult bovine serum with saline (50% v/v) to obtain primary standards in the concentration range of 0.5–100 µg/mL. The calibration curve was obtained by measuring their absorbance at predetermined λ_{max} of 266 nm with a UV–Vis spectrophotometer (UV 1800, Shimadzu, Japan). The concentration of AZT in test samples was calculated using the linear regression equation of the calibration curve in aCSF (Absorbance = 0.00 71 + 0.3457 × Concentration, R^2 = 0.9999) and 50% v/v adult bovine serum with saline (Absorbance = 0.0001 + 0.9955 × Concentration, R^2 = 0.9997). The high value of correlation coefficient (R^2) indicates the linearity of the calibration curve and the curve did not deviate significantly from the origin as indicated by its very low value of intercept. The method was validated for accuracy and precision. When a standard drug solution was assayed repeatedly (n = 3), mean standard error (accuracy) and RSD (precision) were found to be 0.27% and 0.49%, respectively, in aCSF and 0.13% and 0.36%, respectively in 50% v/v adult bovine serum with saline. The purity of AZT in in vitro samples was validated recording UV spectrum using a UV–VIS Spectrophotometer. The spectrum was found comparable with the UV spectrum of pure AZT in the respective medium. The λ_{max} of AZT was appeared at 266.27 nm in the UV spectrum of in vitro samples, which indicated the drug had not been deteriorated and there was no interference from the components of in vitro release medium.

To study the drug release behavior of the NLC formulations in blood serum, a 50% v/v mixture of adult bovine serum and saline was used as dissolution medium. NLC formulation containing a specific amount of encapsulated AZT was mixed with equal volume of the bovine serum–saline mixture and loaded into a dialysis bag. Both ends of the dialysis bag were tied with thread and then immersed into 100 mL of the above serum–saline mixture kept in a beaker and was stirred (100 rpm) continuously on a hot plate-cum-magnetic stirrer. The temperature of the medium

was maintained at 37 °C. 100 μL of the sample was withdrawn at time intervals of 1, 2, 4, 8, and 24 h, which was replaced with fresh medium. The sample was mixed with 1 mL acetonitrile followed by centrifugation at 10,000 rpm for 10 min. The supernatant was collected. This process was repeated three times. Finally, collected acetonitrile was combined in an Eppendorf tube and was evaporated completely by heating it in a hot-air oven at 45 °C. Four milliliters of double distilled water was added to the dried residue and analyzed UV spectrophotometrically at 266 nm against blank formulation sample [5, 43].

The data obtained from serum stability study was fitted in different mathematical models of the kinetics of drug release to find the best fit depending on the regression analysis. The type of formulation (matrix or reservoir type) and the mechanism of drug release (diffusion controlled or erosion controlled) were determined from the observed mathematical models of the kinetics of drug release data [44, 45].

2.10 In Vitro Safety Evaluation of NLC Formulation

In vitro hemolysis: To test hemolysis, blood was drawn from the vein of the healthy human volunteer. The collected blood was put in an ice-cooled heparin tube and centrifuged (4000 rpm for 5 min) to separate the RBCs. After discarding the supernatant, the residue was resuspended in normal saline solution (pH 7.4). The washing steps were repeated three times and finally, RBCs were resuspended in phosphate-buffered saline, and volume was made up to 10 mL. The erythrocyte suspension (0.5 mL) was mixed with SyLn to obtain concentrations of 10, 100, and 500 μg/mL and the mixture was incubated at 37 °C for 1 h in a shaker under gentle agitation. After incubation, the reaction mixture was centrifuged at 4000 rpm for 5 min and the absorbance of the supernatant was read in a spectrophotometer at 540 nm. As a negative hemolysis control (0% hemolysis), erythrocytes were incubated in phosphate-buffered saline in the same condition as the samples. As a positive control to determine the value of 100% hemolysis, erythrocytes were incubated with distilled water, also in the same experimental condition. All the tests were performed in triplicate [46–48]. The percentage of hemolysis was calculated from the measured absorbance values by using Eq. (14.3):

$$\text{Hemolsis}\,(\%) = \frac{A(\text{sample}) - A(\text{negative sample})}{A(\text{positive control}) - A(\text{negative control})} \times 100\% \quad (14.3)$$

2.11 In Vivo Pharmacokinetic and Brain Distribution Study

The animal study was approved by the Institutional Animal Ethical Committee (IAEC) of Dibrugarh University, Dibrugarh, Assam, India (Approval No. IAEC/DU/120 dated 18/02/2016). For the study, 60 healthy young adult male and female

Wistar rats, nulliparous, nonpregnant and weighing 80–150 g were selected. Animals were acclimatized for 15 days before using them for the study. Rats were divided into four major groups (A, B, C, and D) each containing 15 rats. They were then again divided into five subgroups (1, 2, 4, 8, and 24 h) each containing three animals. Identification of the rats was done by putting marker pen marks on the tail. The animals of subgroups were kept in polypropylene cages (55 × 32.7 × 19 cm), bottom covered with paddy husk in an air and temperature controlled room (23 ± 2 °C). Rats were fed with laboratory animal food pellets (Oxbow Regal Rat Food, Rhinelander, Wisconsin, USA) with water ad libitum.

One hour before the experiment, animals were anesthetized by injecting urethane (1.2 g/kg B.W.) intraperitoneally. Group-A animals were injected with 5 mg/kg B.W. AZT solution in water for injection through the tail vein. Group-B animals were injected with SyLN, group-C received SyLN-Peg and group-D received SyLN-HSA with formulation amount equivalent to 5 mg/kg B.W. of AZT. Blood was collected from the hearts of the animals at predefined time intervals after dosing (1, 2, 4, 8, and 24 h.) and was kept in ice-cooled heparinized blood collection tubes. Rats were then sacrificed by cervical dislocation and the chest area was cut open. The brain of the rats was perfused by pumping 0.9% w/v sterile physiological saline solution through the common carotid artery to remove blood from the blood vessels of the brain [49, 50]. Perfusing the brains assures that no AZT is remained in the blood vessels of the brain as free drug or in the NLC formulation and the drug concentration in the brain shown by the analytical procedures is only the amount of AZT that has actually crossed the BBB. The rat brains were then collected by opening the cranium and kept in 15 mL centrifuge tubes after thorough wash of the brain with 0.9% w/v sterile physiological saline solution. Soon after collecting, blood samples were centrifuged at 5000 rpm for 15 min at 20 °C to separate plasma from blood cells. Separated plasma was collected in Eppendorf tubes and kept in −20 °C until further use. The brains were also kept in −20 °C until further use.

Brain Sample Preparation Collected brains were homogenized with 10 mL deionized water in a tissue homogenizer and 100 µL of homogenate was taken in 2 mL Eppendorf tube. Tissue proteins were precipitated by mixing with 1 mL of acetonitrile followed by centrifugation at 13,000 rpm for 15 min. The supernatant was collected. This process was repeated thrice with the residue and the supernatants were finally combined and evaporated at 45 °C in a hot-air oven. It was reconstituted with 100 µL of HPLC mobile phase and 20 µL was injected into the column for the detection of AZT (λ_{max} 265 nm).

Plasma Sample Preparation Plasma samples were deproteinized by mixing 1 mL Acetonitrile with 100 µL of plasma sample followed by centrifugation at 13,000 rpm for 15 min. The supernatant was collected in a tube. This procedure was repeated thrice with the residue. The supernatants were combined and evaporated to dryness at 45 °C in a hot-air oven. It was reconstituted in 100 µL of HPLC mobile phase and 20 µL was injected into the HPLC column for the detection of the AZT (λ_{max} 265 nm) [8].

2.12 Instruments and Chromatographic Conditions

An RP-HPLC system (Agilent Technologies, 1260 Infinity HPLC, California; USA) was used to quantify AZT in plasma and brain samples. The HPLC system was equipped with photodiode array detector set at 265 nm and Agilent Technologies mRP-C18 column (250 mm × 4.60 mm, ID; 5 μm particle size) associated with guard column (50 mm × 4.60 mm, ID; 5 μm particle size). AZT was eluted by isocratic flow at a rate of 1 mL/min at ambient temperature, with a mobile phase comprising a mixture of aqueous Ortho-phosphoric acid solution (0.1% w/v) and Methanol at a ratio of 60:40.

Standard Solutions The AZT was weighed and dissolved in HPLC mobile phase at room temperature to obtain a stock solution of 2.0 mg/mL. Serial dilutions of the stock solutions were made, and 20 μL was injected into the HPLC column to prepare the calibration standards. The calibration curve for AZT was prepared with seven concentrations: 500, 750, 1000, 2500, 5000, 10,000, and 100,000 ng/mL. Stock and working standard solutions were protected from light and stored at −20 °C until used [51].

Assay Validation To validate the assay in rat plasma, the following parameters were investigated: selectivity, linearity, precision and accuracy, limit of quantification, limit of detection and recovery.

Selectivity Selectivity indicates the lack of interfering peaks at the retention times of the assayed drug. The specificity of the method was determined by comparing the chromatograms obtained from the samples containing AZT with those obtained from blank plasma and brain samples.

Recovery and Linearity In the analysis of AZT in rat plasma, the analytical recovery of AZT was determined at concentrations of 500, 2500, and 100,000 ng/mL (n = 3). Samples of the plasma and brain tissue without drug were spiked with known amounts of the drug to achieve the specified concentration. These samples were processed by the analytical method described above and peak areas were compared to that obtained by direct injection of the drug with the mobile phase, that is, the standard curve data. To calculate linearity, calibration curves were constructed by linear regression within the range of 500–100,000 ng/mL of AZT, using seven standard solutions.

The limit of quantification (LOQ) was determined as the lower value in the calibration curve. For the LOD, three samples at concentrations near to the smallest concentration of the standard curve (triplicate) were analyzed in order to obtain the standard deviations.

Precision and Accuracy Precision was determined by the coefficient of variation (CV) and accuracy as the percent relative error (RE). Intraday precision and accuracy data were obtained by analyzing aliquots of plasma samples at low (500 ng/

mL), medium (2500 ng/mL), and high (100,000 ng/mL) levels of the AZT concentration ($n = 3$). Inter-day reproducibility was determined over 3 days.

2.13 Pharmacokinetic Study

Pharmacokinetic analysis was carried out by using the Kinetica Professional software (Version 5.0; Copyright 2017; Adept Scientific, Manchester; UK). Two compartmental intravascular bolus dose analysis methods were employed for the pharmacokinetic parameter analysis.

2.14 The Drug-Targeting Index (DTI)

DTI is considered an important parameter for tissue targeting efficiency. It is basically a comparison between the tissue-targeting efficiency of the developed carrier system and the free drug solution. DTI gives an idea of the performance of the carrier system in delivering the drug to an organ in comparison to the free drug solution. The DTI to the brain can be calculated using the following formula [Eq. (14.4)]:

$$\mathrm{DTI} = \frac{\left\{\dfrac{\mathrm{AUC}\left(\mathrm{Brain}\right)}{\mathrm{AUC}\left(\mathrm{Plasma}\right)}\right\}\left(\mathrm{NLCs}\right)}{\left\{\dfrac{\mathrm{AUC}\left(\mathrm{Brain}\right)}{\mathrm{AUC}\left(\mathrm{Plasma}\right)}\right\}\left(\mathrm{Free\ drug}\right)} \tag{14.4}$$

2.15 Rat Brain Localization and Accumulation of NLCs

In order to confirm and visualize that the intact NLCs had crossed the BBB and localized in the rat brain tissues, ex vivo imaging of rat brain cryosections were performed using the confocal laser scanning microscope (CLSM). Coumarin 6 (C6)-labeled docosanol NLCs (SyLN-C6) were prepared using the same method as described earlier in the section 'Preparation of Docosanol NLCs' with minor modification. C6 was first dissolved in absolute ethanol (1 mg/mL) added to the lipid–ethanol mixture and mixed well. The rest of the formulation process was unaltered. After labeled with C6, the physicochemical properties of SyLN NLCs did not show any significant change in particle size and zeta potential (data not reported). The SyLN-C6 NLCs were injected intravenously in healthy rats through tail vein (Fig. 14.8a). Then, rats were sacrificed by perfusion at 1, 2, 4, 8, and 24 h and brains

were collected (Fig. 14.8b), washed thoroughly with PBS and were stored at −80 °C until further processing. The brain tissues were sliced into sections (6–10 μm in thickness) using a Shandon Cryotome E (Thermo Electron Corporation, USA) and further stained with DAPI (1 μg/mL) for 2 min, washed three times with PBS pH 7.4. The stained tissues were mounted on microscopy slides before analysis by CLSM. The brain tissues were imaged under a confocal laser scanning microscope (TCS SP8, Leica, Germany) using the wavelengths for DAPI (Ex./Em. = 358/461 nm) and C6 (Ex./Em. = 465/505 nm). The images obtained from excitation with both the wavelengths were finally merged using the microscope software [52, 53].

2.16 Statistical Analysis

All pharmacokinetic parameters of AZT solution and NLC formulations were expressed as the mean ± standard deviation (SD). The data were analyzed with Graph Pad Prism (Version 5.03, Copyright 2017, GraphPad Software, Inc.; USA). One-way ANOVA study was done to determine the significant difference between the formulations and AZT solution. The significance of the difference between AZT solution and NLC formulation data was determined with Student's *t*-test. A statistical difference $p > 0.05$ was considered significant.

3 Results and Discussion

3.1 Particle Size and Zeta Potential

The particle size of the optimized NLCs was found to be 54.5 ± 1.3 nm with a PDI of 0.287. The particle size of the PEG4000 and HSA coated formulations were 57.8 ± 2.2 nm (PDI 0.293) and 59.6 ± 1.7 nm (PDI 0.295), respectively. The developed NLCs, both uncoated and coated, were small enough to cross the BBB. Previous literature shows that nanocarriers having a particle size below 100 nm cross the BBB and get into the brain very easily [54]. The uncoated and coated Docosanol NLCs had a small PDI value indicating that the particles are very close to monodispersity, that is, the particle size variation is very small. The reports of the particle size analysis of SyLN, SyLN-Peg, and SyLN-HSA have been given in Fig. 14.1b. The increase in particle size of coated NLCs indicated that the coating materials had been deposited on the surface of the NLC particles.

The zeta potential of the optimized SyLN formulation was found to be −21.6 ± 0.2 mV (Fig. 14.1c). Coating increased the zeta potential by a great margin. The particle size and Zeta potential of SyLN, SyLN-Peg, and SyLN-HSA have been given in Table 14.1. The difference between the zeta potentials of SyLN, SyLN-Peg, and SyLN-HAS was statistically significant (*t*-test, $p > 0.05$).

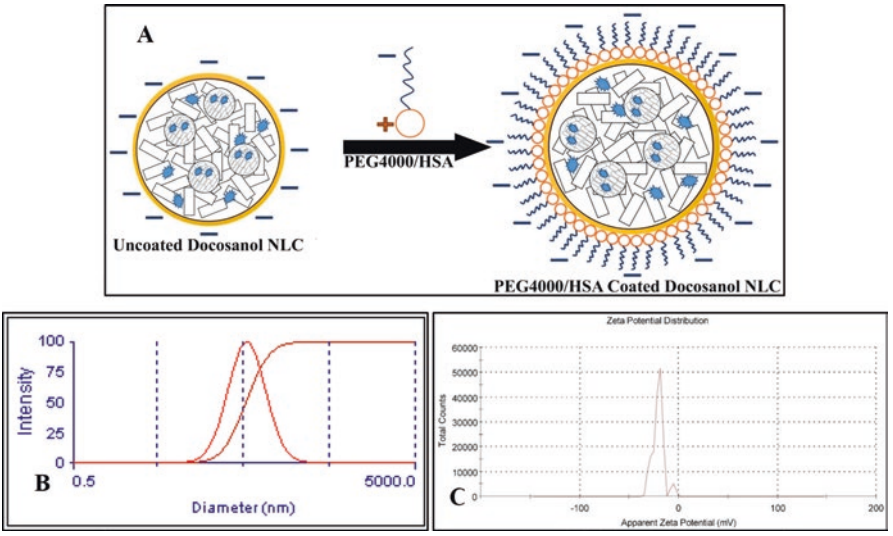


Fig. 14.1 Characterization of Docosanol NLCs: (**a**) Coating of Docosanol NLC with PEG4000/HSA. Freshly prepared Docosanol NLCs were coated with 1%w/v solution of PEG4000 and HSA for 1 h at room temperature by continuous stirring. The coating changes the particle size and zeta potentials of the NLCs confirming that the coating was successful; (**b**) Size distribution of SyLN from DLS measurement. The uncoated formulation shows the nanometric dimension of 54.5 nm with low PDI of 0.287. Coating with PEG4000 and HSA slightly increases the particle size (57.5 nm and 59.6 nm, respectively); (**c**) Zeta potential of SyLN from Zetasizer. The uncoated NLC formulation has a Zeta potential of −21.6 mV. Coating the NLC with PEG4000 and HSA makes the particles more electronegative (−26 mV and −38.5 mV, respectively)

Table 14.1 Particle size, zeta potential, %EE, and %DL of SyLN, SyLN-Peg, and SyLN-HSA

	Size (nm)	Zeta potential (mV)	%EE	%DL
SyLN	54.5 ± 1.3	−21.6 ± 0.2	84.4 ± 0.56	11.32 ± 0.12
SyLN-Peg	57.5 ± 2.2	−26 ± 0.7		
SyLN-HSA	59.6 ± 1.7	−38.5 ± 0.9		

The coating of NLCs involves the physical adsorption of coating materials on NLC surface. The coating might have taken place due to the charge differences between the NLC surface and the coating material. As zeta potential suggests in Table 14.1, the surface of the NLC formulation was negative in nature (i.e., anionic). The freshly prepared NLC formulations have an acidic pH due to the presence of Tween 80 in the medium (the pH of 1% w/v Tween 80 solution is approximately 5.5). Hence, the amine group of HSA ionizes and gives positive ions. These positive ions have a great affinity to interact with the negative ions of the hydroxyl group of Tween 80, present on the surfaces of NLC particles. This interaction of positive and negative ions leads to the coating of the NLC surface [7]. The carboxylic acid group present in the structure of HSA makes the coated NLCs more negative (zeta potential = −38.5). The coating process has been discussed schematically in Fig. 14.1a.

The hydroxyl groups present in the chemical structures of the PEG4000 remain unionized in the acidic medium of the freshly prepared NLCs. PEG4000 has a higher viscosity, which facilitates it to forms a flexible layer on the surface of NLCs leading to the formation of the coating and make them more negative (zeta potential = −26).

The hydrophilic coating on the surface of the NLCs would prevent the adsorption of opsonins via steric hindrance and their subsequent uptake by phagocytic cells [55, 56]. Hence, the surface modified NLCs would remain in the blood for a longer duration of time as compared to an uncoated formulation which may or may not increase the targeting efficacy of the formulation as several reports point out that PEGylation may negatively influence the performance of nanocarriers as a drug carrier [57].

3.2 EE and Drug Loading

The EE and drug loading of AZT in SyLN were found to be 84.4 ± 0.56% and 11.32%, respectively. Higher EE signifies that most of the drug added in the formulation process has entrapped and hence the loss of valuable API is very less. The removal of ingredients that are used in excess quantities to ease the formulation process by gel filtration, like surfactant (Tween 80), led to a higher drug loading.

3.3 Transmission Electron Microscopy (TEM)

The transmission electron microscopy image as shown in Fig. 14.2(I) reveals that the NLC particles are spherical in shape. In the TEM images of SyLN-Peg and SyLN-HSA, a shell-like structure can be seen over the surface of the lipid core. This confirms the coating of the NLC formulations. On higher magnifications, droplet-like spherical structures can be seen inside the NLC particles. These droplets are of the liquid lipid entrapped inside the solid lipid matrix. On further magnification, some patches of nonhomogeneous amorphous substances could be seen inside those droplets. These patches can be of the entrapped AZT inside the liquid lipid droplets. The internal structures of SyLN have been visualized with the help of highly magnified TEM images in Fig. 14.2 (II).

3.4 Compatibility Study of NLC Formulation

The IR spectra of AZT, lyophilized SyLN, and the physical mixture were compared (Fig. 14.3a). The spectra of the formulation and the physical mixture have the same major peak. No additional peak appeared in the IR spectrum of the formulation or

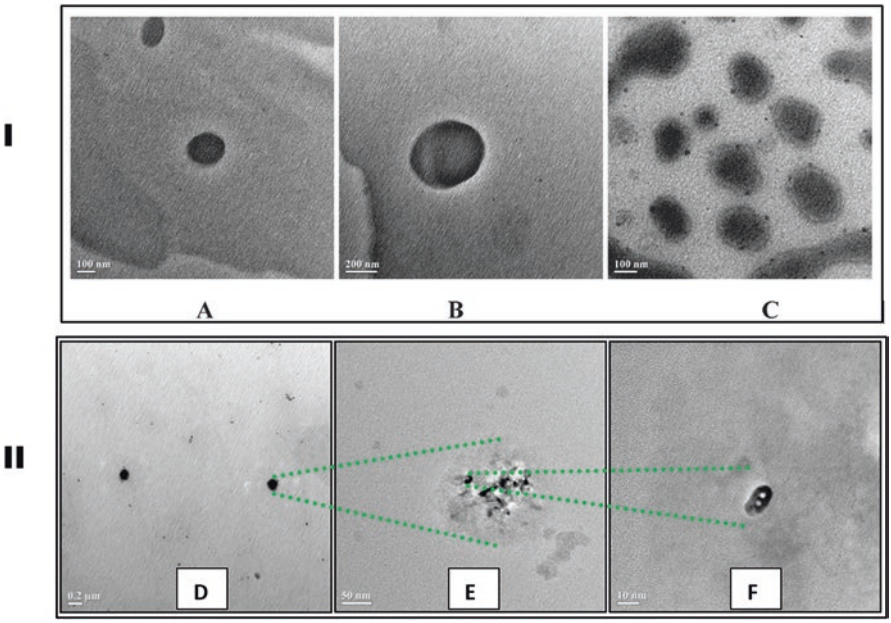


Fig. 14.2 (**I**) TEM micrographs of (A) SyLN, (B) SyLN-Peg, (C) SyLN-HSA. An increased particle size of the coated particles and a shell-like structure on the surface of the NLC particles confirms the coating of the particles. Scale Bar: 100 nm (A, C) and 200 nm (B); (**II**) TEM images of SyLN in higher magnifications. The higher magnifications of NLCs show that the solid lipid cores contain droplets of liquid lipids containing amorphous AZT entrapped in them. Scale Bar: 0.2 μm (D), 50 nm (E) and 10 nm (F)

the physical mixture. This explains that all the components are compatible, as if there was any incompatibility, some additional peaks might have appeared. The interpretation of peaks appearing in the spectra is given in Table 14.2.

On comparing the DSC thermograms of AZT, lyophilized SyLN, and the physical mixture (Fig. 14.3b) it was observed that thermograms of lyophilized SyLN and its physical mixture contain the same endothermic peaks. The peak for AZT was clearly visible in both thermograms. The absence of any unusual peak in the thermograms shows that the components don't react to each other [58].

On comparing the powder X-ray diffractograms of AZT, lyophilized SyLN and the physical mixture (Fig. 14.3c), it was observed that the peak of AZT was absent in the diffractogram of lyophilized SyLN, though it was clearly visible in the diffractogram of the physical mixture. This confirms that AZT might have undergone a state change, from crystalline to amorphous [38], but no new substance has developed during the formulation process of NLCs.

All the above study results confirm that there is no incompatibility between the excipients used in the NLC formulation [59].

Table 14.2 Interpretation of FT-IR spectra of AZT, a physical mixture of AZT and NLC formulation components and docosanol NLC formulation

	Observed peaks in all spectra (cm^{-1})	Standard (cm^{-1})
O–H	3230.94	3600–3200
CH_3 or CH_2	2891.87	2960–2850
RCOH	1669.37	1750–1660
CH_3 or CH_2	1439.19	1470–1430
O–H	1268.56	1410–1260
C–OH	1076.51	1150–1040

3.5 Stability Study of NLC Formulation

The NLC samples kept at 4–8 °C showed no significant change in the particle size, zeta potential, %EE of all the formulations even after 6 months of stability study. However, for the NLC samples kept at room temperature, particle size starts increasing from the very first month and goes on increasing until the end of the study. Entrapment efficiency reduced with time indicating that the formulation was losing the entrapped drug on standing at room temperature. In vitro release studies of stability samples after 6 months of storage shows that the drug release pattern of the NLC samples kept at 4–8 °C remains almost same as the freshly prepared NLCs. The NLC samples kept at room temperature released its drug content much faster than initial. The detailed observation of particle size, %EE and Zeta potential for stability samples has been presented in Table 14.3.

Fig. 14.3 (continued) mixture and the NLC formulation shows the same peaks. Peaks of AZT are present in the spectra of NLC formulation and physical mixture. This indicates that AZT has not undergone any chemical modification during formulation process and is compatible with other components of the NLC formulation; (**b**) DSC thermograms of AZT, NLC formulation and physical mixture of AZT with the NLC formulation components. The thermograms of physical mixture and the NLC formulation show same endothermic peaks at 60, 72, 102 and 345 °C. The peak of AZT at 102 °C is clearly visible in both thermograms. Flattening of the peaks in the NLC formulation thermogram as compared to the physical mixture means that the drug becomes amorphous in the NLC formulation; (**c**) XRD spectra of AZT, NLC formulation and physical mixture of AZT with the NLC formulation components. The spectra of physical mixture and the NLC formulation show same peaks except one. The missing peak in NLC formulation spectrum is of AZT, indicating AZT was converted to amorphous form during the formulation process

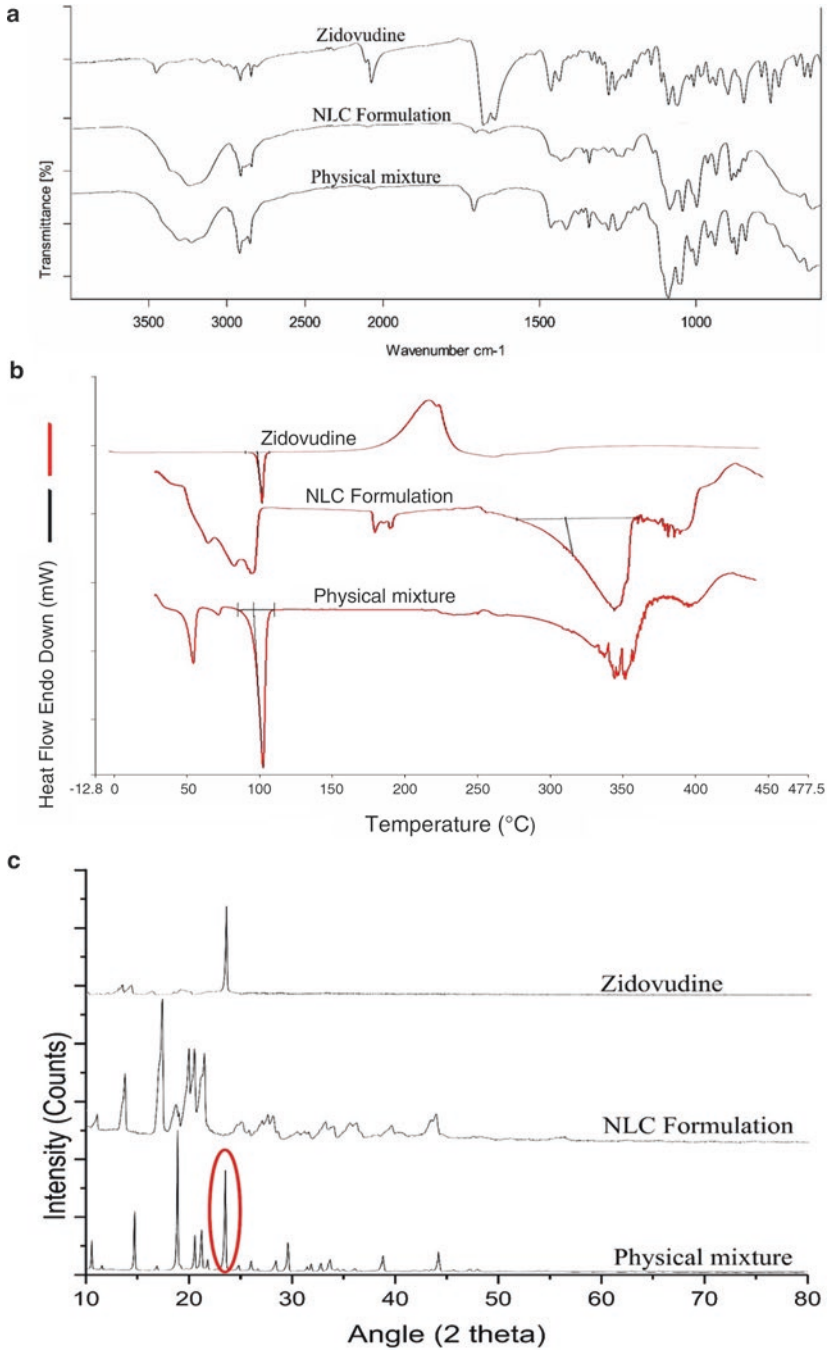


Fig. 14.3 Compatibility studies of NLC formulation: (**a**) FT-IR spectra of AZT, NLC formulation and physical mixture of AZT with the NLC formulation components. The spectrum of physical

Table 14.3 Average particle size and %EE data of stability samples of Docosanol NLCs

		4–8 °C					Room temperature				
		Time (months)					Time (months)				
		0	1	3	6	*PC at 6 months	0	1	3	6	*PC at 6 months
SyLN	Size (nm)	54.5	57	56.6	56.2	3.12	54.5	57	15,414.1	18,787.6	34,372.66
	EE (%)	84.4	83.77	83.34	83.68	2.70	84.4	83.61	81.7	81.87	−3.00
	Zeta potential (mV)	−21.6	**ND	**ND	−21.8	0.93	−21.6	**ND	**ND	−27.5	27.31
SyLN-Peg	Size (nm)	57.5	51.8	51.3	48.4	−15.83	57.5	51.8	8856.6	35,170.1	61,065.39
	EE (%)	84.4	82.87	83.7	84.04	−0.43	84.4	83.54	82.2	79	−6.40
	Zeta potential (mV)	−26	**ND	**ND	−26.3	1.15	−26	**ND	**ND	−31.1	19.62
SyLN-HSA	Size (nm)	59.6	61.7	55.4	55.4	−7.05	59.6	61.7	12,101	11,905.6	19,875.84
	EE (%)	84.4	82.47	83.67	82.27	−2.52	84.4	82.04	81.81	80·3	−4.86
	Zeta potential (mV)	−38.5	**ND	**ND	−38.3	−0.52	−38.5	**ND	**ND	−44.2	14.81

The increase in average particle size and zeta potential of the samples kept at room temperature increased significantly (>5%). However, no significant change was observed in the average particle size and zeta potential of NLC samples kept at cold temperature (<5%). Although the average particle size of SyLN-Peg and SyLN-HSA kept at cold temperature decreases, the size range of particles remained the same

**PC* Percent change, ***ND* Not determined

3.6 In Vitro Drug Release Study

The in vitro drug release study of the developed NLCs in aCSF showed slow release profile of AZT. The percentage of drug released from the different NLC formulations (SyLN, SyLN-Peg, and SyLN-HSA) was between 10.18% and 10.57% at 48 h (Fig. 14.4A). The release of free drug through the dialysis membrane was rapid and nearly 84.88% at 48 h. The observed drug release profile of the docosanol NLCs is significantly different from the previously reported lipid nanocarriers with different drugs (AZT loaded NLC has not been reported yet). The previous studies reported that more than 80% of the entrapped drug get released in vitro within 24 h [37, 38, 60, 61]. In our study, TEM images of SyLN (Fig. 14.2 (II)) confirm that AZT was evenly internalized in the liquid lipid core, which was further entrapped within the solid lipid matrix of NLCs and showed very slow drug release in vitro. Thus, the developed docosanol NLCs in the present study showed better sustained release profile of AZT. However, this does not confirm that the developed nanocarriers would equally perform in vivo, as the environment in vivo is different from in vitro. The different lipolytic enzymes may breakdown the NLCs as soon as it reaches the blood stream making the carrier system short living. To determine how the docosanol NLCs may performs in vivo, stability and drug release properties of the formulations were studied ex vivo in bovine serum saline.

The data obtained from the drug release study with the 50% v/v mixture of bovine serum and saline solution shows that a great amount of drug (83.79%) was released within 24 h from the SyLN formulation. SyLN-Peg and SyLN-HSA show similar types of release properties in this medium (Fig. 14.4B). These drug release data were fit in different mathematical models of the kinetics of drug release (Fig. 14.5). The mathematical models of the kinetics of drug release with the highest R^2 value were the best-fitted model. The R^2 values of all the formulations have been shown in Table 14.4.

R^2 values show that all the formulations are best fitted to zero-order and Korsmeyer–Peppas model. Fitting to zero-order model indicates that the formulations are of matrix type in nature, that is, the drug is evenly distributed in the lipid core. When the data is well fitted to Korsmeyer–Peppas model and the 'n' value is >0.89, the drug release follows the super case II transport and drug release is erosion-controlled [45]. In our study, the observed "n" value is much higher than 0.89 (Table 14.4), indicating AZT release from NLCs is based on erosion of the lipid matrix in blood serum.

3.7 In Vitro Safety Evaluation of NLC Formulation

Assessment of the hemolytic potential against a suspension of erythrocytes illustrated the security of the NLC formulation in vivo. The hemolysis of erythrocytes of NLC formulation having a concentration of 500 μg/mL was 3.98% after incubating

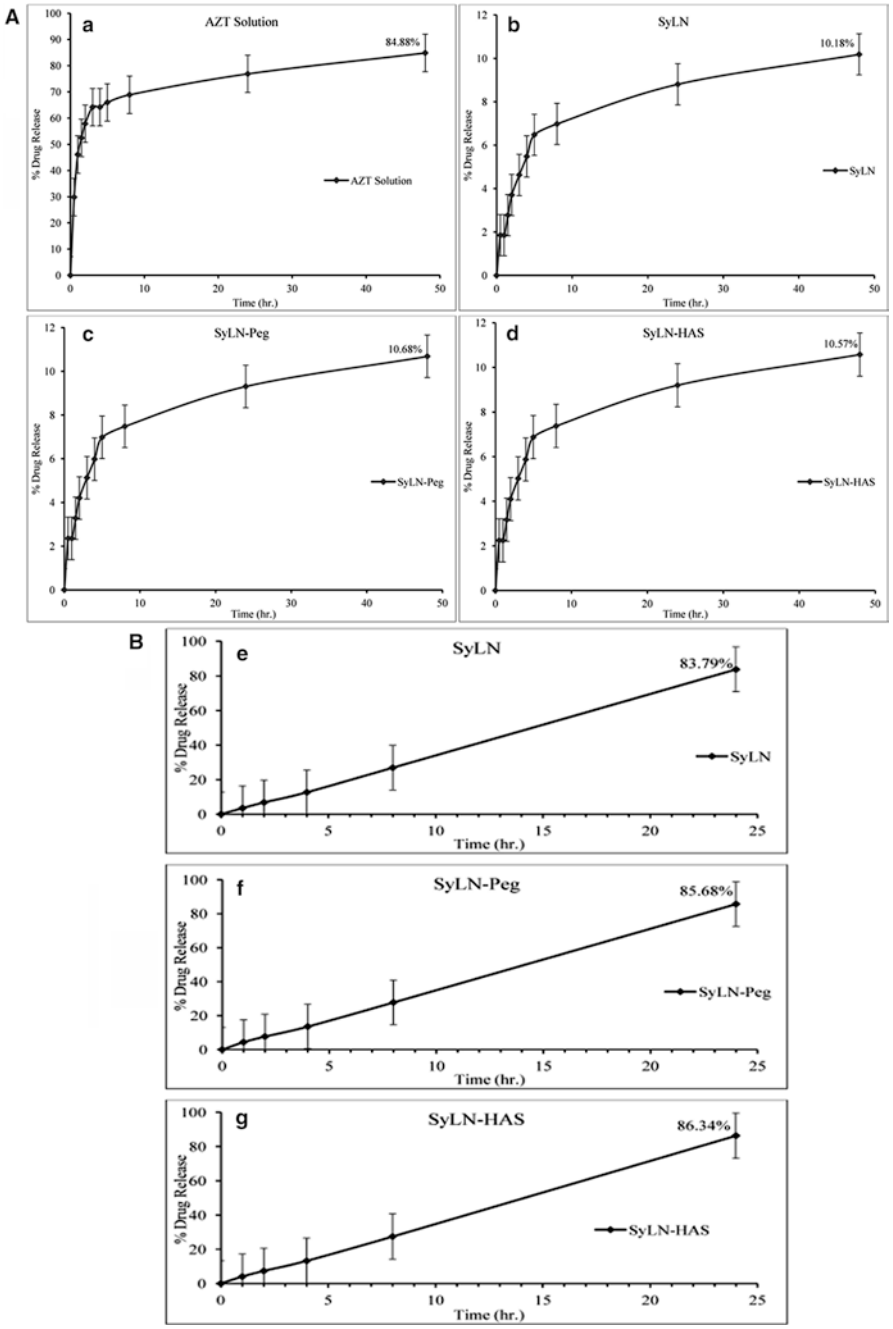


Fig. 14.4 In vitro drug release study of SyLN, SyLN-Peg, and SyLN-HSA in (**A**) aCSF (b–d). As compared to the AZT solution (a), the drug release from the NLC formulations is very less even after 48 h of study. This shows the sustained release properties of the NLC formulations. The coating does not alter the in vitro drug release properties of the NLC formulation to a great extent; (**B**) 50% v/v adult bovine serum with the saline medium (e–g). Both coated and uncoated formulations show similar drug release properties. Though most of the entrapped drugs are released within 24 h from the formulations, some drugs are remained to be released. This shows that the formulation is stable in blood serum for more than 24 h and can release drug for an extended period of time

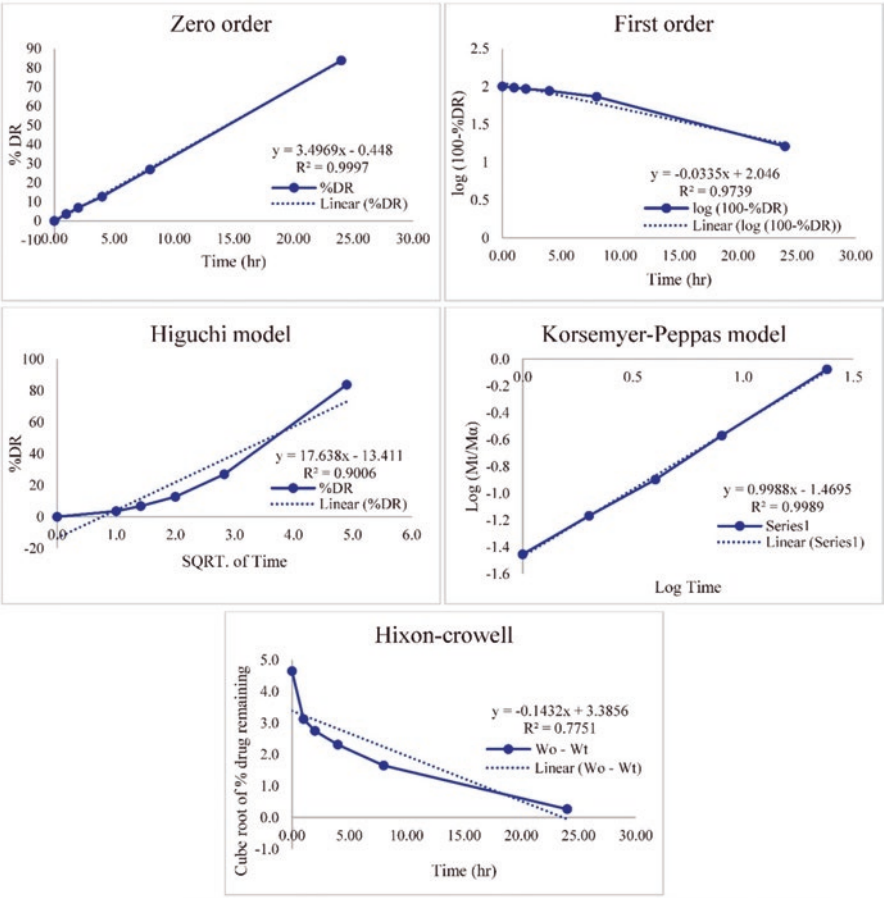


Fig. 14.5 Fitting the drug release data of SyNL in adult bovine serum in the mathematical models of the kinetics of drug release. The respective R^2 of all the models are being shown in the graphs. Data show that the formulation is best fitted in zero order and Korsmeyer–Peppas models. Similarly, the kinetic analysis of drug release data of SyLN-Peg and SyLN-HAS (Figure not shown) confirm that the coated NLCs are also best fitted in Zero order and Korsmeyer–Peppas models

Table 14.4 Kinetic modeling of release data from different NLC formulations

	R^2 values from different kinetic models					"n" value in
Formulation(s)	Zero-order	First order	Korsmeyer–Peppas	Hixon–Crowell	Higuchi	Korsmeyer–Peppas model
SyLN	0.9997	0.9739	0.9989	0.7751	0.9006	0.9988
SyLN-Peg	0.9996	0.9721	0.9961	0.7602	0.9042	0.9372
SyLN-HSA	0.9995	0.9702	0.997	0.7696	0.9002	0.9644

R^2 values show that all the formulations best fit in Zero order and Korsmeyer–Peppas models. "n" value shows that the drug release from all the formulations follows the super case II transport mechanism

at 37 °C for 1 h. The small hemolytic effect of SyLN in such a higher concentration suggested that the formulation was safe for intravenous administration.

3.8 In Vivo Pharmacokinetic and Brain Distribution Study

The reversed-phase HPLC-UV method described, validated, and used for AZT quantification, provides great sensitivity and specificity, and high sample throughput required for pharmacokinetic studies. The chromatograms showed a good baseline separation and the mobile phase used resulted in optimal separation. The method was selective for AZT since it shows that no interfering peaks appeared near the retention time of the compound of interest (Fig. 14.6, I–III) The LOQ and LOD values were low, indicating the good sensitivity of this HPLC method.

The precision of the applied analytical method was very accurate. The observed value of precision for different concentrations ranged from 0.09 to 0.95%. The coefficient of variation did not exceed 15% in the analyses. Accuracy and recovery were also in good agreement with acceptable values for the validation of an analytical procedure (i.e., 100 ± 20%). The observed accuracy value varied from 100.29 to 102.52%. The accuracy of intra and inter day analysis varied between 100.29–103.77% and 100.30–105.23%, respectively. The precision of intra and inter day analysis varied between 0.07–1.31% and 0.07–0.55%, respectively.

The sample preparation used in this study involved only a single step (i.e., deproteinization with acetonitrile). This condition was optimal for sample preparation as it resulted in clean chromatograms.

The mean plasma concentration-time profiles of the AZT solution, SyLN, SyLN-Peg, and SyLN-HSA are presented in Fig. 14.7b. Pharmacokinetic parameters of AZT solution, SyLN, SyLN-Peg, and SyLN-HSA were determined using two-compartmental analysis, and the data are presented in Table 14.5. As shown at all points in Fig. 14.7b, the AZT plasma concentration was higher in rats administered with SyLN-Peg and SyLN-HSA than those administered with AZT solution and SyLN ($p < 0.05$) except the first sampling point. There was no difference in T_{max} of AZT when it was given as NLC formulation. The AUC_{0-t} and $AUC_{0-\infty}$ value of SyLN-HSA have been found to be 1.73 and 1.61 times higher than AZT solution ($p < 0.05$), respectively. But AUC_{0-t} and $AUC_{0-\infty}$ values of SyLN and SyLN-Peg was lesser than that of AZT solution. The half-life of AZT in plasma was decreased with the NLC formulations ($p < 0.05$). Compared with AZT solution, the mean resident time (MRT) was also shortened with NLC formulations ($p < 0.001$). These observations indicated that the AZT is more rapidly removed from the plasma compartment when incorporated in NLC formulations. This may happen in two ways (viz., rapid elimination of AZT loaded NLCs from blood through the excretory routes, or a rapid tissue distribution to the peripheral compartments). In both cases the concentration of AZT in plasma reduces rapidly [62].

The mean concentration versus time profile of AZT solution, SyLN, SyLN-Peg, and SyLN-HSA in the brain have been presented in Fig. 14.7a. The pharmacoki-

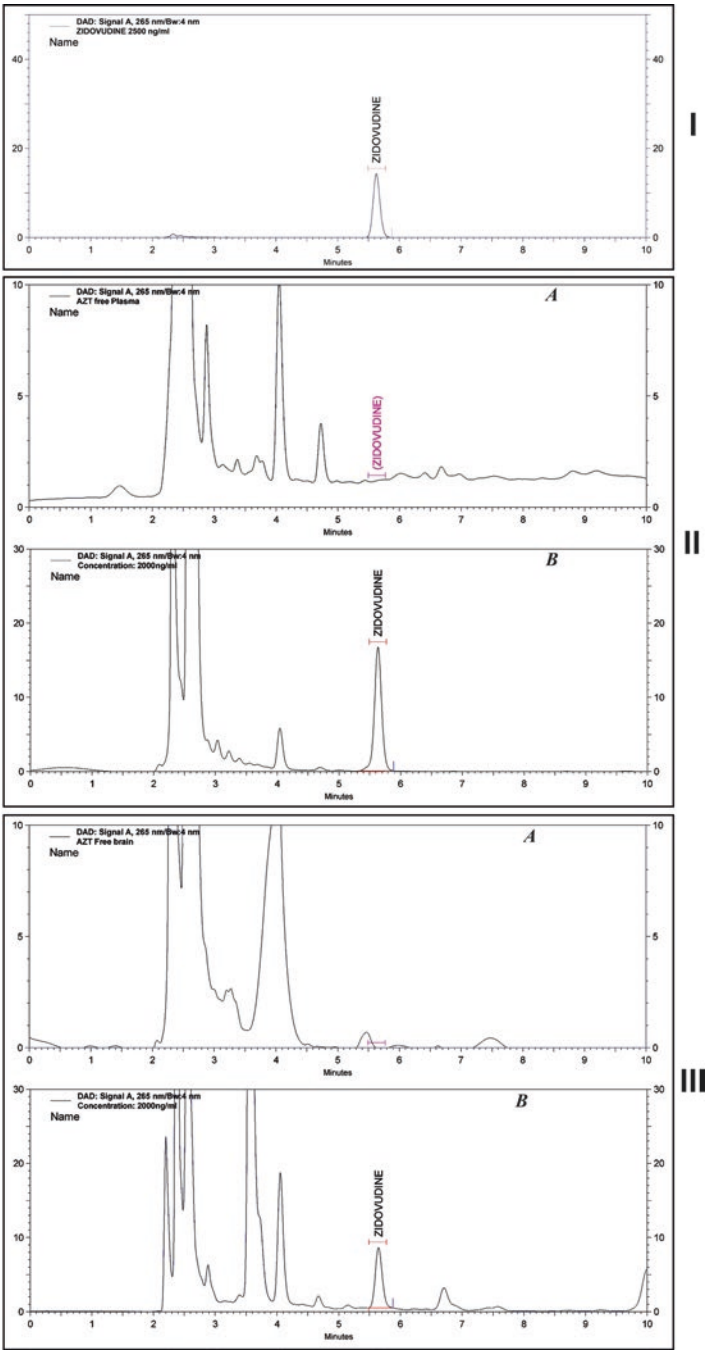


Fig. 14.6 (**I**) HPLC chromatogram of an aqueous solution of AZT. The chromatogram shows a single peak without any interfering peaks; (**II**) HPLC chromatograms demonstrating selectivity with plasma samples (A) Blank rat plasma; (B) Rat plasma spiked with AZT (2000 ng/mL); (**III**) HPLC Chromatograms demonstrating selectivity with rat brain samples (A) Blank rat brain; (B) Rat brain spiked with AZT (2000 ng/mL)

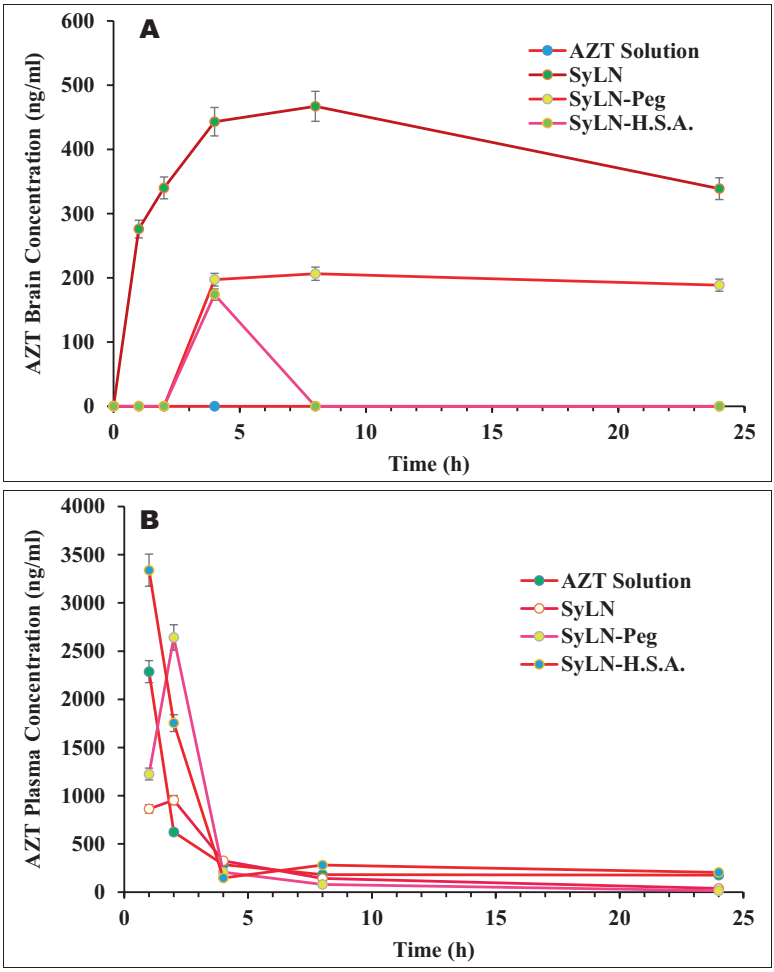


Fig. 14.7 (**a**) Brain pharmacokinetic profile of AZT solution, SyLN, SyLN-Peg, and SyLN-HSA. Uncoated AZT loaded docosanol NLCs (SyLN) increases the brain AZT concentration significantly ($p > 0.05$). Coating the NLC decreases the brain delivering efficiency of the NLC formulation; (**b**) Plasma pharmacokinetic profile of AZT solution, SyLN, SyLN-Peg, and SyLN-HSA. SyLN-HSA shows similar plasma concentration–time profile as AZT solution after intravenous injection and attains C_{max} after 1 h of I.V. injection. SyLN and SyLN-Peg attain C_{max} after 2 h of administration

Table 14.5 Rat plasma pharmacokinetic parameters for intravenous bolus AZT solution, SyLN, SyLN-Peg, and SyLN-HSA

	AZT solution	SyLN	SyLN-Peg	SyLN-HSA
C_{max} (ng/mL)	2287.30 ± 1.47	954.46 ± 11.93	2641.72 ± 11.93	3339.50 ± 11.93
T_{max} (h)	1	2	2	1
AUC_{0-t} ng-h/mL	8715.19 ± 33.98	5293.75 ± 314.23	6996.59 ± 744.62	15,051 ± 169
$AUC_{0-\infty}$ ng-h/mL	10,402.50 ± 56.15	5664.81 ± 478.40	7112.86 ± 866.49	16,701.60 ± 308.19
$t_{1/2}$ (h)	9.31 ± 0.04	6.99 ± 0.98	5.62 ± 1.89	8.20 ± 0.16
MRT (h)	11.51 ± 0.07	7.23 ± 1.17	3.41 ± 0.87	8.02 ± 0.38

Table 14.6 Rat brain pharmacokinetic parameters for intravenous bolus AZT solution, SyLN, SyLN-Peg, and SyLN-HSA

	AZT solution	SyLN	SyLN-Peg	SyLN-HSA
C_{max} (ng/mL)	AZT nondetectable in brain	467.12 ± 9.76	206.44 ± 9.76	173.95 ± 9.76
T_{max} (h)		8	8	4
AUC_{0-t} ng-h/mL		9564.03 ± 235.29	4265.14 ± 213.35	173.95 ± 9.76
MRT (h)		11.8832 ± 0.0037	13.0881 ± 0.0061	4

netic parameters of AZT solution, SyLN, SyLN-Peg, and SyLN-HSA in the brain were determined using two-compartmental analysis and the pharmacokinetic parameters are tabulated in Table 14.6. Some previous studies have shown that AZT enters the brain when administered in solution form [8]. However, in our study, no AZT was detected in the rat brain with intravenous AZT solution in water. This may be due to various reasons like the difference in route of administration, the dose of AZT administered, improper or no brain perfusion, animal's physiological conditions, and application of the different analytical method [63, 64]. In previous studies, the oral AZT solution was administered in rat at 10 mg/kg B.W. The brain was not perfused to remove any traces of AZT from the brain blood vessels. These may be the reasons for detection of AZT in the brain samples [8]. In our study, intravenous AZT solution was administered at 5 mg/kg B.W of rat and the brain was harvested after complete perfusion by pumping 0.9% w/v sterile physiological saline solution through the common carotid artery to remove blood with any traces of AZT from the blood vessels of the brain. The C_{max} for SyLN and SyLN-Peg in collected brain tissue appeared to be 8 h after injection in rats, which is 4 h for SyLN-HSA. Both SyLN and SyLN-Peg enter the brain very rapidly and maintains a constant concentration of AZT in the brain for the entire duration of treatment (Table 14.6). SyLN shows a tendency of decreasing AZT concentration after 24 h. However, for SyLN-Peg, the concentration is almost constant. SyLN-HSA does not enter brain immediately after injection. It enters brain after 2 h of injection and is eliminated from brain very rapidly, as no AZT was detected after 8 h of treatment in the brain. Reduced brain permeability of PEG4000 coated NLC formulation as compared to uncoated formulation may be due to its increased particle size (57.5 ± 2.2 nm) or more negative zeta potential (−26 ± 0.7 mV). The same thing has also happened for the HSA coated formulation. HSA coating increases the particle size (59.6 ± 1.7 nm) and makes the particles drastically more electronegative in nature (−38.5 ± 0.9 mV). It is well known that physiological membranes like BBB are electronegative in nature. Hence, the more electronegative NLCs are repulsed by the BBB [64, 65]. Therefore, more electronegative PEG4000 and HSA coated NLC formulations are poorly permeated through the BBB into rat brain.

Lower plasma concentration of AZT for NLC formulation as compared to AZT solution can be explained using the brain AZT concentration data. The NLC formulations are indeed rapidly distributed to the peripheral tissues including brain, ultimately reducing the AZT plasma concentration. The pharmacokinetic parameters for SyLN, SyLN-Peg, and SyLN-HSA in the brain have been shown in Table 14.6.

The C_{max} for SyLN is higher as compared to the other two coated formulations. AUC value clearly shows that the SyLN is the best among all the formulations in delivering AZT to the brain for a prolonged duration of time.

3.9 The Drug-Targeting Index (DTI)

For SyLN, SyLN-Peg, and SyLN-HSA, the calculated value for brain DTI is "α" (infinity) as the free drug solution was unable to deliver AZT to the brain. Hence the denominator of the Eq. 14.4 becomes zero and making the result of the calculation a "α." This shows that the NLC formulations are much better in delivering AZT to the brain than the free drug solution.

3.10 Rat Brain Localization and Accumulation of NLCs

For a clear assessment of the trafficking and accumulation of NLCs in rat brain in vivo, we took advantage of the CLSM imaging of brain tissues (brain cryosections) from a qualitative point of view after intravenous administration of fluorescent-labeled NLCs. C6, a green fluorescent marker, was incorporated in SyLN NLCs to detect their biodistribution in vivo in rat brain. SyLN-C6 NLCs did not show any significant changes in the physicochemical properties as compared to SyLN NLCs. The selection of SyLN NLCs in brain localization and accumulation study was based on its satisfactory brain pharmacokinetics as shown in Table 14.6. The intravenous injection of SyLN-C6 resulted in a significant increase of SyLN NLCs in the rat brain at each time point. At 1 h post injection, SyLN NLCs were widely distributed in the rat brain (Fig. 14.8c F-2). The quantity of SyLN NLCs in the brain increased as time progressed and maximum brain localization of NLCs was observed at 4 h (Fig. 14.8c H-2). The intensity of fluorescence signals in the brain decreased as the time progressed past 4 h. Moreover, fluorescence signals were still detected in the brain at 24 h post injection (Fig. 14.8c J-2). As shown in the images (Fig. 14.8c, first column), the brain tissues/cells are readily identified by nuclei staining (DAPI, blue color) and the cells in the brain tissues were of less density because of their slow proliferation rate in the normal healthy rat brain. The merged images (Fig. 14.8c, third column) showed that SyLN NLCs were accumulated in the brain tissues and likely internalized into the cells including nucleus. Combining these results, the SyLN NLCs could cross the BBB, accumulated in brain tissues giving controlled delivery of AZT until 24 h.

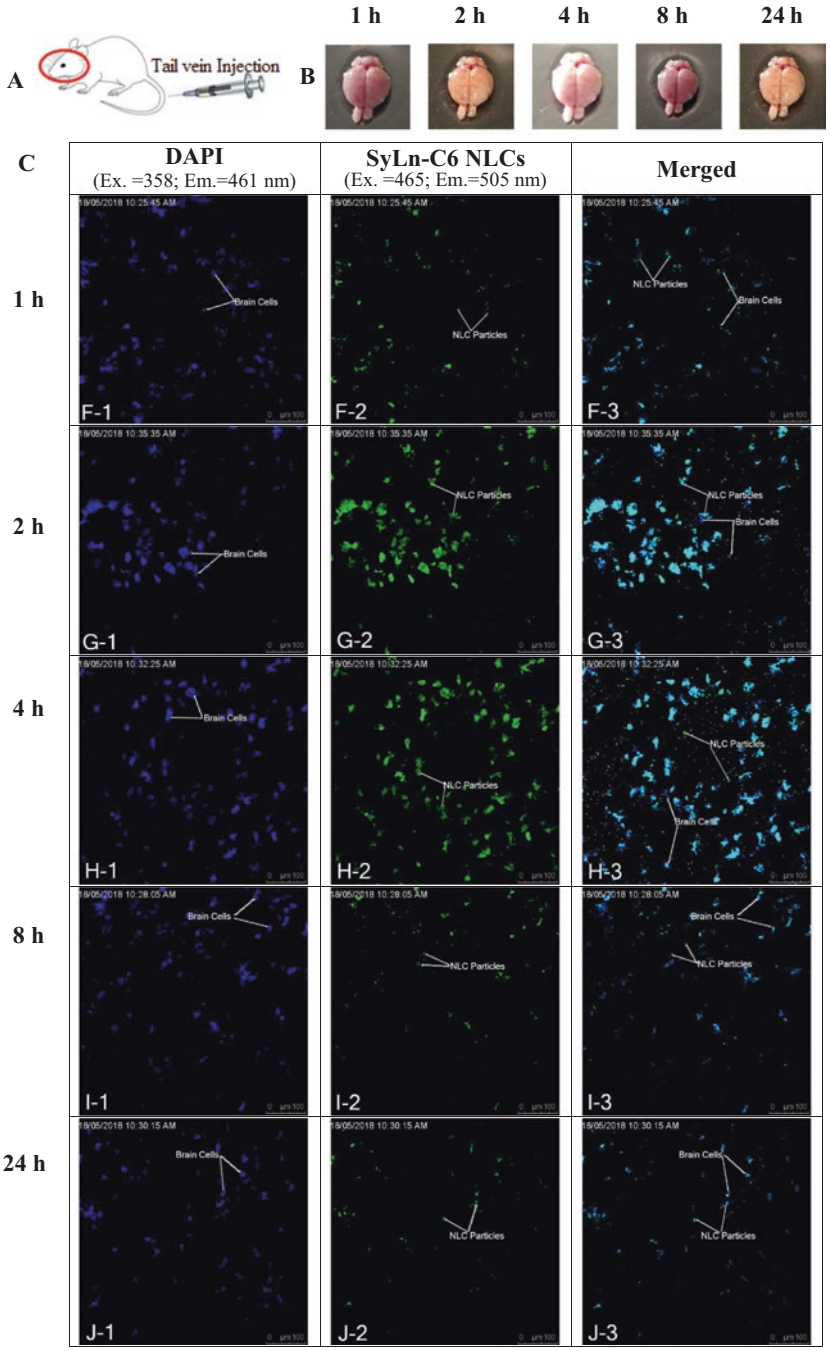


Fig. 14.8 Brain delivery of docosanol NLCs in rat model. (**a**) Wistar albino rat treated with intravenous SyLn-C6 NLCs through tail vein; (**b**) Rat brain collected at 1, 2, 4, 8, and 24 h; (**c**) CLSM images of brain cryosections labeled with DAPI (blue), SyLn-C6 NLCs (green) at 1, 2, 4, 8, and 24 h. Scale Bar: 100 μm

4 Conclusion

The docosanol NLCs were successfully developed by modified emulsion method for effective brain delivery of AZT. The developed NLCs were the best stable in refrigerated condition, in blood serum and safe for intravenous administration. The NLCs showed sustained release profile of AZT in aCSF and in vivo in rat brain. The in vivo plasma and brain pharmacokinetic investigation in rat model revealed that SyLN and SyLN-Peg NLCs rapidly reached the brain and yielded higher MRT, C_{max}, and AUC in rat brain compared to those data with AZT solution. The free drug solution was unable to deliver AZT to the brain. The rat brain pharmacokinetic data and CLSM imaging of rat brain cryosections confirm that the developed NLCs had effectively crossed the BBB delivering AZT in a sustained manner for a prolonged period of time and the SyLN is the best among all the formulations investigated in delivering AZT to the brain. This may provide an effective therapeutic strategy to combat the challenges of HIV infection in the brain. Further, preclinical and clinical development studies are warranted.

Acknowledgments The authors gratefully acknowledge the experimental/analytical support of Guwahati Biotech Park, Technology Complex, IIT Guwahati and The Sophisticated Analytical Instrument Facility (SAIF), NEHU Shillong, and College of Veterinary Science, Guwahati. This work was financially supported by the Department of Biotechnology, Ministry of Science & Technology, Government of India under Grant No. BT/504/NE/TBP/2013.

Declaration All figures and tables are original and self-made.

References

1. Koyuncu, O. O., Hogue, I. B., & Enquist, L. W. (2013). Virus infections in the nervous system. *Cell Host and Microbe, 13*, 379–393. https://doi.org/10.1016/j.chom.2013.03.010.
2. Schnell, G., Joseph, S., Spudich, S., Price, R. W., & Swanstrom, R. (2011). HIV-1 replication in the central nervous system occurs in two distinct cell types. *PLoS Pathogens, 7*, e1002286. https://doi.org/10.1371/journal.ppat.1002286.
3. Klecker, R. W., Collins, J. M., Yarchoan, R., Thomas, R., Jenkins, J. F., Broder, S., et al. (1987). Plasma and cerebrospinal fluid pharmacokinetics of 3′-azido-3′-deoxythymidine: A novel pyrimidine analog with potential application for the treatment of patients with AIDS and related diseases. *Clinical Pharmacology and Therapeutics, 41*, 407–412.
4. Fan, H., Liu, G., Huang, Y., Li, Y., & Xia, Q. (2014). Development of a nanostructured lipid carrier formulation for increasing photo-stability and water solubility of phenylethyl resorcinol. *Applied Surface Science, 288*, 193–200. https://doi.org/10.1016/j.apsusc.2013.10.006.
5. Lim, W. M., Rajinikanth, P. S., Mallikarjun, C., & Kang, Y. B. (2014). Formulation and delivery of itraconazole to the brain using a nanolipid carrier system. *International Journal of Nanomedicine, 9*, 2117–2126. https://doi.org/10.2147/IJN.S57565.
6. De Clercq, E. (2010). Antiretroviral drugs. *Current Opinion in Pharmacology, 10*, 507–515. https://doi.org/10.1016/j.coph.2010.04.011.
7. Kuo, Y.-C., & Chung, J.-F. (2011). Physicochemical properties of nevirapine-loaded solid lipid nanoparticles and nanostructured lipid carriers. *Colloids and Surfaces. B, Biointerfaces, 83*, 299–306. https://doi.org/10.1016/j.colsurfb.2010.11.037.

8. Purvin, S., Vuddanda, P. R., Singh, S. K., Jain, A., & Singh, S. (2014). Pharmacokinetic and tissue distribution study of solid lipid nanoparticles of zidovudine in rats. *Journal of Nanotechnology, 2014*, 1–7. https://doi.org/10.1155/2014/854018.
9. Singh, S., Dobhal, A. K., Jain, A., Pandit, J. K., & Chakraborty, S. (2010). Formulation and evaluation of solid lipid nanoparticles of a water soluble drug: Zidovudine. *Chemical and Pharmaceutical Bulletin (Tokyo), 58*, 650–655.
10. Uronnachi, E. M., Ogbonna, J. D., Kenechukwu, F. C., Chime, S. A., Attama, A. A., & Okore, V. C. (2014). Formulation and release characteristics of zidovudine-loaded solidified lipid microparticles. *Tropical Journal of Pharmaceutical Research, 13*, 199–199. https://doi.org/10.4314/tjpr.v13i2.5.
11. Deepak Sunil, B., Rajendra, D., & Narendra, D. (2010). Liposomal drug delivery system for zidovudine: Design and characterization. *International Journal of Drug Development and Research, 2*, 8–14.
12. Shibata, A., McMullen, E., Pham, A., Belshan, M., Sanford, B., Zhou, Y., et al. (2013). Polymeric nanoparticles containing combination antiretroviral drugs for HIV type 1 treatment. *AIDS Research and Human Retroviruses, 29*, 746–754. https://doi.org/10.1089/aid.2012.0301.
13. Das, S., Ng, W. K., & Tan, R. B. H. (2012). Are nanostructured lipid carriers (NLCs) better than solid lipid nanoparticles (SLNs): Development, characterizations and comparative evaluations of clotrimazole-loaded SLNs and NLCs? *European Journal of Pharmaceutical Sciences, 47*, 139–151. https://doi.org/10.1016/j.ejps.2012.05.010.
14. Doktorovová, S., Araújo, J., Garcia, M. L., Rakovský, E., & Souto, E. B. (2010). Formulating fluticasone propionate in novel PEG-containing nanostructured lipid carriers (PEG-NLC). *Colloids and Surfaces. B, Biointerfaces, 75*, 538–542. https://doi.org/10.1016/j.colsurfb.2009.09.033.
15. Joshy, K. S., & Sharma, C. P. (2012). Blood compatible nanostructured lipid carriers for the enhanced delivery of azidothymidine to brain. *Journal of Computational and Theoretical Nanoscience, 6*(1), 47–55.
16. Marcelletti, J. F. (2002). Synergistic inhibition of herpesvirus replication by docosanol and antiviral nucleoside analogs. *Antiviral Research, 56*, 153–166.
17. Nanjwade, B. K., Kadam, V. T., & Manvi, F. V. (2013). Formulation and characterization of nanostructured lipid carrier of ubiquinone (Coenzyme Q10). *Journal of Biomedical Nanotechnology, 9*, 450–460.
18. Pope, L. E., Marcelletti, J. F., Katz, L. R., Lin, J. Y., Katz, D. H., Parish, M. L., et al. (1998). The anti-herpes simplex virus activity of n-docosanol includes inhibition of the viral entry process. *Antiviral Research, 40*, 85–94. https://doi.org/10.1016/S0166-3542(98)00048-5.
19. Souto, E. B., Mehnert, W., & Muller, R. H. (2006). Polymorphic behaviour of Compritol888 ATO as bulk lipid and as SLN and NLC. *Journal of Microencapsulation, 23*, 417–433. https://doi.org/10.1080/02652040600612439.
20. Pope, L. E., Marcelletti, J. F., Katz, L. R., & Katz, D. H. (1996). Anti-herpes simplex virus activity of n-docosanol correlates with intracellular metabolic conversion of the drug. *Journal of Lipid Research, 37*, 2167–2178.
21. *TOXNET: 1-DOCOSANOL* [WWW Document]. (n.d.). Retrieved October 19, 2017, from http://toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs+hsdb:@term+@DOCNO+5739
22. Iglesias, G., Hlywka, J. J., Berg, J. E., Khalil, M. H., Pope, L. E., & Tamarkin, D. (2002a). The toxicity of behenyl alcohol: I. Genotoxicity and subchronic toxicity in rats and dogs. *Regulatory Toxicology and Pharmacology, 36*, 69–79. https://doi.org/10.1006/rtph.2002.1566.
23. Iglesias, G., Hlywka, J. J., Berg, J. E., Khalil, M. H., Pope, L. E., & Tamarkin, D. (2002b). The toxicity of behenyl alcohol: II. Reproduction studies in rats and rabbits. *Regulatory Toxicology and Pharmacology, 36*, 80–85. https://doi.org/10.1006/rtph.2002.1566.
24. Aburahma, M. H., & Badr-Eldin, S. M. (2014). Compritol 888 ATO: A multifunctional lipid excipient in drug delivery systems and nanopharmaceuticals. *Expert Opinion on Drug Delivery, 11*, 1865–1883. https://doi.org/10.1517/17425247.2014.935335.
25. Chinsriwongkul, A., Chareanputtakhun, P., Ngawhirunpat, T., Rojanarata, T., Sila-on, W., Ruktanonchai, U., et al. (2012). Nanostructured lipid carriers (NLC) for parenteral delivery of an anticancer drug. *AAPS PharmSciTech, 13*, 150–158. https://doi.org/10.1208/s12249-011-9733-8.

26. Gönüllü, Ü., Üner, M., Yener, G., Karaman, E. F., & Aydoğmuş, Z. (2015). Formulation and characterization of solid lipid nanoparticles, nanostructured lipid carriers and nanoemulsion of lornoxicam for transdermal delivery. *Acta Pharmaceutica, 65*, 1–13. https://doi.org/10.1515/acph-2015-0009.
27. Patel, D., Dasgupta, S., Dey, S., Ramani, Y. R., Ray, S., & Mazumder, B. (2012). Nanostructured lipid carriers (NLC)-based gel for the topical delivery of aceclofenac: Preparation, characterization, and in vivo evaluation. *Scientia Pharmaceutica, 80*, 749–764. https://doi.org/10.3797/scipharm.1202-12.
28. Azhar Shekoufeh Bahari, L., & Hamishehkar, H. (2016). The impact of variables on particle size of solid lipid nanoparticles and nanostructured lipid carriers; a comparative literature review. *Advanced Pharmaceutical Bulletin, 6*, 143–151. https://doi.org/10.15171/apb.2016.021.
29. Shaji, J., & Jain, V. (2010). Solid lipid nanoparticles: A novel carrier for chemotherapy. *International Journal of Pharmacy and Pharmaceutical Sciences, 2*, 8–17.
30. Uner, M., & Yener, G. (2007). Importance of solid lipid nanoparticles (SLN) in various administration routes and future perspectives. *International Journal of Nanomedicine, 2*, 289–300.
31. Wang, L., Luo, Q., Lin, T., Li, R., Zhu, T., Zhou, K., et al. (2015). PEGylated nanostructured lipid carriers (PEG-NLC) as a novel drug delivery system for biochanin A. *Drug Development and Industrial Pharmacy, 41*, 1204–1212. https://doi.org/10.3109/03639045.2014.938082.
32. Tamjidi, F., Shahedi, M., Varshosaz, J., & Nasirpour, A. (2014). Design and characterization of astaxanthin-loaded nanostructured lipid carriers. *Innovative Food Science and Emerging Technologies, 26*, 366–374. https://doi.org/10.1016/j.ifset.2014.06.012.
33. Kashanian, S., & Rostami, E. (2014). PEG-stearate coated solid lipid nanoparticles as levothyroxine carriers for oral administration. *Journal of Nanoparticle Research, 16*, 2293. https://doi.org/10.1007/s11051-014-2293-6.
34. Tsai, M. J., Wu, P. C., Huang, Y. B., Chang, J. S., Lin, C. L., Tsai, Y. H., et al. (2012). Baicalein loaded in tocol nanostructured lipid carriers (tocol NLCs) for enhanced stability and brain targeting. *International Journal of Pharmaceutics, 423*, 461–470. https://doi.org/10.1016/j.ijpharm.2011.12.009.
35. Pardeike, J., Weber, S., Haber, T., Wagner, J., Zarfl, H. P., Plank, H., et al. (2011). Development of an Itraconazole-loaded nanostructured lipid carrier (NLC) formulation for pulmonary application. *International Journal of Pharmaceutics, 419*, 329–338. https://doi.org/10.1016/j.ijpharm.2011.07.040.
36. Yuan, H., Wang, L.-L., Du, Y.-Z., You, J., Hu, F.-Q., & Zeng, S. (2007). Preparation and characteristics of nanostructured lipid carriers for control-releasing progesterone by melt-emulsification. *Colloids and Surfaces. B, Biointerfaces, 60*, 174–179. https://doi.org/10.1016/j.colsurfb.2007.06.011.
37. Thatipamula, R., Palem, C., Gannu, R., Mudragada, S., & Yamsani, M. (2011). Formulation and in vitro characterization of domperidone loaded solid lipid nanoparticles and nanostructured lipid carriers. *Daru, 19*, 23–32.
38. Gaba, B., Fazil, M., Khan, S., Ali, A., Baboota, S., & Ali, J. (2015). Nanostructured lipid carrier system for topical delivery of terbinafine hydrochloride. *Bulletin of Faculty of Pharmacy, Cairo University, 53*, 147–159. https://doi.org/10.1016/j.bfopcu.2015.10.001.
39. Tita, B., Ledeti, I., Bandur, G., & Tita, D. (2014). Compatibility study between indomethacin and excipients in their physical mixtures. *Journal of Thermal Analysis and Calorimetry, 118*, 1293–1304. https://doi.org/10.1007/s10973-014-3986-x.
40. Gartziandia, O., Egusquiaguirre, S. P., Bianco, J., Pedraz, J. L., Igartua, M., Hernandez, R. M., et al. (2016). Nanoparticle transport across in vitro olfactory cell monolayers. *International Journal of Pharmaceutics, 499*, 81–89. https://doi.org/10.1016/j.ijpharm.2015.12.046.
41. Praveen, S., Gowda, D. V., Srivastava, A., & Osmani, R. A. M. (2016). Formulation and evaluation of nanostructured lipid carrier (NLC) for glimepiride. *Der Pharmacia Lettre, 8*, 304–309. https://doi.org/10.20959/wjpps20164-6398.
42. Patlolla, R. R., Chougule, M., Patel, A. R., Jackson, T., Tata, P. N. V., & Singh, M. (2010). Formulation, characterization and pulmonary deposition of nebulized celecoxib encapsulated nanostructured lipid carriers. *Journal of Controlled Release, 144*, 233–241. https://doi.org/10.1016/j.jconrel.2010.02.006.

43. D'Souza, S., & Souza, S. (2014). A review of in vitro drug release test methods for nano-sized dosage forms. *Advances in Pharmacy, 2014*, 1–12. https://doi.org/10.1155/2014/304757.
44. Ahmad, A. M. (2007). Recent advances in pharmacokinetic modeling. *Biopharmaceutics and Drug Disposition, 28*, 135–143. https://doi.org/10.1002/bdd.
45. Dash, S., Murthy, P. N., Nath, L., & Chowdhury, P. (2010). Kinetic modeling on drug release from controlled drug delivery systems. *Acta Poloniae Pharmaceutica, 67*, 217–223. https://doi.org/10.1016/S0928-0987(01)00095-1.
46. Hu, X., Yang, F., Liao, Y., Li, L., & Zhang, L. (2017). Cholesterol–PEG comodified poly (N -butyl) cyanoacrylate nanoparticles for brain delivery: In vitro and in vivo evaluations. *Drug Delivery, 24*, 121–132. https://doi.org/10.1080/10717544.2016.1233590.
47. Khatik, R., Dwivedi, P., Shukla, A., Srivastava, P., Rath, S. K., Paliwal, S. K., et al. (2014). Development, characterization and toxicological evaluations of phospholipids complexes of curcumin for effective drug delivery in cancer chemotherapy. *Drug Delivery, 23*, 1–12. https://doi.org/10.3109/10717544.2014.936988.
48. Li, C., Shen, Y., Sun, C., Nihad, C., & Tu, J. (2014). Immunosafety and chronic toxicity evaluation of monomethoxypoly(ethylene glycol)-b-poly(lactic acid) polymer micelles for paclitaxel delivery. *Drug Delivery, 23*, 1–8. https://doi.org/10.3109/10717544.2014.920429.
49. Bondonna, T. J., Jacquet, Y., & Wolf, G. (1977). Perfusion-fixation procedure for immediate histologic processing of brain tissue. *Physiology and Behavior, 19*, 345–347. https://doi.org/10.1016/0031-9384(77)90351-1.
50. Gage, G. J., Kipke, D. R., & Shain, W. (2012). Whole animal perfusion fixation for rodents. *Journal of Visualized Experiments, 65*, 1–9. https://doi.org/10.3791/3564.
51. Mainardes, R. M., Palmira, D., & Gremiao, M. (2009). Reversed phase HPLC determination of zidovudine in rat plasma and its pharmacokinetics after a single intranasal dose administration. *Biological Research, 42*, 357–364. https://doi.org/10.4067/S0716-97602009000300010.
52. Yuan, Z. Y., Hu, Y. L., & Gao, J. Q. (2015). Brain localization and neurotoxicity evaluation of polysorbate 80-modified chitosan nanoparticles in rats. *PLoS One, 10*, e0134722. https://doi.org/10.1371/journal.pone.0134722.
53. He, C., Cai, P., Li, J., Zhang, T., Lin, L., Abbasi, A. Z., et al. (2017). Blood-brain barrier-penetrating amphiphilic polymer nanoparticles deliver docetaxel for the treatment of brain metastases of triple negative breast cancer. *Journal of Controlled Release, 246*, 98–109.
54. Shilo, M., Sharon, A., Baranes, K., Motiei, M., Lellouche, J.-P. M., & Popovtzer, R. (2015). The effect of nanoparticle size on the probability to cross the blood-brain barrier: An in-vitro endothelial cell model. *Journal of Nanobiotechnology, 13*, 19. https://doi.org/10.1186/s12951-015-0075-7.
55. Drobek, T., Spencer, N. D., & Heuberger, M. (2005). Compressing PEG brushes. *Macromolecules, 38*, 5254–5259. https://doi.org/10.1021/ma0504217.
56. Storm, G., Belliot, S. O., Daemen, T., & Lasic, D. D. (1995). Surface modification of nanoparticles to oppose uptake by the mononuclear phagocyte system. *Advanced Drug Delivery Reviews, 17*, 31–48. https://doi.org/10.1016/0169-409X(95)00039-A.
57. Amoozgar, Z., & Yeo, Y. (2012). Recent advances in stealth coating of nanoparticle drug delivery systems. *Wiley Interdisciplinary Reviews. Nanomedicine and Nanobiotechnology, 4*, 219–233. https://doi.org/10.1002/wnan.1157.
58. Łaszcz, M., Kosmacińska, B., Korczak, K., Śmigielska, B., Glice, M., Maruszak, W., et al. (2007). Study on compatibility of imatinib mesylate with pharmaceutical excipients. *Journal of Thermal Analysis and Calorimetry, 88*, 305–310. https://doi.org/10.1007/s10973-006-8001-8.
59. Manikandan, M., Kannan, K., & Manavalan, R. (2013). Compatibility studies of camptothecin with various pharmaceutical excipients used in the development of nanoparticle formulation. *International Journal of Pharmacy and Pharmaceutical Sciences, 5*, 315–321.
60. Nagaich, U., & Gulati, N. (2016). Nanostructured lipid carriers (NLC) based controlled release topical gel of clobetasol propionate: Design and in vivo characterization. *Drug Delivery and Translational Research, 6*, 289–298. https://doi.org/10.1007/s13346-016-0291-1.
61. Ribeiro, L. N. M., Breitkreitz, M. C., Guilherme, V. A., da Silva, G. H. R., Couto, V. M., Castro, S. R., et al. (2017). Natural lipids-based NLC containing lidocaine: From pre-formulation to in vivo studies. *European Journal of Pharmaceutical Sciences, 106*, 102–112. https://doi.org/10.1016/j.ejps.2017.05.060.

62. Barre, J., Urien, S., Albengres, E., & Tillement, J. P. (1988). Plasma and tissue binding as determinants of drug body distribution. Possible applications to toxicological studies. *Xenobiotica, 18*(Suppl 1), 15–20.
63. Sane, R., Agarwal, S., & Elmquist, W. F. (2012). Brain distribution and bioavailability of elacridar after different routes of administration in the mouse. *Drug Metabolism and Disposition, 40*, 1612–1619. https://doi.org/10.1124/dmd.112.045930.
64. Lockman, P. R., Koziara, J. M., Mumper, R. J., & Allen, D. D. (2004). Nanoparticle surface charges alter blood–brain barrier integrity and permeability. *Journal of Drug Targeting, 12*, 635–641. https://doi.org/10.1080/10611860400015936.
65. Voigt, N., Henrich-Noack, P., Kockentiedt, S., Hintz, W., Tomas, J., & Sabel, B. A. (2014). Surfactants, not size or zeta-potential influence blood-brain barrier passage of polymeric nanoparticles. *European Journal of Pharmaceutics and Biopharmaceutics, 87*, 19–29. https://doi.org/10.1016/j.ejpb.2014.02.013.

Chapter 15
Brain-Targeted Drug Delivery with Surface-Modified Nanoparticles

Sunita Lahkar and Malay K. Das

Abstract Medical treatment of CNS disorders remains unsuccessful as most of the drugs could not penetrate through the blood brain barrier (BBB). Although several strategies were developed to overcome these problems, still the treatment remains ineffective. To overcome these problems, nanomedicines which are based on noninvasive strategies are an emerging trend for brain-targeted drug delivery. The advantages of nanoparticles such as small size, lipophilicity, target specificity, and controlled delivery of drug satisfy the requisites for brain targeting. However, it suffers from opsonization and phagocytosis, which can be bypassed by surface modification of nanoparticles. The carrier/transporter-mediated transcytosis, adsorptive-mediated transcytosis, receptor-mediated transcytosis are the different mechanism followed by surface-modified nanoparticles to cross the BBB. However, nanoparticles may cause neurotoxicity due to its accumulation, oxidative stress and protein aggregation. Still nanoparticles are a promising carrier for drug targeting to the brain. The present chapter highlights the significance and recent development of drug targeting to the brain with surface-modified nanoparticles, the mechanism of transport and nanotoxicity.

Keywords Brain-targeted nanoparticles · Noninvasive nanomedicines · Surface-functionalized magnetic nanoparticles · Carrier-mediated transcytosis · Receptor-mediated transcytosis · Low-density lipoprotein receptor · Neurotoxicity of surface-functionalized nanoparticles · Nanotoxicity

S. Lahkar · M. K. Das (✉)
Drug Delivery Research Laboratory, Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh, Assam, India
e-mail: mkdps@dibru.ac.in

Y. V Pathak (ed.), *Surface Modification of Nanoparticles for Targeted Drug Delivery*, https://doi.org/10.1007/978-3-030-06115-9_15

1 Introduction

According to World Health Organization (WHO) report, neurological disorders ranging from epilepsy, Alzheimer disease, brain tumor, HIV encephalopathy, cerebrovascular diseases, and neurodegenerative disorders affect up to one billion people worldwide. About 6.8 million people die of neurological disorders every year. It signifies an inefficient delivery of CNS drugs to the brain. In the nineteenth century, a German physician Paul Ehrlich found out the existence of a physical barrier between brain and blood, and then it was not until 1960s that the researcher could find out the existence of BBB [1]. BBB provides the most distressing fact about the drug delivery to the brain. The other barrier present is the blood–cerebrospinal fluid barrier (BCSFB) and CSF–brain barrier (CSFB). The BBB is considered to be the major barrier due to its large surface area which is considered to be the main site for crossing of endogenous substances into the CNS [2]. BBB have a tendency to impair the drug distribution and refers to the major challenge for the development of CNS drugs. In spite of the complexity of the BBB, the lack of efficient technologies also limits the development of CNS drugs [3]. Although conventional therapies are available, yet the treatment fails. Briefly, BBB located at the choroid plexus epithelium controls the exchange of molecules between the blood and the cerebrospinal fluid. It is composed of tight junctions of protein complexes of endothelial cells, the capillary basement membrane, astrocytes end feet present over the basal lamina and pericytes present in the abluminal side of the endothelial cells, in the perivascular space, between the capillary wall and astrocytes end feet. The tight junctions of the endothelial cells are nonfenestrated, contains low number of endocytic vesicles, high electrical resistance, higher mitochondrial volume and specialized transport system. BBB restricts the entry of 98% of small molecules and 100% of macromolecules. Only lipophilic molecules, small molecular size (<500 Da) could penetrate through the BBB [4]. BBB restricts the penetration of most of the large-sized, hydrophilic drugs such as oligonucleotides, peptides, and antibiotics. The presence of the tight junctions between the endothelial cells at the BBB promotes a very high electrical resistance of around 1500–2000 Ω cm^2 in the brain compared with 3.33 Ω cm^2 in other body tissues. Still BBB allows the transport of chemical and biological endogenous substances to cross the membrane [5]. However, several endogenous substances are transported to the brain and toxic compounds are excreted from the cerebral and vascular compartment by the influx transport system in the endothelial cell membrane [6]. The passive transport is responsible for the influx of molecules having low molecular weight, good lipophilicity and low protein binding through the BBB. The small molecules such as hormones, O_2, and CO_2 are transported by passive transport mechanism [7], whereas active transport comprises transporter-mediated transcytosis and receptor-mediated endocytosis. The transporter-mediated transcytosis is for the influx of small hydrophilic molecules through the carriers present on the endothelial cell membranes. As such, glucose carrier GLUT1 and amino acid carrier LAT1 are responsible for the transport of glucose and amino acids through the BBB, whereas the membrane receptors present

on the endothelial cells are responsible for the transport of transferrin, insulin, and lipoprotein through the BBB by receptor-mediated endocytosis [8]. The drug delivery through the BBB remains the most challenging area of research, which attracts researchers to investigate on several strategies for an efficient CNS drug delivery. Basically, two strategies are studied—invasive and noninvasive. Invasive strategies involve either disruption of the BBB to allow drug delivery or direct injection of the drug into the brain. The disruption of the BBB involves an intra-arterial injection of hyperosmotic solution of mannitol which causes shrinkage of endothelial cells, resulting in opening the tight junctions for few hours, and thus facilitating delivery of the drug to the brain. Other substances such as high-dose ethanol, DMSO, alkylating agents like etoposide and melphalan, immune adjuvants, and cytokines have all been used to disrupt the BBB. No doubt such techniques can deliver drug to the brain, but as invasive strategies, they suffer from side effects such as seizures, bradycardia, and hypotension [9]. Recently, focused ultrasound with microbubbles is reported to be nondestructive delivery of drug to the brain. In this technique, microbubbles, 1–10 μm sized diameter, are introduced into the blood circulation prior to ultrasound administration. The microbubbles disrupt targeted areas of BBB without causing any neural damage, which reduces the intensity of the ultrasound needed to open the tight junctions. The second invasive CNS drug delivery based on injecting the drug through injection or catheter requires opening of the skull [10]. The major drawback is the penetration to the nontarget brain tissue, brain tissue damage, bleeding, and chance of infection. Alternatively, wafers such as Gliadel wafers which are biodegradable impregnated with chemotherapeutics can be implanted. Reliable on the diffusion of the drug from the injection and implant sites, the concentration of drug distribution at the site of action cannot be controlled due to an exponential decrease in the concentration of the drug with distance from the injection or implantation site [11]. On the other hand, convection-enhanced delivery developed with positive hydrostatic pressure to deliver drug at farther distances from the site of administration did not show a significant result with Gliadel wafers, as reported [12]. The invasive technique facilitates an increase in permeability of the BBB which is reported to be harmful. As reported, similar permeability enhancement and disruption of the BBB occurs during cerebral ischemia and hypoxia. It leads to the leakage of the serum proteins into the brain which triggers the activation of astrocytes and brain immune system, leading to neuronal hyperexcitability and neurodegeneration. Similar results may be observed with multiple sclerosis and encephalitis where the disruption of the BBB leads to the leakage of plasma components into the blood vessels and surrounding tissues results neuronal damage and other disabling cerebrovascular conditions—lacunar stroke, leukoaraiosis, and dementia [13]. The drawbacks associated with the invasive techniques are based on high cost and chemotherapy either by osmotic agents or direct injection or Gliadel wafers or convection enhanced delivery may lead to BBB dysfunctioning and multiple brain diseases [14]. Alternatively, noninvasive strategies were developed to overcome the potential disadvantages of invasive strategies. In addition to the passive transport of small-sized lipophilic molecules, other transport mechanism constitutes paracellular aqueous pathway for water-soluble agents, carrier or transporter-mediated

transcytosis that relies on the transport mechanism of glucose and amino acid, receptor-mediated transcytosis that mimics the endogenous molecules such as insulin and lipoproteins to act on the specific receptor in the endothelial cell membrane and adsorptive-mediated transport that allows polycationic substances such as cationized albumin to attach to the negatively charged plasma membrane [3]. Another noninvasive strategy follows the conversion of water-soluble/polar drugs into lipid soluble ones by linking with some lipid/nonpolar moiety. Esterification or amidation of the hydroxyl, amino, and carboxylic groups of the drug enhances the lipid solubility and membrane permeability of the drug. This concept is known as prodrug. Prodrug as such is pharmacologically inactive compounds which crosses the BBB and metabolizes into the parent drug. The receptor-mediated transport or carrier-mediated transport have been exploited significantly in delivering the prodrug across the BBB. One example is the antiparkinsonism drug, L-DOPA acting on the L-amino acid transporter system. The prodrug suffers from the drawback of adverse pharmacokinetics, increase molecular weight of the drug [15]. Another noninvasive strategy, nanomedicine has gained lots of attention in the development of CNS-targeted drug delivery due to its ability for targeted drug delivery and sustained release of drug [16]. This chapter highlights the significance and recent development of drug targeting to the brain with surface-modified nanoparticles, the mechanism of transport and nanotoxicity.

2 Nanoparticles for Brain Drug Delivery

2.1 Nanoparticles Technology

Nanoparticles can be defined as colloidal particles of size range 1–100 nm which adsorb the drug to their surface or entrap the drug within their matrix. Other noninvasive techniques could deliver an inadequate drug to the brain and also affect the nontarget sites causing toxicity, whereas nanoparticles are target specific and deliver only the required quantity of the drug to the site of action without affecting the nontarget sites and thus reduces toxicity like other noninvasive techniques. In order to target drugs to the brain, nanoparticles should be nontoxic, should be biodegradable, have small particle size (<100 nm), have prolonged blood circulation without agglomeration, target specificity, and good drug loading [17]. A prolonged blood circulation is essential to recognize the therapeutic site of action so as to have an efficient drug release. However, the opsonization or removal of nanoparticulate drug carriers from the body by the mononuclear phagocytic system (MPS), also known as the reticuloendothelial system (RES), obstructs the efficient drug delivery at the site. When nanoparticles enter systemic circulation after intravenous administration, they undergo opsonization and phagocytosis, leading to an inadequate distribution in the brain and poor drug availability in the brain, thus making the therapy inefficient.

As such opsonization can be described as the process of attachment of opsonin to the surface of undesirable particles during systemic circulation, thereby making it visible to phagocytic cells. Without the presence of opsonin on their surface, phagocytes may not be able to recognize the foreign particles. Thus, opsonization and phagocytosis are the two methods of clearance of foreign particles from the bloodstream. The common opsonin present in the blood serum constitutes immunoglobulins and components of the complement system such as C3, C4, and C5 [18]. Other blood serum protein includes laminin, fibronectin, C-reactive protein, type I collagen, and many others. As such, opsonins are inactive proteins, but when it comes in contact with foreign particles, it undergoes conformational changes from inactive protein to active proteins which in turn are easily identified by phagocytes. Phagocytes' surface contains receptors which can easily identify the modified conformation of the opsonin and thus can easily alter the functioning of foreign bodies. The ingestion of nanoparticles by phagocytes takes place. The breakdown of the phagocytosed materials takes place due to the secretion of enzymes and other oxidative-reactive chemical factors, such as superoxides, oxyhalide molecules, nitric oxide, and hydrogen peroxide. Nanoparticles are taken rapidly by RES present in liver, spleen, bone marrow and distributed rapidly into liver (60–90)% and spleen (2–10)% and to a minor degree into bone marrow [19]. A low concentration of nanoparticles can enter the brain due to their uptake by RES following intravenous administration. In order to avoid opsonization and phagocytosis, several techniques are hypothesized to modify the surface of nanoparticles such as coated nanotechnology and ligand-based nanotechnology [19]. Surface-modified or -functionalized nanoparticles could not adsorb opsonin on the surface and may not be recognized by phagocytes. Thus, avoidance of opsonization and phagocytosis by the RES or MPS prevents its clearance from the bloodstream, leading to the prolonged circulation of nanoparticles in the blood, adequate delivery of the drug to the brain and maintaining the therapeutic concentration of the drug in the brain. Thus, nanocarriers as a noninvasive strategy are significant for brain-targeted delivery of drugs [20].

3 Types and Significance of Surface-Functionalized Nanocarriers

3.1 Liposomes

Liposomes are small vesicles composed of one or more phospholipid bilayers surrounding an aqueous space. Both hydrophilic and hydrophobic drugs can be incorporated in liposomes and their physicochemical characteristics can be manipulated to control drug delivery and tissue uptake of the drug. Zhao et al. reported that RDP peptide-conjugated liposome (RCL) could deliver curcumin to the intracranial glioma mice model transplanted with U251MG cells. RCL could enter the cells by

acetylcholine (Ach) receptor-mediated, energy-dependent endocytosis [21]. In another study, transferrin-conjugated PEGylated-liposome could internalize anti-cancerous drug, resveratrol to the U-87 glioblastoma cells by the receptor-mediated endocytosis through the transferrin receptor present on the endothelial cell [22] as reported by Jhaveri et al. In another study, Kim et al. followed transferrin mediated transcytosis pathway for successful targeting of temozolomide using transferrin grafted nanoliposomes in glioblastoma multiform tumor model in mice [23].Qu et al. reported that rabies virus glycoprotein (RVG29)-functionalized liposomes efficiently enhanced the entrapment efficiency of dopamine-derived *N*-3,4-bis(pivaloyloxy)-dopamine (BPD) in murine brain endothelial cells and dopaminergic cells and high penetration efficiency across the blood brain barrier (BBB) in vitro. RVG acts on the Ach receptor present on the brain endothelial cells and dopaminergic cells to facilitate the transport of BPD across the BBB [24]. Sonali et al. developed transferrin-conjugated theranostic D-alpha-tocopheryl polyethylene glycol 1000 succinate monoester (TPGS) liposomes which successfully targeted docetaxel and quantum into brain cancer cells [25]. T7 (a seven-peptide ligand of transferrin receptors) and ^{D}A7R (a D-peptide ligand of vascular endothelial growth factor receptor 2) dual peptides-modified PEGylated liposomes were able to code-liver doxorubicin (DOX) and vincristine (VCR) to C6 tumor mice model by receptor-mediated endocytosis as reported by Zhang et al. [26]. However, liposomes suffer from drawbacks such as low solubility, oxidation and hydrolysis of the phospholipids, leakage of the encapsulated drugs, and instability which limits its use in targeted drug delivery.

3.2 Polymeric Nanoparticles

Polymeric nanoparticles, made from biodegradable and nonbiodegradable polymers, are spherical, branched, or shell structure colloidal solid particles with a size range of 10–1000 nm in which drugs are incorporated by dissolution, entrapment, adsorption, and attachment or by encapsulation. The polymeric nanoparticles are an ideal platform for targeted and controlled drug delivery. Biodegradable polymeric nanoparticles have got a wide application in brain-targeted delivery of drugs, as it can be manipulated to fulfill the criteria needed for brain-targeted delivery due to small size, nontoxicity, biodegradability, etc. Poly(lactic acid) (PLA), poly(ε-caprolactone) (PCL), poly(aspartic acid), poly(butylcyanoacrylate) (PBCA), poly(glycolic acid) (PGA), poly(D,L-lactide-*co*-glycolide) (PLGA), and poly(amino acids) are the most commonly used polymers in CNS delivery [27]. As such surface modification is essential to avoid opsonization and phagocytosis by macrophages. Till now, several research works have been carried out which successfully target different drugs across the BBB using biodegradable polymeric nanoparticles. In 1995, Kreuter et al. were the first to develop dalargin-loaded PBCA nanoparticles coated with polysorbate 80 that successfully targeted dalargin to the brain and also enhanced the penetration by threefold than the nanoparticles without surface

coating [28]. Later on, polysorbate 80 was further used to enhance drug transport of several drugs like loperamide and doxorubicin. Later PEGylated poly(hexa-decyl cyanoacrylate) (PHDCA) nanoparticles were found to penetrate the brain to a greater extent than the P80 formulation which might be due to passive diffusion or intake by macrophages [29]. PLA, PGA, and their copolymer, PLGA are extensively used in brain-targeted delivery of different drugs. In a study, H102 peptide, an antialzheimeric drugs was targeted successfully. A tocopherol PEGylated PLGA nanoparticles targeted oxcarbazepine, an antiepileptic drug, across in vitro models of the blood–brain barrier (hCMEC/D3 cells) and human placental trophoblast cells (BeWo b30 cells) [30]. Glutathione–PEG conjugate-coated PLGA nanoparticles showed higher permeation through the coculture of rat brain endothelial (RBE4) and C6 astrocytoma cells. The glutathione on the surface of the nanoparticles are found to bind to the glutathione transporters present on the BBB and deliver the drug by carrier or transporters mediated transcytosis [31]. Another report, by Ahmed et al., showed the efficient targeting of Rutin in rat model, an antioxidant, to the brain using chitosan nanoparticles through intranasal administration [32]. Although, FDA approved polymers are recommended for nanoparticulate drug delivery, yet some drawbacks exist during nanoformulation which are to be considered for further modification. Other than these, polymeric nanoparticles suffer from other disadvantages such as high cost, inability of autoclave sterilization, low-scale production, and presence of organic solvent residue. But, despite its drawbacks, it is still a potential drug delivery vector for targeting drugs to the brain [33].

3.3 Solid Lipid Nanoparticles (SLNs)

Later on, SLNs came into play for drug targeted delivery system. It has a size range of 1–100 nm, composed of a monolayer of phospholipid surrounding a solid hydrophobic core of lipids, such as monoglycerides, diglycerides, and triglycerides or fatty acids. They are stable and biodegradable under physiological conditions with high encapsulation efficiency both for hydrophilic and hydrophobic drugs [33]. Solid lipid nanoparticles can be applied for targeted drug delivery, controlled drug delivery and also can be surface-functionalized with polymeric coating or ligand grating for targeting drugs significantly. Other advantages include large-scale production, long-term stability, avoidance of organic solvents, easy scale-up and sterilization, less cost than polymeric/surfactant-based carriers, and easy validation and regulatory approval [34]. These make solid lipid nanoparticles an attractive approach for targeted drug delivery. The anticancer drug, camptothecin was the first drug delivered using solid lipid nanoparticles which showed stronger inhibition of melanoma cell proliferation than the free drug after a 24 h incubation period. It was hypothesized that solid lipid nanoparticles endocytosed by the cancer cells, leading to greater drug uptake and thus presenting SLNs as an attractive option for cancer therapy [35]. Later surface coating by polysorbate 80 on solid lipid nanoparticles carried out by Kreuter et al. [36] also showed satisfactory results. Several lipophilic

drugs, peptides, or proteins were delivered using solid lipid nanoparticles. Other antiepileptic drugs successfully brain-targeted include rizatriptan benzoate. An anticancer drug, carmustine (BCNU), loaded in transferrin (Tx) and lactoferrin (Lf)-functionalized PEGylated solid lipid nanoparticles could penetrate human microvascular endothelial cells (BMECs) ten times more than that of PEGylated solid lipid nanoparticles. Transferrin (Tx) prevents the efflux transporter system, while lactoferrin (Lf) undergoes receptor-mediated endocytosis (Kou et al. [37]). Another drug, resveratrol, a neuroprotective compound could penetrate in the hcmec/D3 monolayers 1.8-fold higher when incorporated in apolipoprotein E-conjugated solid lipid nanoparticles by endocytosis (Neves et al. [38]). Similarly, the concentration of drugs in multiple sclerosis-induced mice was 4–8 times higher when loaded in PEGylated solid lipid nanoparticles surface-modified with anticontactin 2 or antineurofascin than that of unmodified solid lipid nanoparticles [39]. Bruun et al. encapsulated *si*RNA in cationic angiopep-functionalized SLNs with >95% efficiency for delivery to glioma cells [40]. However, several limitations of SLNs due to poor drug loading capacity, drug expulsion after polymeric transition during storage, relatively high water content of the dispersions, the low capacity to load hydrophilic drugs due to partitioning effects during the production process does not limits its use as a drug delivery system [34]. In spite of these drawbacks, SLNs have got good potential for CNS-targeted drug delivery.

3.4 Magnetic Nanoparticles

In the recent years, magnetic nanoparticles (MNPs) gained special interest in brain-targeted delivery, since brain cells are quite sensitive to MNPs, compared to, liver and heart cells. At present magnetic nanoparticles have lots of applications: as a contrast agent for magnetic resonance imaging (MRI), to induce hyperthermia in cancer therapy, for cell labeling and cell separation, in targeted therapeutics, in magnetofection, etc. As a contrast agent in MRI and targeted therapeutics, superparamagnetic iron oxide nanoparticles (SPIONs) have been focused on with a wide range of applications. Magnetic nanoparticles under the influence of an externally applied low frequency magnetic field can elevate the physiological temperature, 38–39 °C, which facilitates the penetration into the blood–brain barrier. SPIONs degrade to Fe^{3+}in the body, which undergoes cell metabolism and ultimately eliminated from the body and also the particles due to very small size <30 nm, will not be attracted to each other, and so the risk of agglomeration in a medical setting is minimized. SPIONs can diagnose and directed to the diseased cell under magnetic field, can also generate radiation to treat the cells [41]. Anti-IL-1β monoclonal antibody (mAb)-functionalized SPIONs were used to render MRI diagnoses and simultaneously provide targeted therapy with the neutralization of IL-1β overexpressed in epileptogenic zone of an acute rat model of temporal lobe epilepsy. Similarly, they are also used to target metastases cells in human [42]. The dual application of MNPs as a diagnostics and treatment agent can be called a theranostics. A wide range of

applications of magnetic nanoparticles with their mechanism of drug delivery are discussed in the next section.

4 Mechanism of Surface-Functionalized Nanoparticle Drug Delivery

Surface-functionalized nanoparticles can noninvasively deliver neurotherapeutics to the CNS by modifying the endogenous molecules transport mechanism. Basically, three types of transport mechanisms for CNS drug delivery exist—adsorptive-mediated transcytosis, receptor-mediated transcytosis, and transport or carrier-mediated transcytosis. These transport mechanisms can be manipulated by the nanoparticles with surface modification either by coating with polymers or attachment of ligands to deliver drug to the brain [19].

4.1 Carrier/Transporter-Mediated Transcytosis (CMT)

Carrier-mediated transcytosis is based on the conformational change of membrane transport proteins add direct energy conversion such as ATP hydrolysis to move endogenous solutes such as glucose and amino acids along their concentration gradient. These transport proteins are present on the luminal and abluminal side of the brain endothelial cell membrane in the BBB. GLUT1and GLUT3 are the transporter protein for the intake of D-glucose and glucose analogs from the blood into the brain while LAT1 is the neutral amino acid transported protein in the membrane. There are two drug transport mechanism in CMT, either by chemical modification of the drug into a "pseudonutrient" to resemble these endogenous substances as in the transport of L-DOPA or the conjugation of the nanocarriers with a natural substrate to allow endogenous transport mechanism for the drug [43].

Glucose Transporter-Mediated Transcytosis The glucose transport to the brain involves the interaction of solutes, transporters, enzymes and cell signaling processes in the brain. The GLUT1 and GLUT3 are the sodium independent facilitative glucose transporters which are involved in the catabolism of the glucose to create a concentration gradient for the transport of glucose by GLUT1 from the blood toward the brain interstitial fluid [44]. Other glucose transporter (GLUT) and sodium-dependent transporters (SGLTs) also contribute in the transportation of glucose across the BBB [45]. It is found that the endothelial cells at the blood-brain barrier could transport around ten times their weight of glucose per minute to support the glucose requirements of the brain [46]. This provides good potential to mediate glucose transporter mechanism in drug delivery system. Till now, several neuroactive drugs conjugated with glucose to target GLUT1 are efficiently transported across the BBB. These drugs include neuroactive enkephalin peptides, antidepressant

drug—7-chlorokynurenic acid, and anti-inflammatory drugs (NSAIDs) —ketoprofen and indomethacin. Also, glucose conjugated to nanocarriers could deliver several drugs to the brain [47]. In one study, biodistribution of the fluorescent model drug, coumarin-6 loaded liposomes composed of phospholipids and glucose-derived cholesterols with different linker lengths (GLU200-LIP, GLU400-LIP, GLU1000-LIP, and GLU2000-LIP) were evaluated in vivo in mice brain. The liposomes exhibited the strongest brain delivery potential with GLU1000-LIP [48]. Another drug, docetaxel was delivered significantly when loaded in glucose-modified liposomes than control liposomes as observed in mice brain [49]. Another reported that GLUT1 and GLUT3 responsible for the cellular uptake of liposomes modified with *p*-aminophenyl-α-D-mannopyranoside by transporter-mediated transcytosis [50]. Similarly, dehydroascorbic acid-derivatized micelles have been developed for treating the highly aggressive cancer malignant glioma and have shown accumulation within tumor cells and therefore potential for delivering drugs to cancer sites in the brain and central nervous system via GLUT1 [47]. Similarly, doxorubicin, an anticancerous drug, was brain-targeted to GLUT1 and accumulated in glioma cells when loaded in Pluronic P105 polymeric micelles [51]. Nanoparticles of poly(ethylene glycol)-co-poly(trimethylene carbonate) functionalized with 2-deoxy-D-glucose were dual targeted to GLUT1 for drug delivery in glioma treatment [52].

Large Neutral Amino Acids Transporters Large neutral amino acid transporters (LAT1) are an endogenous nutrient transporter present in the luminal and abluminal cell membrane of the brain capillary endothelial cells (BCECs). The brain uptake of neutral amino acids such as phenylalanine, leucine, and tyrosine are regulated by LAT1. LAT1 has gained popularity for brain-targeted drug delivery either as "prodrug" or substrate conjugated to the drug delivery system resembling the endogenous neutral amino acids [43]. One example of "prodrug concept" is the delivery of L-DOPA. Dopamine as such cannot cross the BBB, but when delivered in the form of L-DOPA, LAT1 facilitates the uptake of L-DOPA, where dopamine is released by decarboxylation. Drugs such as baclofen, α-methyl-DOPA, and gabapentin are also transported by this technique [53]. Technique based on Trojan horse is used to deliver drug through LAT1. LAT1 substrate such as L-cysteine conjugate 6-mercaptopurine increases the lipophilicity of the drug, otherwise a polar molecule could not target to the LAT1 into the brain [54]. Similarly, LAT1 substrate tyrosine is coupled with the NSAID ketoprofen to form a zwitterionic prodrug which facilitates the release of conjugate drug by the action of esterase enzyme present in the brain parenchyma [47]. In another study, phenylalanine-coupled solid lipid nanoparticles were found to be capable for increased accumulation of efavirenz in the brain and cerebrospinal fluid to inhibit viral loads in neurodegenerative disorders which could be attributed to the presence of LAT1 transporters which facilitate transport of phenylalanine to the brain via carrier or transporter-mediated transcytosis [55]. Fernandez et al. reported that saxagliptin (SAX), a dipeptidyl peptidase-4 enzyme inhibitor molecule used in the therapy of Alzheimer disease is hydrophilic and not permeable across the BBB. An attempt of incorporating the drug in chitosan

nanoparticles, conjugated with L-valine showed a higher accumulation of 53 ng/mL SAX from the nanoparticles than the pure SAX after 24 h, as obtained after in vivo study in rat [56].

SMVT/SLC5A6 (Sodium-Dependent Multivitamin Transporter) Sodium-dependent multivitamin transporter (SMVT/SLC5A6) is a significant transporter requisite for the uptake of the vitamins—biotin and pantothenate, which are highly expressed in placenta, intestine, brain, liver, lung, kidney, and heart [57]. Thus exploiting the SMVT mechanism may transport several drugs to the brain. Biotin-labeled solid lipid nanoparticles penetration in an in vitro blood–brain barrier model hCMEC/D3 brain endothelial cell was compared to biotinylated glutathione-labeled nanoparticles. Biotin as a ligand increased the uptake and the transfer of nanoparticles across brain endothelial cells by SMVT supporting its use as a brain targeting vector [58].

Thiamine Transporter Thiamine (a water-soluble vitamin B1), a micronutrient essential for normal cell growth and development is reported to transport to the brain by thiamine transporter-mediated transcytosis. Thiamine was used as a surface ligand conjugated with solid lipid nanoparticles, composed of emulsifying wax and Brij 78, were reported to transport to the brain as tested in situ by rat perfusion technique [33]. The mechanism involves an interaction with the thiamine transporter, which is responsible for a facilitated transport or an increased passive diffusion of the nanoparticles toward the BBB.

ChT/SLC5A7 (Choline Transporter) Choline is an endogenous compound required in the synthesis of the neurotransmitter acetylcholine and the membrane phospholipid phosphatidylcholine. Choline transporters (ChT) are responsible for the cellular uptake of acetylcholine and the membrane phospholipid phosphatidylcholine [59]. Based on the sodium dependence and the affinity for choline, there are two choline transporter systems. The sodium-dependent and choline low-affinity transporter is expressed widely in the body, whereas the sodium-independent and choline high affinity transporter is expressed in the presynaptic cholinergic nerve ending. The sodium independent transporter is needed for the choline transfer across BBB [60]. Herein, a choline derivative was used as a ligand in the formulation of doxorubicin and gene complexed nanoscale codelivery system showed higher cellular uptake efficiency and cytotoxicity than unmodified codelivery system in U87 MG cells [61].

SVCT2/SLC23A2 (Sodium-Coupled Vitamin C Transporter 2) Sodium-dependent transporter for vitamin C (SVCT2), expressed by neuroepithelial cells of the choroid plexus are involved in the transport of the reduced form of ascorbic acid or vitamin C. Modification of the drugs in a form to target SVCT2 may facilitate the drug delivery to the brain. Recently, the anticholinesterase galantamine used for the treatment of neurodegenerative disorder, Alzheimer disease was incorporated in ascorbic acid grafted PLGA-*b*-PEG nanoparticles to increase the cellular uptake of nanoparticles in SVCT2 expressing NIH/3 T3 cells. A significantly higher

therapeutic and sustained action by drug-loaded PLGA-*b*-PEG-Asc NPs than free drugs and drug-loaded plain PLGA as well as PLGA-*b*-mPEG NPs was observed in an in vivo pharmacodynamic study [62]. The result also showed a higher biodistribution of the drug to the brain than other formulations.

MCT1/SLC16A1 (Monocarboxylate Transporter 1) MCT1 is a proton-coupled transporter expressed in endothelial cells in the BBB responsible for the transport of monocarboxylates lactate as well as the ketone body β-hydroxybutyrate across the BBB [63]. Venishetty et al. [64] studied the β-hydroxybutyrate-grafted docetaxel-loaded solid lipid nanoparticles to increase the drug distribution to brain. The result showed an increased uptake and cytotoxicity of β-hydroxybutyrate-grafted nanoparticles in brain endothelial cells as compared to unmodified nanoparticles as β-Hydroxybutyrate-grafted nanoparticles could effectively increase docetaxel distribution across the BBB by Monocarboxylate Transporter 1 (MCT1).

OCTN2/SLC22A5 (Novel Organic Cation Transporter 2) Another transporter OCTN2 is highly expressed in blood–brain barrier capillary endothelial cells. This transporter is overexpressed in glioblastoma multiforme T98G cells. Therefore, nanocarriers modified to target OCTN2 offers a potential platform for the brain-targeted delivery of chemotherapeutics. Kou et al. reported that the conjugation of L-carnitine significantly increased the uptake of paclitaxel loaded nanoparticles in BBB endothelial cell line hCMEC/D3 and glioma cell line T98G improving its antigliomic activity [65].

4.1.1 Inhibition of Efflux Transporter System

P-Gp/ABCB1 (P-Glycoprotein/ATP-Binding Cassette Transporter Family, Member B1 ATP-binding cassette (ABC) transporters are membrane transporters, which bind and hydrolyze ATP to drive the efflux of various compounds out of cells. Several drugs are moved out of the cells by efflux transport such as paclitaxel, docetaxel and doxorubicin. One of the ABC membrane transporters is *P*-glycoprotein (*P*-gp/ABCB1/MDR1). As involved in efflux transport, *P*-gp is doubtful in facilitating drug transport to the brain [66]. However, investigation is being done using *P*-gp as a substrate coupling with nanoparticles which reported to increase significantly the uptake of nanoparticles by brain. Such substrates are azithromycin, clarithromycin [67]. An increase in drug delivery to the brain can be obtained by using *P*-gp inhibitors, which inhibits *P*-gp overexpressed cells. Based on this strategy, doxorubicin uptake and transfection efficiency were significantly enhanced in rat brain endothelial by using *si*RNA-chitosan nanoparticles. It may be due to the *si*RNA-mediated silencing of the *P*-gp gene to improve the delivery of drug to the brain [68]. Another report showed that amisulpride when coadministered with A-cyclosporine showed an increase and prolonged antipsychotic effect as observed in vivo, which is due to the inhibition of *P*-gp efflux transport. Several polymers which are *P*-gp inhibitors are used for surface modification of nanocarriers. Natural

polymers such as xanthan and gellan gum, as well as alginates, could inhibit the action of the *P*-gp efflux pump at concentrations of 0.05% and 0.5 mg/mL, respectively. An increased concentration of *P*-gp substrates such as vinblastine and doxorubicin was shown in the presence of xanthan gum, whereas an increased intracellular concentration of doxorubicin was obtained in everted gut sac cells. Other synthetic polymers inhibitors of *P*-gp efflux transporter such as PEG 400, g-Pluronic P85, and D-α-tocopheryl polyethylene glycol 1000 succinate could enhance the digoxin concentration in the brain, whereas Pluronic P85 inhibits the *P*-gp transporter, causing reduction in ATP and inhibition of ATPase enzymes as well as lipid membrane fluidization. Another polymer, poloxamer 188 reported to transport doxorubicin across the BBB when coated on PBCA nanoparticles against intracranial glioblastoma in rat. Other drug transported was acyclovir [69]. Glutathione, an antioxidant, when used as a coating material of PLGA nanoparticles could efficiently delivered paclitaxel across the brain due to the inhibition of *P*-gp as observed by ATPase assay. Similarly, doxorubicin was also transported by the same mechanism using glutathione-coated PLGA PEG nanoparticles [19].The carrier or transporter-mediated transport system can deliver several therapeutics or neuroactive agents to the brain by manipulating the endogenous transport. However, this strategy suffers from drawbacks due to an increase molecular size of the moiety as the required criteria is that the drug/ligand must be very small and similar in structure to the nutrient [70].

4.2 Adsorptive-Mediated Transcytosis (AMT)

The adsorptive-mediated transcytosis relies on the electrostatic interaction between a positively charged molecule and the negatively charged brain endothelial cell membrane. Originally, Pardridge et al. demonstrated the capability of cationized albumin nanoparticles in delivering drugs and peptides to the cerebral parenchyma [71]. Later, the cell-penetrating peptides (CPPs) developed as positively charged peptides were developed, which could penetrate the brain endothelial cell membranes by adsorptive-mediated transcytosis [72]. Another example is the HIV-1 trans-activating transcriptor (TAT) peptide. TAT-derived CPPs bind to the surface of the cell, induce macropinocytosis, allow large molecules such as large chained peptides to transport across the BBB [73]. In another study, Liu et al. incorporated ciprofloxacin–HCl in the PEGylated nanoparticles conjugated with TAT peptides was found to cross the BBB [74].The application of AMT in drug delivery is limited due to its lack of tissue or site specificity which lead to an accumulation of undesired drug in the nonspecific or nontargeted tissue causing toxicity as well as low therapeutic concentration of the drug in the brain. Overall, it makes the treatment ineffective [75].

4.3 Receptor-Mediated Transcytosis (RMT)

Receptor-mediated transcytosis mainly responsible for the transport of large molecules such as insulin, lipoproteins, and transferrin to the brain. Surface-modified nanoparticles by coating or natural ligand conjugation mimic the normal endogenous substances, which acts on the specific receptor to deliver drugs to the brain. Nanoparticles surface-functionalized with polymers are based on RMT to deliver several therapeutics to the brain.

Low-Density Lipoproteins (LDL) Receptor Widely distributed in the brain endothelial cell membrane, LDL receptors (LDLr) are proven to be an effective tool for the delivery of several drugs. As such, coated nanotechnology based polysorbate 80-coated nanoparticles are proven to be efficiently acted on LDLr to deliver drugs to the brain. Polysorbate 80 coating covalently couple with apolipoprotein E, A-I, or B-100 in the bloodstream, which mimics the endogenous lipoprotein and act on the LDLr present in the brain endothelial cell membrane and deliver the drug by receptor-mediated transcytosis. Polysorbate 80-coated nanoparticles are able to deliver several drugs to the brain such as dalargin, gemcitabine, nerve growth factor, gallic acid, doxorubicin, and rivastigmine [19]. As a part of our research, based on the receptor-mediated transcytosis technique, an attempt has been made to investigate the ability of polysorbate 80-coated 6-carboxyflourescein (6CF) tagged kokum butter solid lipid nanoparticles (P806CFNvKLNs) in delivering Nevirapine (Nv), an antiretroviral drug to the brain in Swiss Wistar rat model. Conventionally, Nevirapine, a nonnucleoside reverse transcription inhibitor, suffers from the drawbacks of poor aqueous solubility, hepatotoxicity, and patient incompliance on frequent dosing, and also undergoes first pass metabolism and enzymatic degradation when orally administered in highly active antiretroviral therapy (HAART). The P806CFNvKLNs was administered in the tail vein of the rat, which were sacrificed by cervical dislocation and the cryosection of rat brain was taken to check the distribution of the P806CFNvKLNs at different time intervals of 1 h, 2 h, 4 h, 6 h, and 24 h under confocal laser scanning microscopy (CLSM) as shown in Fig. 15.1. Initially, at 1 h, the P806CFNvKLNs were in the surroundings of the BCECs. At 2 h, it undergoes receptor-mediated endocytosis and entered the BCECs and remained in the BCECs for 24 h showing higher intensity at 4 h. After 24 h, the P806CFNvKLNs moved out of the BCECs into the brain parenchymal cells indicating receptor-mediated transcytosis into brain parenchyma. The result showed that the P806CFNvKLNs may have acted on the LDLr and underwent receptor-mediated endocytosis showing uniform distribution of nanoparticles in the BCECs and parenchymal cells.

Other study showed that polysorbate 80-coated chitosan nanoparticles could target doxycycline HCl, antipsychotic drug to the brain as observed in brain micro vascular endothelial cells by Yadav et al. [76]. Curcumin showed its antidepressant activity in in vivo pharmacophore model when administered in polysorbate 80-coated PLGA nanoparticles [77]. Similarly, curcumin showed cytotoxicity on U87MG brain tumor cells, when loaded in P80-coated PEGylated PLGA

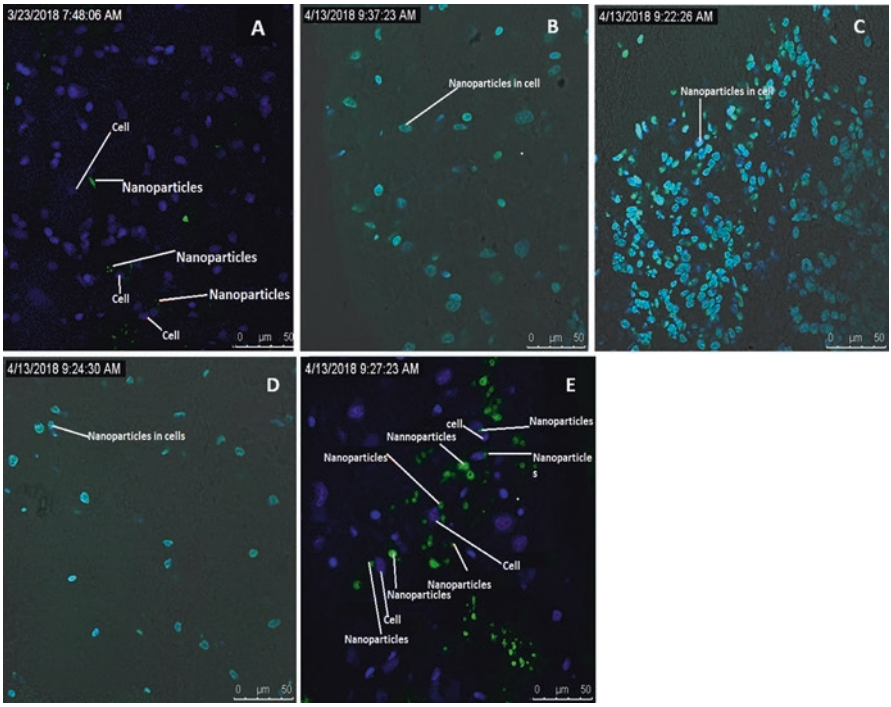


Fig. 15.1 (**a**) P806CFNvKLNs surrounding BCECs in Swiss Wister rat brain 1 h post injection, (**b**) LDLr mediated endocytosis of P806CFNvKLNs in Swiss Wister rat BCECs 2 h post injection, (**c**) P806CFNvKLNs in Swiss Wister rat BCECs 4 h post injection, (**d**) P806CFNvKLNs in Swiss Wister rat BCECs 6 h post injection, and (**e**) LDLr-mediated transcytosis of P806CFNvKLNs into the brain parenchyma after 24 h post injection

nanoparticles showing effective drug delivery to glioblastoma multiforme (GBM) tumor cells [78]. Acetylpuerarin is an acetylated derivative of puerarin could permeate through the BBB and showed its brain protective activity in rat when incorporated in P80-coated PLGA nanoparticles [79]. P80-coated PLGA nanoparticles also proven to deliver Bacoside, a neuroprotectant, to the brain for the treatment of neurodegenerative disorders as observed in Wister albino rats [80].

Angiopeptides Another receptor that has recently been targeted for brain drug delivery is the low-density lipoprotein receptor-related protein (LRP), a member of the low-density lipoprotein receptor family. It is highly expressed on BBB and involved in BBB transcytosis of several proteins and peptides, including lactoferrin, melanotransferrin and receptor associated protein. Angiopep-2, a 19 amino acid peptide, was reported as a ligand targeted to this receptor [81]. Still, a peptide conjugated with three molecules of paclitaxel linked to Angiopep-2 called ANG1005 showed to have activity against glioblastoma and to lengthen the survival of mice with intracerebral tumors [82]. Angiopep-conjugated poly(ethylene glycol)-copoly(3-caprolactone)nanoparticles (ANG-PEG-NP) enhanced

significantly the uptake by brain capillary endothelial cells (BCECs) compared with that of PEG-NP which might be due to LRP receptor-mediated transcytosis process [83].

Leptin Receptor Leptin is a 146-amino-acid polypeptide secreted into the bloodstream by adipocytes. Leptin, a specific receptor, ObR is overexpressed in the hypothalamus and other parts of the brain. Leptin, secreted in the bloodstream undergoes passive diffusion across the BBB by receptor-mediated transcytosis as it acts on the receptor, ObR, present on the luminal side of the brain capillary endothelial cells. Endogenous leptin binds to the ObR receptor only after meal, is responsible to reduce obesity by decreased food intake and retarding weight gain. This particular characteristic of leptin provides an ample potential to utilize leptin-mediated transcytosis pathway for brain-targeted delivery of drugs [84]. On the other hand, leptin-modified nanocarriers may not be effective in obese individuals due to its impaired activity. Till now, researchers have developed several leptin derived peptides for drug targeting to the brain. The gene transfection efficiency of Dendrigraft poly-L-lysine (DGL) plasmid DNA nanoparticles conjugated with leptin 30 were studied on brain capillary endothelial cells (BCECs), which express leptin receptors. The result showed the nanoparticles could transport across in vitro BBB model effectively by ligand-receptor-mediated endocytosis leading to enhanced gene transfection ability of DGLePEGeLeptin30/DNA NPs with low cytotoxicity. Hence, these observations would support the potential of the ObR receptor to serve as a transport system in brain delivery of drugs [85].

Transferrin Receptor Another receptor highly expressed in brain capillary endothelial cells is the transferrin receptors. It provides a wide scope of research for RMT. The transferrin or an antibody against transferrin is either coated or attached to nanocarriers to allow for the delivery of large molecules to the brain [19]. Nevirapine, an antiretroviral drug was successfully targeted to the brain using transferrin-conjugated PLGA nanoparticles [86]. Similarly, lactoferrin, a protein of the transferrin family, also induced uptake in an in vitro and in vivo model, when attached to PEG-PLA nanoparticles [87]. The disadvantages with transferrin are that the endogenous transferrin present in the blood competes with the transferrin-functionalized nanocarriers for the same receptor site in BBB, which may inhibit the uptake of nanocarrier reducing the efficacy of the drug. Thus, antibody against transferrin is in use to overcome its drawback. One such antibody is ox26 reported to bind to an extracellular epitome of transferrin receptor preventing the competition with natural endogenous transferrin for binding the same site. Ox26 nanocarrier conjugation can be done by covalent chemical linkages. One such method is sulfhydrylmaleimide coupling method [19]. Tempol was targeted to the brain by conjugation of ox26 antibody to the maleimide-grafted PLGA nanoparticles by NHSPEG 3500 maleimide cross-linker [88]. Ox26-conjugated PEGylated cationic solid lipid nanoparticles could enhance the penetration of baicalin, a flavone glycoside 1.12-fold higher than unmodified solid lipid nanoparticles as observed in vivo [89].Transferrin receptors are overexpressed in tumor cells. This concept was

proven by the enhancement of cellular internalization of temozolomide in glioblastoma cells, U215 and U87, when incorporated in ox26-conjugated PLGA nanoparticles [90]. Similarly, amphotericin B, a drug for candidal meningitis, got a low therapeutic level in the brain due to poor penetration. However, delivery attempt were made by modifying the transferrin receptor-mediated transcytosis. An anti-TfR antibody (OX26)-modified amphotericin-loaded PLA (poly[lactic acid])–PEG (polyethylene glycol)-based micellar drug delivery system was constructed which showed significant reduction of CNS fungal burden and an increase of mouse survival time [91]. In another study, loperamide, an antinociceptive drug showed significant antinociceptive effects in the tail-flick test in ICR (CD-1) mice after intravenous injection when incorporated in human serum albumin nanoparticles covalently bound with ox26, monoclonal antibody [92].

Melanotransferrin Receptors Another receptor known as melanotransferrin, is a high-level homolog of human serum transferrin and lactoferrin was first observed in melanoma cell membrane. Basically, it is an iron-bound protein crucially involve in the transport of iron from the blood plasma across the BBB by RMT, which is irrelevant to transferrin and transferrin receptor. Due to less plasma concentration of the endogenous melanotransferrin, it favors its utility in RMT CNS drug delivery. Melanotransferrin antibody and tamoxifen-conjugated solid lipid nanoparticles were able to release etoposide to the human-brain microvascular endothelial cells (HBMECs) and to restrain the proliferation of malignant U87MG cells [93]. Other drugs such as paclitaxel, an anticancer drug accumulation in the brain was 10 times higher than the free drug when conjugated with melanotransferrin [41].

Mannosyl Receptors Some receptors are distributed widely on the cell wall of the macrophages, which is used by macrophages for phagocytosis and endocytosis such as fibronectin, mannosyl, lectin, and galactosyl. Manipulation of these receptors by nanocarriers conjugated with ligands such as mannosyl, immunoglobulin, fibronectin, and galactosyl can target and deliver several drugs to the brain by RMT [19]. Mannan-coated gelatin nanoparticles could recognize the mannosyl receptors predominantly present in the macrophages cell of the brain and successfully target didanosine, an anti-HIV drug to the brain [94].

Insulin Receptors Insulin is transported to the brain by the transcytosis mechanism by acting on the insulin receptors present on the brain endothelial cell membrane. Insulin is not a suitable ligand for RMT due to the existence of competition with the natural endogenous insulin present in the blood circulation may reduce the therapeutic concentration of insulin in the brain and/or changes in receptor activity, of which the latter may have negative consequences for glucose metabolism. Thus, antibody against insulin receptors is in use for RMT, which act on different epitome of the insulin receptor other than the endogenous insulin binding site. One such is 83–14 mouse monoclonal antibody (mAb) for receptor-mediated endocytosis performed in primates (Rhesus monkey) [19]. Another report showed that the human anti-insulin receptor monoclonal antibody (HIRMAb) was

able to transport a TNFα decoy receptor (TNFR), which would neutralize the effects of TNFα in inflamed brain regions, into the brain of Rhesus monkeys following intravenous administration [95]. Similarly, loperamide-loaded human serum albumin nanoparticles with covalently bound insulin or the anti-insulin receptor monoclonal antibody, 29B4 stimulated significant antinociceptive effects in the tail-flick test in ICR (CD-1) mice after intravenous injection, showing that insulin or the antibodies covalently coupled to human serum albumin nanoparticles are able to transport loperamide across the blood–brain barrier (BBB), which the drug usually is unable to cross [96].

Rabies Virus Glycoprotein (RVG) RVG, a short peptide, is found on the surface of rabies virus. Rabies virus follows a decisive pathway to enter CNS. It enters the CNS by acting on the α1 subunits of nicotinic acetylcholine receptors (nAcR), which is widely distributed in CNS as reported by Lentz et al. Rabies virus glycopeptide is responsible for cellular entry and virus fusion. Modification of rabies virus CNS pathway can be a promising tool for the targeted drug delivery to the brain. One of the widely used RVG peptide is a 29-amino-acid peptide derived from rabies virus glycoprotein (RVG29). CNS targeting peptide such as RVG 29 is a promising strategy, underlined by its wide application as a targeting peptide for CNS-targeting strategies. It is reported to inhibit the binding of snake-venom toxin α-bungarotoxin (BTX) to the AchR in solution [97]. The RVG29 peptide has been exploited as a brain-targeted ligand to deliver gene into the brain by conjugation on the surface of polyethylene glycol-polyamidoamine (PEG-PAMAM) [98]. Another study showed the RVG29 peptide-conjugated albumin nanoparticles could deliver itraconazole to the immortalized mouse brain endothelial cells [99]. Zou et al. reported that rabies virus glycoprotein (RVG) peptide conjugated paclitaxel loaded PLGA nanoparticles could deliver anticancer drug, paclitaxel, to the human glioma of mice model [100]. RVG-peptide-linked trimethylated chitosan could efficiently delivered *si*RNA to the mouse neuroblastoma Neuro2a cells with increased serum stability, negligible cytotoxicity, and higher cellular uptake than the unmodified *si*RNA/TMC—mPEG complexes in acetylcholine receptor positive Neuro2a cells [101].

Diphtheria Toxin Receptor (DTR) A membrane-bound precursor of heparin-binding epidermal growth factor (HB-EGF), also known as the diphtheria toxin receptor (DTR) is a well-characterized endogenous transport receptor on the BBB for the targeting of drugs. Usually, it is based on endogenous transport mechanism of molecules by receptor-mediated endocytosis. Based on receptor-mediated endocytosis, several molecules of proteins, essential nutrients, etc. are transported through it. The absence of any endogenous ligands expected to prevent the competition with DTR and the blockage of transport of essential nutrients to the brain. As it is predominantly expressed on the BBB, neurons, and glial cells, it is overexpressed during diseased conditions, providing an opportunity for targeted drug delivery [102]. Pharmacologically active compounds (like heparin and protease

inhibitors) can modulate its biological activity. A nontoxic mutant of diphtheria toxin known as CRM197 is used as the receptor-specific carrier protein because of its use as a safe and effective carrier protein in human vaccines for a long time [103]. A study by Tosi et al. showed the BBB crossing efficiency of polymeric poly-lactide-co-glycolide (PLGA) NPs modified with a mutated form of diphtheria toxin (CRM197). CRM197 PLGA nanoparticles are able to reach the CNS by receptor-mediated endocytosis after interaction with diphtheria toxin receptor (DTR) [104]. In another study, CRM197-linked PEGylated polyethylenimine small interference RNA (CRM197-PEG-PEI/*si*RNA) could deliver therapeutic *si*RNA in glioblastoma cells [105].

Tetanus Toxin Tetanus toxin is a 150,000 molecular weight protein produced by the anaerobic bacterium *Clostridium tetani.* Tetanus toxin is transported to the brain as it binds to the gangliosides, a component of the neuronal membranes. Van Heyningen identified that tetanus toxin bound to the membrane glycolipids, gangliosides, and demonstrated that it bound best to certain specific disialogangliosides and trisialogangliosides. Later on, it was found that a retrograde transport from distal axon terminals to the neuronal cell body can carry both heavy and light chains of tetanus toxin in motor nerves. It is evident that retrograde axonal transport of several substances from the peripheral neurons to motor neurons can penetrate the CNS effectively bypassing the BBB. Hence, exploitation of retrograde neuronal transport may enhance the possibility to target nanoparticles to the CNS [106]. Out of the two fragments, the C-terminal heavy chain fragment is nontoxic, attached with the light chain which is endowed pathogenic enzymatic characteristics. The heavy chain fragments are transported along axons as it acts on the GT1b, the gangliosides receptor for tetanus toxin. As reported, an intramuscular injection of superoxide dismutase tetanus toxin into the mouse tongue reached higher order motor neurons. Also, an enhanced CNS uptake was obtained for intraperitoneal injection of tetanus toxin protein hybrid. Also, tetanus toxin-conjugated PLGA PEGylated nanoparticles showed six fold increase in binding to N18-RE-105 neuroblastoma cells. However, its immunogenicity due to vaccination is a major concern [107]. To overcome this problem, peptides analogous to tetanus toxin having similar binding efficiency and subsequent cellular processing was identified, Tet1. Tet1 is transported in a retrograde manner. Another peptide, G23, with a sequence identical to Tet1 was identified that revealed effective targeting by a transcytotic pathway in human brain capillary endothelial cells and mediates efficient transport of G23-coated polymersomes across an in vitro BBB model [108]. Following intracarotid artery injection in mice studies provided an evidence for in vivo transfer across the BBB of intact peptide-targeted drug delivery into brain parenchyma. Further research confirmed the binding of G23 peptide and Tet1 to both GM1 and GTb1 gangliosides indicating that both gangliosides involve in transcytotic transport of G23-coupled polymersomes across the BBB [109].

5HT Receptors 5-hydroxytryptamine or 5-HT or serotonin receptors, a group of G protein coupled receptor and ligand gated ion channels are located both in the CNS

and peripheral nervous system. Serotonin receptor modulates the secretion of several neurotransmitters such as acetylcholine, epinephrine/norepinephrine, dopamine, glutamate, GABA as well as many hormones such as oxytocin, prolactin, vasopressin, cortisol, corticotropin, and substance P. The natural endogenous serotonin is responsible for the activation of these receptors, which is transported across the BBB by receptor-mediated transcytosis. Several pharmaceutical drugs such as antidepressants, antipsychotics, anorectics, antiemetics, and antimigraine drugs are successfully targeted to these receptors [110]. Another subtype of 5HT or serotonin receptor is serotonergic 1B receptor subtype (S1BRS; 5-hydroxytryptamine (1B) receptor) expressed by brain endothelial cells.S1BRS could play a crucial role in the generation of and treatment for depression, regulation addictive drugs responses. The major concern is the reuptake of S1BRS by the CNS. Thus S1BRS antagonism could extend SSRI induced effect on 5-hydroxytryptamine levels in the frontal cortex. Using this strategy, Carmustine (BCNUs) solid lipid nanoparticles modulated with S1BRSA could target S1BRS expressed on the human brain microvascular endothelial cells (HBMECs) for BBB penetration [111]. Other drugs such as etoposide, antitumor drug were successfully transferred across BBB using 5-HT moduline-grafted cationic solid lipid nanoparticles. It acts on the 5-HT_{1B} receptors present on the brain endothelial cells and transported the drug by receptor-mediated endocytosis [112]. Similarly, an antimigraine drug, sumatriptan succinate, loaded in chitosan solid lipid nanoparticles could be successfully targeted to brain via oral delivery [113].

Other peptide used to target drugs based on ligand nanotechnology are trans-Golgi network (TGN) and QSH. Based on this technique, Zhang et al. developed a dual-functional nanoparticulate drug delivery system based on a PEGylated poly(lactic acid) polymer containing two targeting peptides, TGN and QSH, conjugated to the surfaces of the nanoparticles. TGN specifically targets ligands at the BBB, while QSH has good affinity for $A\beta_{1–42}$, which is the main component of amyloid plaques, in Alzheimer disease. These nanoparticles were delivered to amyloid plaques with enhanced and precisely targeted delivery in the brains of Alzheimer disease model mice [114].

4.4 Surface-Functionalized Magnetic Nanoparticles

As discussed in the previous section, magnetic nanoparticles [i.e., SPIONs or iron oxide nanoparticles (IONs)] have gained lots of attention as a vector in drug delivery. The dual functioning of magnetic nanoparticles both as a diagnostic agent and targeting got significance in brain-targeted drug delivery. Magnetic nanoparticles follow three mechanisms to cross the BBB. Firstly, natural ligands such as peptides, antibody-conjugated magnetic nanoparticles target to a specific receptor in the brain. Secondly, application of an external magnetic field directs the nanoparticle-incorporated drugs to the brain. Thirdly, application of low radiofrequency waves in the presence of an external magnetic field generates heat to open the tight junction

of the brain capillary endothelial cells which allow the diffusion of the drug across the BBB. The second and third strategies differentiate the magnetic nanoparticles from other nanoparticles while the first strategy based on attachment of ligands on the surface is similar with those other nanoparticles. Whereas combination of ligand-based strategies with the application of magnetic field may be useful for drug targeting to the brain [41].

Ligand-Functionalized Magnetic Nanoparticles Transport Across BBB The feasibility of drug delivery using natural ligand-conjugated or -coupled magnetic nanoparticles is investigated and reported. One such is the modification of magnetic nanoparticles surface by peptides. Angiopep-2(ANG) conjugated to the surface of Pluronic F127 water-dispersible poly(acrylic acid)-bound iron oxide complex could cross the BBB due to its dual targeting ability, one to recognize the low density lipoprotein receptor protein, which is overexpressed in both BBB and glioblastoma cells and secondly, it acts on the clathrin-mediated receptor on the U87 surface. Thus, ANG-conjugated iron oxide complex undergoes receptor-mediated transcytosis to cross BBB. Another peptide, trans-activator of transcription protein (TAT) when used to functionalize liposomes was able to increase permeability to the brain and also accumulated significantly in the spinal cord site [41]. Kaluzoba et al. reported that cetuximab, an epidermal growth factor inhibitor, when conjugated with IONs resulted in a significant antitumor activity in rat against glioblastoma (GBM), GBM stem like cells (GSCs) that was greater than with cetuximab alone due to more efficient, CD133-independent cellular targeting and uptake, EGFR signaling alterations, EGFR internalization, and apoptosis induction in EGFR-expressing GSCs and neurospheres [115].

Transport of Magnetic Nanoparticles Across the Brain Cell by External Magnetic Field Application The application of external magnetic field is directly proportional to the particle size of magnetic nanoparticles. So an increase in particle size and strong magnetization are essential for attractive forces to exist. Most of the anti-HIV drugs not able to cross the BBB provoke the occurrence of diseases called neuroAIDS [41]. Jain et al. reported to modify the transport of endogenous macrophage system based on the monocytes/neutrophils mediated delivery of Arg-Gly-Asp (RGD) anchored magnetic nanoparticles transport to an in vitro BBB model by an externally applied magnetic field [41]. Thomsen et al. studied the capacity of fluorescent IONs to pass through human brain capillary endothelial cells facilitated by an external magnetic field. In another study, transferrin-conjugated magnetic liposomes passage to the BBB was studied, both in the presence and absence of an external magnetic field. In the presence of external magnetic field, transferrin-conjugated magnetic liposomes showed an increase in transmigration due to a synergistic effect than that in the absence of magnetic field [116].

Magnetic nanoparticles therapy increased the survival of glioma-bearing rats by enhancing the brain concentration of paclitaxel, an antigliomic drug [117]. Kong et al. reported that polystyrene nanospheres encapsulated magnetic nanoparticles in the presence of an external magnetic field showed accumulation of nanospheres in

an in vitro brain model as observed by confocal laser scanning microscopy [118]. Another report, demonstrated that the application of the external magnetic field could target magnetic liposome to the glioma multiforme in vivo, quantitative determination by MRI, electron spin resonance spectroscopy to determine the magnetic liposomes crossing the BBB, confocal laser scanning microscopy showed enhanced accumulation of magnetic liposomes in brain parenchyma [119].

Heating by Low Radiofrequency to Increase of BBB Permeability Application of an external altering magnetic field in the presence of radiofrequency generates heat energy, which induce hyperthermia. A moderate heat is required to open up the BBB tight junction which is reversible. Tabatabaei et al. initially observed the permeability of magnetic nanoparticles in rat brain due to generation of moderate heat in the presence of an externally applied magnetic field [41]. Dan et al. also observed the that the SPIONs cross linked with IONs were able to permeate through an in vitro BBB model (bEnd.3 and Madin-Darby canine kidney II cells) which was activated by hyperthermia dissipated by externally applied magnetic field in a controlled manner and in a specific area [120]. Thus, it can be concluded that the hyperthermia generated due to an external applied magnetic field could have the potential to deliver SPIONs across the BBB for diagnostic and therapeutic activity.

Strategies Following Both Attachment of Ligand and Application of External Magnetic Field Recently, Nair showed that magnetic nanoparticles loaded with AZTTP (the triphosphate, active form of AZT) and encapsulated in liposomes could migrate across an established BBB model (primary human brain microvascular endothelial cells and astrocytes) when induced by application of an external magnetic field. Moreover, it showed the phagocytosis of the magnetic liposomes by human monocytes, and its transmigration across the BBB in the presence of an externally applied magnetic field in vitro [121]. A list of drugs/moiety delivered across the BBB is given in Table 15.1. Before implementing magnetic nanoparticles for drug delivery, toxicity studies need to be carried out in the specific cell type of interest. On a study, it was found that the unmodified SPIONs are responsible for cell death in dermal fibroblasts while lung cells appeared not to be affected. Secondly, it is the strength of applied magnetic field to control the concentration at the site of action, as the magnetic gradient decreases with an increase in distance from the site of application. Next is the small size, which reduces the magnetic strength making it a challenge for the particle to maintain its concentration at or near the site of action while withstanding the resistance of blood flow. Also, the effect of differences in physiological conditions (such as weight, cardiac output, blood volume), before extrapolating from animal study to human are to be considered. Thus, drug delivery using magnetic nanoparticles for the treatment of metastatic neoplasm or small tumors remains a challenge [122]. However, proper modification of magnetic nanoparticles has a significant potential or recognition as well as efficient drug targeting to the sites where the conventional dosage cannot reach.

Table 15.1 Drugs delivered across the BBB using magnetic nanoparticles

Strategies	Magnetic nanoparticles	Mechanism	Drugs/moiety	Application	References
Ligand conjugation	Lactoferrin-conjugated graphene oxide Iron oxide nanocomposites	Acting on lactoferrin receptor overexpressed in glioma cells and brain endothelial cells	Doxorubicin	Efficacy and stronger cytotoxicity against C6 glioma cells	[123]
	Transferrin-conjugated fluorescein magnetic nanoparticles	Acting on the transferrin receptors in brain endothelial cells	Fluorescein (FITC)	Wide distribution in brain parenchyma as observed by TEM and CLSM	[124]
	Anti-IL-1β monoclonal antibody (mAb) SPIONs	Neutralization of IL-1β overexpressed in epileptogenic zone of temporal lobe epilepsy (TLE)	Anti-IL-1 monoclonal antibody	Diagnosis and treatment of TLE by MRI	[42]
	Chlorotoxin loaded methotrexate PEGylated iron oxide nanoparticles	Receptor-mediated endocytosis through lysosomes in brain cytoplasm	Methotrexate	MRI diagnosis, accumulation and cytotoxicity in tumor cells in vivo	[125]
Application of magnetic field	Hybrid chitosan–dextran SPIONs	Application of magnetic field	Chitosan-dextran SPIONs	Magnetic resonance contrast enhancing properties for the delineation of the brain tumor	[126]
	cmHsp70.1 monoclonal antibody-conjugated SPIONs	SPIONs	Targeting membrane Hsp70 expressed in tumor cells	MRI diagnosis of tumor	[127]
	pEGFP/p53-loaded SPIONs	P53 gene	Targeting Tp53 to glioblastoma (U87) cells across a simulated BBB model that comprised KB cells	Induction of apoptosis in the cancerous cells	[128]

(continued)

Table 15.1 (continued)

Strategies	Magnetic nanoparticles	Mechanism	Drugs/moiety	Application	References
Application of heat and magnetic field	Doxorubicin graphene oxide-PEGylated iron oxide hybrid nanocomposite	Photothermal therapy induced magnetic field	Doxorubicin	MRI diagnosis, drug targeting,cytotoxic effect	[129]
	Hydroxyapatite (HAP)-conjugated SPIONs	Magnetic hyperthermia	SPIONs	Magnetic hyperthermia induced destructive effects onto cancer cells only while minimizing the risks imposed onto healthy ones observed in U87 human brain cancer cells and human mesenchymal stem cells (MSCs)	[130]

5 Neurotoxicity of Surface-Functionalized NPs

Drug delivery using nanoparticles as a novel carrier gained popularity as it can target the drug to the site generally unreachable by conventional dosage forms as well as control the drug delivery. As such, it can be used for targeting the drugs across the BBB due to small size, surface alteration characteristics, stability, target specificity, etc. Surface modification or functionalization is responsible to bypass the endogenous opsonization and phagocytosis in the bloodstream, which otherwise would lead to an inefficient drug delivery to the brain. As discussed in the previous section, several strategies adopted for surface functionalization of nanoparticles are successful in targeting several drugs, peptides, hormones across the BBB [19]. Still, chances of potential neurotoxicity exist during its passage to the brain. One such is the neuron injury due to oxidative nanoparticles upon metabolism releases free radical, reactive oxygen species (ROS), which causes oxidative stress including DNA damage. ROS are associated with neurodegenerative diseases like Alzheimer disease, Parkinson disease [131]. A study by Yuan et al. reported the neurotoxicity due to polymeric nanoparticles. Polysorbate 80-modified chitosan nanoparticles showed apoptosis and necrosis of neurons, slight inflammatory response in the frontal cortex and decrease of GFAP expression in the cerebellum when injected in rat with no obvious changes observed for oxidative stress. Oxidative stress due to generation of free radicals may cause cell death [132]. Smaller sized NH_2 polystyrene nanospheres gained access to the cell organelles (mitochondria) causing cell death due to free radical generated as reported. Another study showed that high dose

administration of poly-butyl cyanoacrylate nanoparticles caused depression in mice. Magnetic nanoparticles are also responsible for neurotoxicity due to the release of ROS [133] while neurotoxicity due to iron oxide magnetic nanoparticles is due to iron accumulation, oxidative stress and protein aggregation in the brain. Iron accumulation in the neurons releases ROS, which causes oxidative stress. Iron accumulation and the consequent oxidative stress leads to protein aggregation including Aβ and α-synuclein, which play a critical role in Alzheimer and Parkinson diseases, respectively. Ultimately, iron accumulation, oxidative stress and protein aggregation leads to cell death. A study carried out by Borysov et al. showed that the unmodified ferritin magnetic nanoparticles significantly reduced L-[14C] glutamate transport in synaptosomes and acidification of synaptic vesicles with no change in the membrane potential of synaptosome. While coated magnetic nanoparticles with polysaccharides such as dextran, had no significant effect on synaptic vesicle acidification, the initial velocity of L-[14C] glutamate uptake, ambient level of L-[14C] glutamate and the synaptosomes plasma membrane potential [134]. The neurotoxic potential for iron oxide nanoparticles was observed in rat brain striatum by incubating dopaminergic neurons with radioactive iron oxide nanoparticles. The result showed that it leads to neuron viability, trigger oxidative stress and caused apoptosis [135].For lipid nanoparticles it is found that composition of lipid and surfactant plays an important role in toxicity. So optimization and regulation of composition is a key factor in reducing toxicity [136]. In order to have less neurotoxicity, a proper evaluation of physicochemical characteristics such the morphology, surface area, surface charge, coating, purity, material solubility, and the materials used for formulation should be considered. Other parameters, such as the dosage, administration route, concentration of the drug in the target organ, duration of action, and the degradation time of the biodegradable materials are most important and fundamental problems to be considered in the evaluation of nanoparticle neurotoxicology [131, 137]. A list of neurotoxicity study of different nanoparticles is shown in Table 15.2.

Table 15.2 Neurotoxicity study of nanoparticles

Types of nanoparticles	Neurotoxicity study	Results	References
Zinc oxide nanoparticles	Zinc oxide nanoparticles induced neurotoxicity in different age group C57BL/6 J mice	Neurotoxicity in old aged mice is due to the suppression of hippocampal cAMP response element binding protein (CREB), phosphorylated CREB, synapsin I, and cAMP	[138]
Iron oxide nanoparticles	Toxicity assessment of iron oxide nanoparticles in Zebrafish (Danio rerio) early life stages	≥10 mg/L of iron oxide nanoparticles induced developmental toxicity in Zebra fish embryos, causing mortality, hatching delay and malformation	[139]

(continued)

Table 15.2 (continued)

Types of nanoparticles	Neurotoxicity study	Results	References
Superparamagnetic iron oxide nanoparticles (SPIONs)	Characterization of superparamagnetic iron oxide nanoparticle-induced apoptosis in dopaminergic neuronal PC12 cells and mouse hippocampus and striatum	It showed a dose-dependent cytotoxic in PC12 cells at 60–200 μg/mL but not at 10–50 μg/mL, reduced cell viability, decreased the PC12 cells capacity to extend neuritis in response to nerve growth factor (NGF), increased PC12 cell apoptosis. Also decreased the TH^+ fiber density in both the dorsal striatum and the hippocampus	[140]
Oleic acid-coated iron oxide nanoparticles	Neurotoxicity assessment of oleic acid-coated iron oxide nanoparticles in human neuronal cells SH-SY5Y cells	Moderate cytotoxicity related to cell membrane impairment, cell cycle disruption and cell death induction, especially notable in serum-free medium	[141]
Iron oxide nanoparticles	Study based on iron oxide nanoparticles induces cell cycle-dependent neuronal apoptosis in mice	The result showed an increased level of oxidants, β amyloid accumulation, reduced level of cdk5, which indicates cell apoptosis and DNA damage due to overexpression of RNA Pol II and PARP cleavage	[142]
Silica-coated iron oxide nanoparticles	Study of the cytotoxicity and genotoxicity of silica-coated ION was evaluated on human A172 glioblastoma cells	The result showed certain cytotoxicity, related to cell cycle disruption and cell death. Scarce genotoxic effects and no alteration of the DNA repair process were observed	[143]
Iron oxide nanoparticles	Toxicity evaluation of magnetic iron oxide nanoparticles with egg albumin and subsequent toxicity on chicken embryo	Histology of brain tissue revealed degeneration of neurons (50–60%) at 10–100 μg/ml dose range of IONs	[144]

6 Conclusion

Nanoparticles have got significant potential in targeting therapeutics to the brain in the treatment of several diseases such as Alzheimer disease, cancer, Parkinson disease, epilepsy, and HIV encephalopathy. Till now several drugs are targeted to the brain based on several strategies as discussed. A prior challenge in drug targeting to the brain is the avoidance of opsonization and phagocytosis in blood, which can now be easily avoided by surface-functionalized nanoparticles, facilitating an efficient drug delivery to the brain. Still, neural cell death associated with oxidative

stress including neurodegenerative diseases may occur due to ROS released by nanoparticles in neurons. Thus, it is essential to carry out neurotoxicity or cytotoxicity studies for nanoformulations. However, results obtained in animal studies may not be extrapolated to human beings due to differences in physiological conditions of the body, genetic factors, and differences in transport mechanisms. Thus, studies dealing with pharmacokinetic and pharmacodynamic effects of nanoparticles on the physiological conditions of the body have to be considered for efficient drug targeting to the brain with reduced neurotoxicity. Reduction of neurotoxicity can be achieved by proper evaluation of the physicochemical characteristics of nanoparticles as well as their pharmacokinetic and pharmacodynamic parameters.

Acknowledgements The authors acknowledge Department of Pharmaceutical Sciences, Dibrugarh University, Assam for their partial help with carrying out the work.

Conflicts of Interest The authors have no conflicts of interest.

Declaration All figures and tables are original and self-made.

References

1. Nearly 1 in 6 of world's population suffers from neurological disorders—UN report. Retrieved July 10, 2018, from https://news.un.org/en/story/2007/02/210312-nearly-1-6-worlds-population-suffer-neurological-disorders-un-report
2. Saunders, N. R., Habgood, M. D., Mollgard, K., et al. (2016). The biological significance of brain barrier mechanisms: Help or hindrance in drug delivery to the central nervous system? *F1000 Research, 5*, 1–15.
3. Lu, C. T., Zhao, Y. Z., Wong, H. L., et al. (2014). Current approaches to enhance CNS delivery of drugs across the brain barriers. *International Journal of Nanomedicine, 9*, 2241–2257.
4. Selin, Y., Shellef, L., Knyazer, B., et al. (2015). Anatomy and physiology of the blood-brain barrier. *Seminars in Cell and Developmental Biology, 38*, 2–6.
5. Stamatovic, S. M., Keep, R. F., & Andjelkovic, A. V. (2008). Brain endothelial cell-cell junctions: How to "open" the blood brain barrier. *Current Neuropharmacology, 6*(3), 179–192.
6. Covarrubias, L. S., Slosky, L. M., & Thompson, B. J. (2014). Transporters at CNS barrier sites: Obstacles or opportunities for drug delivery? *Current Pharmaceutical Design, 20*(10), 1422–1449.
7. Chen, Y., & Liu, L. (2012). Modern methods for delivery of drugs across the blood–brain barrier. *Advanced Drug Delivery Reviews, 64*, 640–665.
8. Pardridge, W. M. (2012). Drug transport across the blood–brain barrier. *Journal of Cerebral Blood Flow and Metabolism, 32*(11), 1959–1972.
9. Hersh, D. S., Wadajkar, A. S., & Roberts, N. (2016). Evolving drug delivery strategies to overcome the blood brain barrier. *Current Pharmaceutical Design, 22*(9), 1177–1193.
10. Chen, P. Y., Yeh, C. K., Hsu, P. H., et al. (2017). Drug-carrying microbubbles as a theranostic tool in convection-enhanced delivery for brain tumor therapy. *Oncotarget, 8*(26), 42359–42371.
11. Bota, D. A., Desjardins, A., Quinn, J. A., et al. (2007). Interstitial chemotherapy with biodegradable BCNU (Gliadel®) wafers in the treatment of malignant gliomas. *Therapeutics and Clinical Risk Management, 3*(5), 707–715.

12. Zhou, Z., Singh, R., & Souweidane, M. M. (2017). Convection-enhanced delivery for diffuse intrinsic Pontine Glioma treatment. *Current Neuropharmacology, 15*(1), 116–128.
13. Meairs, S. (2015). Facilitation of drug transport across the blood–brain barrier with ultrasound and microbubbles. *Pharmaceutics, 7*(3), 275–293.
14. Azad, A. D., Pan, J., & Connolly, L. D. (2015). Therapeutic strategies to improve drug delivery across the blood-brain barrier. *Neurosurgical Focus, 38*(3), E9.
15. Jornada, D. H., Fernandes, G. F. D. S., & Chiba, D. E. (2016). The prodrug approach: A successful tool for improving drug solubility. *Molecules, 21*(42), 1–31.
16. Desai, N. (2012). Challenges in development of nanoparticle-based therapeutics. *The AAPS Journal, 14*(2), 282–295.
17. Wim, H. H. D. J., & Paul, J. A. B. (2008). Drug delivery and nanoparticles: Applications and hazards. *International Journal of Nanomedicine, 3*(2), 133–149.
18. Nie, S. (2010). Understanding and overcoming major barriers in cancer nanomedicine. *Nanomedicine (London), 5*(4), 523–528.
19. Lahkar, A., & Das, M. K. (2013). Surface modified polymeric nanoparticles for brain targeted delivery. *Current Trends in Biotechnology and Pharmacy, 7*(4), 914–931.
20. Choi, S. W., Kim, W. S., & Kim, J. H. (2003). Surface modification of functional nanoparticles for controlled drug delivery. *Journal of Dispersion Science and Technology, 24*(3–4), 475–487.
21. Zhao, M., Zhao, M., & Fu, C. (2018). Targeted therapy of intracranial glioma model mice with curcumin nanoliposomes. *International Journal of Nanomedicine, 13*, 1601–1610.
22. Aditi, J., Deshpande, P., & Pattni, B. (2018). Transferrin-targeted, resveratrol-loaded liposomes for the treatment of glioblastoma. *Journal of Controlled Release, 277*, 89–101.
23. Kim, S. S., Rait, A., & Kim, E. (2015). Encapsulation of temozolomide in a tumor-targeting nanocomplex enhances anti-cancer efficacy and reduces toxicity in a mouse model of glioblastoma. *Cancer Letters, 369*(1), 250–258.
24. Qu, M., Lin, Q., He, S., et al. (2018). A brain targeting functionalized liposomes of the dopamine derivative N-3, 4-bis(pivaloyloxy)-dopamine for treatment of Parkinson's disease. *Journal of Controlled Release, 277*, 173–182.
25. Sonali, S., Singh, R. P., Singh, N., et al. (2016). Transferrin liposomes of docetaxel for brain targeted cancer applications: Formulation and brain theranostics. *Drug Delivery, 23*(4), 1261–1271.
26. Zhang, Y., Zhai, M., & Chen, Z. (2017). Dual-modified liposome codelivery of doxorubicin and vincristine improve targeting and therapeutic efficacy of glioma. *Drug Delivery, 24*(1), 1045–1055.
27. Reddy, Y. D., Dhachinamoorthi, D., & Chandra Sekhar, K. B. (2015). A brief review on polymeric Nanoparticles for drug delivery and targeting. *Journal of Medical and Pharmaceutical Innovation, 2*(7), 19–32.
28. Khalin, I., Alyautdin, R., Wong, T. W., et al. (2016). Brain-derived neurotrophic factor delivered to the brain using poly(lactide-co-glycolide) nanoparticles improves neurological and cognitive outcome in mice with traumatic brain injury. *Drug Delivery, 23*(9), 3520–3528.
29. Calvo, P., Gouritin, B., Chacun, H., et al. (2001). Long-circulating PEGylated polycyanoacrylate nanoparticles as new drug carrier for brain delivery. *Pharmaceutical Research, 18*(8), 1157–1166.
30. Lopalco, A., Hasem, A., Denora, N., et al. (2015). Oxcarbazepine-loaded polymeric nanoparticles: Development and permeability studies across in vitro models of the blood–brain barrier and human placental trophoblast. *International Journal of Nanomedicine, 10*, 1985–1996.
31. Geldenhuy, W., Wehrung, D., & Groshev, A. (2015). Brain-targeted delivery of doxorubicin using glutathione-coated nanoparticles for brain cancers. *Pharmaceutical Development and Technology, 20*(4), 497–506.
32. Ahmad, N., Ahmad, R., Naqvi, A. A., et al. (2016). Rutin-encapsulated chitosan nanoparticles targeted to the brain in the treatment of cerebral ischemia. *International Journal of Biological Macromolecules, 91*, 640–655.

33. Blasi, P., Giovagnoli, S., Schoubben, A., et al. (2007). Solid lipid nanoparticles for targeted brain drug delivery. *Advanced Drug Delivery Reviews, 59*, 454–477.
34. Ramteke, K. H., Joshi, S. A., & Dhole, S. N. (2012). Solid lipid nanoparticle: A review. *IOSR Journal of Pharmacy, 2*(6), 34–44.
35. Yang, S., Zhu, J., Lu, Y., et al. (1999). Body distribution of Camptothecin solid lipid nanoparticles after oral administration. *Pharmaceutical Research, 16*(5), 751–757.
36. Kreuter, J. (1994). Nanoparticles. In *Colloidal drugs delivery systems* (pp. 219–342). New York: Dekker.
37. Kuo, Y. C., & Cheng, S. J. (2016). Brain targeted delivery of carmustine using solid lipid nanoparticles modified with tamoxifen and lectoferrin for antitumor proliferation. *International Journal of Pharmaceutics, 499*(1–2), 10–19.
38. Neves, A. R., Queiroz, J. F., & Reis, S. (2016). Brain-targeted delivery of resveratrol using solid lipid nanoparticles functionalized with apolipoprotein E. *Nano, 14*(27), 1–11.
39. Gandomi, N., Varshochian, R., Atyabi, F., et al. (2017). Solid lipid nanoparticles surface modified with anti-Contactin2 or anti-Neurofascin for brain targeted delivery of medicines. *Pharmaceutical Development and Technology, 22*(3), 426–435.
40. Bruun, J., Larsen, T. B., Jølck, R. I., et al. (2015). Investigation of enzyme-sensitive lipid nanoparticles for delivery of siRNA to blood-brain barrier and glioma cells. *International Journal of Nanomedicine, 10*, 5995–6008.
41. Busquets, M. A., Espargaró, A., Sabaté, R., et al. (2015). Magnetic nanoparticles cross the blood-brain barrier: When physics rises to a challenge. *Nanomaterials, 5*, 2231–2248.
42. Fu, T., Kong, Q., Sheng, H., et al. (2016). Value of functionalized superparamagnetic iron oxide nanoparticles in the diagnosis and treatment of acute temporal lobe epilepsy on MRI. *Neural Plasticity, 2016*, 1–12.
43. Tsuji, A., Tamai, I. I., et al. (1999). Carrier-mediated or specialized transport of drugs across the blood-brain barrier. *Advanced Drug Delivery Reviews, 36*(2–3), 277–290.
44. Du, D., Chang, N., Sun, S., et al. (2014). The role of glucose transporters in the distribution of p-aminophenyl-α-d-mannopyranoside modified liposomes within mice brain. *Journal of Controlled Release, 182*, 99–110.
45. Vemula, S., Roder, K. E., Yang, T., et al. (2009). A functional role for sodium-dependent glucose transport across the blood-brain barrier during oxygen glucose deprivation. *The Journal of Pharmacology and Experimental Therapeutics, 328*(2), 487–495.
46. Vivo, D. C. D., Trifiletti, R. R., Jacobson, R. I., et al. (1991). Defective glucose transport across the blood-brain barrier as a cause of persistent Hypoglycorrhachia, seizures, and developmental delay. *The New England Journal of Medicine, 325*, 703–709.
47. Rautio, J., Laine, K., Gynther, M., et al. (2008). Prodrug approaches for CNS delivery. *The AAPS Journal, 10*(1), 92–102.
48. Xie, F., Yao, N., Qin, Y., et al. (2012). Investigation of glucose-modified liposomes using polyethylene glycols with different chain lengths as the linkers for brain targeting. *International Journal of Nanomedicine, 7*, 163–175.
49. Li, X., Qu, B., Jin, X., et al. (2013). Design, synthesis and biological evaluation for docetaxel-loaded brain targeting liposome with "lock-in" function. *Journal of Drug Targeting, 22*(3), 251–261.
50. Peng, H., Du, D., Zhang, J., et al. (2013). Liposomes modified with p-aminophenyl-α-D-mannopyranoside: A promising delivery system in targeting the brain. *Therapeutic Delivery, 4*(12), 1475–1477.
51. Niu, J., Wang, A., Ke, Z., et al. (2014). Glucose transporter and folic acid receptor-mediated Pluronic P105 polymeric micelles loaded with doxorubicin for brain tumor treating. *Journal of Drug Targeting, 22*(8), 712–723.
52. Jiang, X., Xin, H., Ren, Q., et al. (2014). Nanoparticles of 2-deoxy-D-glucose functionalized poly(ethylene glycol)-co-poly(trimethylene carbonate) for dual-targeted drug delivery in glioma treatment. *Biomaterials, 35*(1), 518–529.

53. Pinho, M. J., Serrao, M. P., Gomes, P., et al. (2004). Over-expression of renal LAT1 and LAT2 and enhanced L-DOPA uptake in SHR immortalized renal proximal tubular cells. *Kidney International, 66*(1), 216–226.
54. Wang, B., Navath, R. S., Romero, R., et al. (2009). Anti-inflammatory and anti-oxidant activity of anionic dendrimer-N-acetyl cysteine conjugates in activated microglial cells. *International Journal of Pharmaceutics, 377*, 159–168.
55. Kharya, P., Jain, A., Gulbake, A., et al. (2013). Phenylalanine-coupled solid lipid nanoparticles for brain tumor targeting. *Journal of Nanoparticle Research, 15*(11), 1–12.
56. Fernandes, J., Ghate, M. V., Mallik, B. S., et al. (2018). Amino acid conjugated chitosan nanoparticles for the brain targeting of a model dipeptidyl peptidase-4 inhibitor. *International Journal of Pharmaceutics, 547*(1–2), 563–571.
57. Vadlapudi, A. D., Vadlapatla, R. K., & Mitra, A. K. (2012). Sodium dependent multivitamin transporter (SMVT): A potential target for drug delivery. *Current Drug Targets, 13*(7), 994–1003.
58. Veszelka, S., Meszaros, M., Kiss, L., et al. (2017). Biotin and glutathione targeting of solid nanoparticles to cross human brain endothelial cells. *Current Pharmaceutical Design, 23*(28), 4198–4205.
59. Michel, V., Yuan, Z., Ramsubir, S., et al. (2006). Choline transport for phospholipid synthesis. *Experimental Biology and Medicine (Maywood, N.J.), 231*(5), 490–504.
60. Lockman, P. R., & Allen, D. D. (2002). The transport of choline. *Drug Development and Industrial Pharmacy, 28*(7), 749–771.
61. Li, J., Yang, H., Zhang, Y., et al. (2015). Choline derivate-modified doxorubicin loaded micelle for glioma therapy. *ACS Applied Materials and Interfaces, 7*(38), 21589–21601.
62. Gajbhiye, K. R., Gajbhiye, V., Siddiqui, I. A., et al. (2017). Ascorbic acid tethered polymeric nanoparticles enable efficient brain delivery of galantamine: An *in vitro-in vivo* study. *Scientific Reports, 7*, 1–12.
63. Vijay, N., & Morris, M. E. (2014). Role of monocarboxylate transporters in drug delivery to the brain. *Current Pharmaceutical Design, 20*(10), 1487–1498.
64. Venishetty, V. K., Samala, R., Komuravelli, R., et al. (2013). β-Hydroxybutyric acid grafted solid lipid nanoparticles: A novel strategy to improve drug delivery to brain. *Nanomedicine, 9*(3), 388–397.
65. Kou, L., Hou, Y., Yao, Q., et al. (2017). L-carnitine-conjugated nanoparticles to promote permeation across blood-brain barrier and to target glioma cells for drug delivery via the novel organic cation/carnitine transporter OCTN2. *Artificial Cells, Nanomedicine and Biotechnology, 46*(7), 1–12.
66. P-glycoprotein. Retrieved July 10, 2018, from https://en.wikipedia.org/wiki/P-glycoprotein
67. Srivalli, K. M. R., & Lakshmi, P. K. (2012). Overview of P-glycoprotein inhibitors: A rational outlook. *BJPS, 48*(3), 353–367.
68. Malmo, J., Sandvig, A., Varum, K. M., et al. (2013). Nanoparticle mediated P-glycoprotein silencing for improved drug delivery across the blood-brain barrier: A *si*RNA-Chitosan approach. *PLoS One, 8*(1), 1–8.
69. Hoosain, F. G., Choonara, Y. E., Tomar, L. K., et al. (2015). Bypassing P-glycoprotein drug efflux mechanisms: Possible applications in Pharmaco resistant schizophrenia therapy. *BioMed Research International, 2015*, 484963.
70. Tam, V. H., Sosa, C., Liu, R., et al. (2016). Nanomedicine as a non-invasive strategy for drug delivery across the blood brain barrier. *International Journal of Pharmaceutics, 515*(1–2), 331–342.
71. Pardridge, W. M. (2002). Drug and gene targeting to the brain with molecular Trojan horses. *Nature Reviews. Drug Discovery, 1*, 131–139.
72. Mae, M., & Langel, U. (2006). Cell-penetrating peptides as vectors for peptide, protein and oligonucleotide delivery. *Current Opinion in Pharmacology, 6*(5), 509–514.
73. Madani, F., Lindberg, S., Langel, U., et al. (2011). Mechanisms of cellular uptake of cell-penetrating peptides. *Journal of Biophysics, 2011*, 414729.

74. Liu, L., Guo, K., Lu, J., et al. (2008). Biologically active core/shell nanoparticles self-assembled from cholesterol-terminated PEG-TAT for drug delivery across the blood-brain barrier. *Biomaterials, 29*, 1509–1517.
75. Allhenn, D., Boushehri, M. A., & Lamprecht, A. (2012). Drug delivery strategies for the treatment of malignant gliomas. *International Journal of Pharmaceutics, 436*, 299–310.
76. Yadav, M., Parle, M., Sharma, N., et al. (2017). Brain targeted oral delivery of doxycycline hydrochloride encapsulated Tween 80 coated chitosan nanoparticles against ketamine induced psychosis: Behavioral, biochemical, neurochemical and histological alterations in mice. *Drug Delivery, 24*(1), 1429–1440.
77. Yusuf, M., Khan, M., Khan, R. A., et al. (2016). Polysorbate-80-coated, polymeric curcumin nanoparticles for in vivo anti-depressant activity across BBB and envisaged biomolecular mechanism of action through a proposed pharmacophore model. *Journal of Microencapsulation, 33*(7), 646–655.
78. Das, M. K., Hussain, K., & Pathak, Y. V. (2013). Brain targeted delivery of Curcumin using P80-PEG-coated poly(lactide-co-glycolide) nanoparticles. *Asian Journal of Chemistry, 25*, S297–S301.
79. Sun, D., Xue, A., Zhang, B., et al. (2015). Polysorbate 80-coated PLGA nanoparticles improve the permeability of acetylpuerarin and enhance its brain-protective effects in rats. *The Journal of Pharmacy and Pharmacology, 67*(12), 1650–1662.
80. Jose, S., Sowmya, S., Cinu, T. A., et al. (2014). Surface modified PLGA nanoparticles for brain targeting of Bacoside-a. *European Journal of Pharmaceutical Sciences, 63*, 29–35.
81. Demeule, M., Currie, J. C., Bertrand, Y., et al. (2008). Involvement of the low-density lipoprotein receptor-related protein in the transcytosis of the brain delivery vector angiopep-2. *Journal of Neurochemistry, 106*(4), 1534–1544.
82. Thomas, F. C., Taskar, K., Rudraraju, V., et al. (2009). Uptake of ANG1005, a novel Paclitaxel derivative, through the blood-brain barrier into brain and experimental brain metastases of breast cancer. *Pharmaceutical Research, 26*(11), 2486–2494.
83. Xin, H., Jiang, X., Gu, J., et al. (2011). Angiopep-conjugated poly(ethylene glycol)-co-poly(ε-caprolactone) nanoparticles as dual-targeting drug delivery system for brain glioma. *Biomaterials, 32*(18), 4293–4305.
84. Inagaki, O. K., Mayuzumi, H., Kato, S., et al. (2014). Enhancement of leptin receptor signaling by SOCS3 deficiency induces development of gastric tumors in mice. *Oncogene, 33*(1), 74–84.
85. Liu, Y., Li, J., Shao, K., et al. (2010). A leptin derived 30-amino-acid peptide modified pegylated poly-L-lysine dendrigraft for brain targeted gene delivery. *Biomaterials, 31*(19), 5246–5257.
86. Kou, Y. C., Lin, P. I., & Wang, C. C. (2011). Targeting nevirapine delivery across human brain microvascular endothelial cells using transferrin-grafted poly(lactide-*co*-glycolide) nanoparticles. *Nanomedicine, 6*(6), 1011–1106.
87. Hu, K., Li, J., Shen, Y., et al. (2009). Lactoferrin-conjugated PEG-PLA nanoparticles with improved brain delivery: *In vitro* and *in vivo* evaluations. *Journal of Controlled Release, 134*(1), 55–61.
88. Carroll, R. T., Bhatia, D., Geldenhuys, W., et al. (2010). Brain-targeted delivery of tempol-loaded nanoparticles for neurological disorders. *Journal of Drug Targeting, 18*(9), 665–674.
89. Zhang, S., Wang, J., & Pan, J. (2016). Baicalin-loaded PEGylated lipid nanoparticles: Characterization, pharmacokinetics, and protective effects on acute myocardial ischemia in rats. *Drug Delivery, 23*(9), 3696–3703.
90. Ramalho, M. J., Sevin, E., Gosselet, F., et al. (2018). Receptor mediated PLGA nanoparticles for glioblastoma multiforme treatment. *International Journal of Pharmaceutics, 545*(1–2), 84–92.
91. Tang, X., Liang, Y., Zhu, Y., et al. (2015). Anti-transferrin receptor-modified amphotericin B-loaded PLA-PEG nanoparticles cure candidal meningitis and reduce drug toxicity. *International Journal of Nanomedicine, 10*, 6227–6241.

92. Ulbrich, K., Hekmatara, T., Herbert, E., et al. (2009). Transferrin- and transferrin-receptor-antibody-modified nanoparticles enable drug delivery across the blood–brain barrier (BBB). *European Journal of Pharmaceutics and Biopharmaceutics, 71*(2), 251–256.
93. Kuo, Y. C., & Wang, I. H. (2016). Enhanced delivery of etoposide across the blood-brain barrier to restrain brain tumor growth using melanotransferrin antibody- and tamoxifen-conjugated solid lipid nanoparticles. *Journal of Drug Targeting, 24*(7), 645–654.
94. Kaur, A., Jain, S., & Tiwary, A. K. (2008). Mannan-coated gelatin nanoparticles for sustained and targeted delivery of didanosine: *In vitro* and *in vivo* evaluation. *Acta Pharmaceutica, 58*(1), 61–74.
95. Boado, R. J., Hui, E. K. W., Lu, J. Z., et al. (2010). Selective targeting of a TNFR decoy receptor pharmaceutical to the primate brain as a receptor-specific IgG fusion protein. *Journal of Biotechnology, 146*(1–2), 84–91.
96. Ulbrich, K., Knobloch, T., & Kreuter, J. (2011). Targeting the insulin receptor: Nanoparticles for drug delivery across the blood-brain barrier (BBB). *Journal of Drug Targeting, 19*(2), 125–132.
97. Oswald, M., Geissler, S., & Goepferich, A. (2017). Targeting the central nervous system (CNS): A review of rabies virus-targeting strategies. *Molecular Pharmaceutics, 14*(7), 2177–2196.
98. Liu, Y., Huang, R., Han, L., et al. (2009). Brain-targeting gene delivery and cellular internalization mechanisms for modified rabies virus glycoprotein RVG29 nanoparticles. *Biomaterials, 30*(25), 4195–4202.
99. Chen, W., Zhan, C., Gu, B., et al. (2011). Targeted brain delivery of itraconazole via RVG29 anchored nanoparticles. *Journal of Drug Targeting, 19*(3), 228–234.
100. Zou, L., Tao, Y., Payne, G., et al. (2017). Targeted delivery of nano-PTX to the brain tumor-associated macrophages. *Oncotarget, 8*(4), 6564–6578.
101. Gao, Y., Wang, Z. Y., Zhang, J., et al. (2014). RVG-peptide-linked trimethylated chitosan for delivery of *si*RNA to the brain. *Biomacromolecules, 15*(3), 1010–1018.
102. Gaillard, P. J., Brink, A., & de Boer, A. G. (2005). Diphtheria toxin receptor-targeted brain drug delivery. *International Congress Series, 1277*, 185–198.
103. Buzzi, S., Rubboli, D., Buzzi, G., et al. (2004). CRM197 (nontoxic diphtheria toxin): Effects on advanced cancer patients. *Cancer Immunology, Immunotherapy, 53*(11), 1041–1048.
104. Tosi, G., Vilella, A., Veratti, P., et al. (2015). Exploiting bacterial pathways for BBB crossing with PLGA Nanoparticles modified with a mutated form of diphtheria toxin (CRM197): *In Vivo* experiments. *Molecular Pharmaceutics, 12*(10), 3672–3684.
105. Hobel, S., Appeldoorn, C. C. M., Gaillard, P. J., et al. (2011). Targeted CRM197-PEG-PEI/*si*RNA complexes for therapeutic RNA*i* in Glioblastoma. *Pharmaceuticals (Basel), 4*(12), 1591–1606.
106. Chen, C., Zhuji, F., JPK, J., et al. (2009). Gangliosides as high affinity receptors for tetanus neurotoxin. *The Journal of Biological Chemistry, 284*(39), 26569–26577.
107. Francis JW, Bastia E, Matthews CC, et al. Tetanus toxin fragment C as a vector to enhance delivery of proteins to the CNS. Brain Research 2004; 1011(1):7-13.
108. Georgieva, J. V., Hoekstra, D., & Zuhorn, I. S. (2014). Smuggling drugs into the brain: An overview of Ligands targeting transcytosis for drug delivery across the blood–brain barrier. *Pharmaceutics, 6*(4), 557–583.
109. Stojanov, K., Georgieva, J. V., Brinkhuis, R. P., et al. (2012). *In vivo* biodistribution of prion- and GM1-targeted polymersomes following intravenous administration in mice. *Molecular Pharmaceutics, 9*(6), 1620–1627.
110. 5-HT receptor. Retrieved July 11, 2018, from https://en.wikipedia.org/wiki/5-HT_receptor
111. Kuo, Y. C., & Wang, C. C. (2015). Carmustine-loaded catanionic solid lipid nanoparticles with serotonergic 1B receptor subtype antagonist for *in vitro* targeted delivery to inhibit brain cancer growth. *Journal of Taiwan Institute of Chemical Engineers, 46*, 1–14.

112. Kuo, Y. C., & Hong, T. Y. (2014). Delivering etoposide to the brain using catanionic solid lipid nanoparticles with surface 5-HT-moduline. *International Journal of Pharmaceutics, 465*(1–2), 132–142.
113. Hansraj, G. P., Singh, S. K., & Kumar, P. (2015). Sumatriptan succinate loaded chitosan solid lipid nanoparticles for enhanced anti-migraine potential. *International Journal of Biological Macromolecules, 81*, 467–476.
114. Zhang, C., Zheng, X., & Wan, X. (2014). The potential use of H102 peptide-loaded dual-functional nanoparticles in the treatment of Alzheimer's disease. *Journal of Controlled Release, 192*, 317–324.
115. Kaluzova, M., Bouras, A., Machaidze, R., et al. (2015). Targeted therapy of glioblastoma stem-like cells and tumor non-stem cells using cetuximab-conjugated iron-oxide nanoparticles. *Oncotarget, 6*(11), 8788–8806.
116. Thomsen, L. B., Thomsen, M. S., & Moos, T. (2015). Targeted drug delivery to the brain using magnetic nanoparticles. *Therapeutic Delivery, 6*(10), 1145–1155.
117. Tian, J., Yan, C., Liu, K., et al. (2017). Paclitaxel-loaded magnetic nanoparticles: Synthesis, characterization, and application in targeting. *Journal of Pharmaceutical Sciences, 106*(8), 2115–2122.
118. Kong, S. D., Lee, J., Ramachandran, S., et al. (2012). Magnetic targeting of nanoparticles across the intact blood–brain barrier. *Journal of Controlled Release, 164*(1), 49–57.
119. Belhadj, Z., Zhan, C., Ying, M., et al. (2017). Multifunctional targeted liposomal drug delivery for efficient glioblastoma treatment. *Oncotarget, 8*(40), 66889–66900.
120. Dan, M., Bae, Y., Pittman, T. A., et al. (2015). Alternating magnetic field-induced hyperthermia increases iron oxide nanoparticle cell association/uptake and flux in blood-brain barrier models. *Pharmaceutical Research, 32*, 1615–1625.
121. *Magnetic nano delivery of therapeutic agents across the blood brain barrier*. Retrieved July 11, 2018, from http://www.florida-institute.com/comp-tech/magnetic-nanodelivery-of-therapeutic-agents-across-blood-brain-barrier
122. Markides, H., Rotherham, M., & El Haj, A. J.. (2012). Biocompatibility and toxicity of magnetic nanoparticles in regenerative medicine. *Journal of Nanomaterials, 2012*, 1–12. Article ID 614094.
123. Song, M. M., Xu, H. L., Liang, J. X., et al. (2017). Lactoferrin modified graphene oxide iron oxide nanocomposite for glioma-targeted drug delivery. *Materials Science and Engineering. C, Materials for Biological Applications, 77*, 904–911.
124. Yan, F., Wang, Y., He, S., et al. (2013). Transferrin-conjugated, fluorescein-loaded magnetic nanoparticles for targeted delivery across the blood-brain barrier. *Journal of Materials Science. Materials in Medicine, 24*(10), 2371–2379.
125. Conroy, S., Chen F Zachary, S., et al. (2008). Tumor-targeted drug delivery and MRI contrast enhancement by chlorotoxin-conjugated iron oxide nanoparticles. *Nanomedicine (London, England), 3*(4), 495–505.
126. Shevtsov, M., Nikolaev, B., Marchenko, Y., et al. (2018). Targeting experimental orthotopic glioblastoma with chitosan-based superparamagnetic iron oxide nanoparticles (CS-DX-SPIONs). *International Journal of Nanomedicine, 13*, 1471–1482.
127. Shevtsov, M. A., Nikolaev, B. P., Ryzhov, V. A., et al. (2015). Ionizing radiation improves glioma-specific targeting of superparamagnetic iron oxide nanoparticles conjugated with cmHsp70.1 monoclonal antibodies (SPION-cmHsp70.1). *Nanoscale, 7*(48), 20652–20664.
128. Eslaminejad, T., Nematollahi-Mahani, S. N., & Ansari, M. (2017). Glioblastoma targeted gene therapy based on pEGFP/p53-loaded superparamagnetic iron oxide nanoparticles. *Current Gene Therapy, 17*(1), 59–69.
129. Ma, X., Tao, H., Yang, K., et al. (2012). A functionalized graphene oxide-iron oxide nanocomposite for magnetically targeted drug delivery, photothermal therapy, and magnetic resonance imaging. *Nano Research, 5*(3), 199–212.

130. Pernal, S., Wu, V. M., & Uskoković, V. (2017). Hydroxyapatite as a vehicle for the selective effect of superparamagnetic iron oxide nanoparticles against human glioblastoma cells. *ACS Applied Materials and Interfaces, 9*(45), 39283–39302.
131. Hu, Y. L., & Gao, J. Q. (2010). Potential neurotoxicity of nanoparticles. *International Journal of Pharmaceutics, 394*, 115–121.
132. Yuan, Z. Y., Hu, Y. L., & Gao, J. Q. (2015). Brain localization and neurotoxicity evaluation of Polysorbate 80-modified chitosan nanoparticles in rats. *PLoS One, 10*(8), 1–14.
133. Manke, A., Wang, L., & Rojanasakul, Y. (2013). Mechanisms of nanoparticle-induced oxidative stress and toxicity. *BioMed Research International, 2013*, 1–15. Article ID 942916.
134. Borysov, A., Krisanova, N., Chunihin, O., et al. (2014). A comparative study of neurotoxic potential of synthesized polysaccharide coated and native ferritin based magnetic nanoparticles. *Croatian Medical Journal, 55*, 195–205.
135. Wu, J., Ding, T., & Sun, J. (2013). Neurotoxic potential of iron oxide nanoparticles in the rat brain striatum and hippocampus. *Neurotoxicology, 34*, 243–253.
136. Marcato, P. D. (2008). Durán N new aspects of nanopharmaceutical delivery systems. *Journal of Nanoscience and Nanotechnology, 8*(5), 2216–2229.
137. Shwe, T. T. W., & Fujimaki, H. (2011). Nanoparticles and neurotoxicity. *International Journal of Molecular Sciences, 12*, 6267–6280.
138. Tian, L., Lin, B., Wu, L., et al. (2015). Neurotoxicity induced by zinc oxide nanoparticles: Age-related differences and interaction. *Scientific Reports, 5*, 1–12.
139. Zhu, X., Tian, S., & Cai, Z. (2012). Toxicity assessment of iron oxide nanoparticles in Zebrafish (*Danio rerio*) early life stages. *PLoS One, 7*(9), 1–6.
140. Yutong, L., Juan, L., Kaige, X., et al. (2018). Characterization of superparamagnetic iron oxide nanoparticle-induced apoptosis in PC12 cells and mouse hippocampus and striatum. *Toxicology Letters, 292*, 151–161.
141. Bertolaz, N. F., Costa, C., Brandao, F., et al. (2018). Neurotoxicity assessment of oleic acid-coated iron oxide nanoparticles in SH-SY5Y cells. *Toxicology, 407*, 81–91.
142. Manickam, V., Dhakshinamoorthy, V., & Perumal, E. (2018). Iron oxide Nanoparticles induces cell cycle-dependent neuronal apoptosis in mice. *Journal of Molecular Neuroscience, 64*(3), 352–362.
143. Bertolaz, N. F., Costa, C., Brandao, F., et al. (2018). Toxicological assessment of silica-coated iron oxide nanoparticles in human astrocytes. *Food and Chemical Toxicology, 118*, 13–23.
144. Patel, S., Jana, S., Chetty, R., et al. (2017). Toxicity evaluation of magnetic iron oxide nanoparticles reveals neuronal loss in chicken embryo. *Drug and Chemical Toxicology, 27*, 1–8.

Chapter 16
Surface Modification of Resorcinarene-Based Self-Assembled Solid Lipid Nanoparticles for Drug Targeting

Sanjoy Das and Malay K. Das

Abstract Supramolecular chemistry associates the dual concepts of self-arranging and molecular perception to generate novel nanocarrier systems. Nanoparticles have numerous benefits over other drug delivery carriers. Solid lipid nanoparticles (SLNs) have acquired significant attention as a potential substitutive carrier system to usual colloidal carriers like liposomes, emulsion, as well as polymeric nanoparticles. SLNs are the novel fundamental approaches to alter the oral bioavailability problems of the poorly aqueous soluble drug. However, due to the hydrophobicity of SLNs, they are essentially stabilized to prevent aggregation and diminish the liability of clearance by the macrophage system. Therefore, coating the SLNs surface by a highly hydrophilic moiety leads to prevent aggregation and severe interaction with healthy cells. Resorcinarenes are synthetic supramolecular macrocycles with bowl-shaped head and several hydrogen-bonding tails which are capable of developing additional host-guest complexes through the self-associate process. Resorcinarenes are the most frequently studied macrocycles for the buildup of supramolecular SLN systems, because the bowl-shaped head of the resorcinarene molecules can enable them to adhere readily to the SLN surface, permitted them to interact with the substances outside the coating but prevented them from touching each other, leading to meaningful impact on the stability aspects and physical–functional properties of nanoparticles. Significantly, resorcinarenes and its water-soluble components show good biodegradability, biocompatibility, and nontoxicity, which are essential requirements for applications in any type of drug delivery carriers. This chapter highlights the recent development in resorcinarene-based lipid nanocarriers for drug delivery and targeting.

Keywords Self-assembled SLNs · Metal nanoparticles · Surface-modified resorcinarene-based SLNs · Surface-modified resorcinarene-based metal nanoparticles · Drug delivery · Drug targeting

S. Das · M. K. Das (✉)
Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh, India
e-mail: mkdps@dibru.ac.in

Y. V Pathak (ed.), *Surface Modification of Nanoparticles for Targeted Drug Delivery*, https://doi.org/10.1007/978-3-030-06115-9_16

1 Introduction

In current years, the meaningful effort has been committed to establishing nanotechnology for drug delivery, since it offers a convenient means of transporting low molecular weight drugs and biomolecules like genes, proteins, and peptides to cells or tissues and protects them against enzymatic deterioration [1–3]. Nanoparticles with their typical features like narrow particle size, broad surface area, and the capability to change their exterior properties have numerous benefits over other drug delivery carriers [4, 5]. Targeted drug delivery to the specific organ is highly attractive for local or systemic treatment or diagnosis of various diseases [6, 7]. Drug targeting indicates for discriminating and efficient localization of therapeutically active ingredient at target tissues or cells, while lowering its connection to nontarget cells or organs, leading to effective treatment at the therapeutically effective doses and minimize the toxic adverse effects [8, 9].

Solid lipid nanoparticles (SLNs) have acquired significant attention as a potential substitutive carrier system to usual colloidal carriers like liposomes, emulsion, as well as polymeric nanoparticles [10, 11]. The SLNs are the attractive contraption that is beneficial because the solid compartment of the lipids provides more flexibility in controlling the release process of the entrapped drug [12, 13]. SLNs are prepared by solid lipid instead of using liquid lipid and are distributed in water or an aqueous surfactant solution, giving particle size in the ranges between 50 and 1000 nm [14, 15]. SLNs are one of the essential approaches to alter the oral bioavailability problems of the low water or aqueous soluble drugs, resulting in protection of entrapped drugs from deterioration, the release of drug in a delayed or controlled manner, and good desirability [16–18]. SLNs are generally prepared using natural solid lipids, water, emulsifiers, and co-solvent [19]. In addition to these natural solid lipids, macrocyclic synthetic supramolecules have been exhibited to be a good candidate to replace these amphiphiles [20].

Supramolecular chemistry associates the dual concepts of self-configuration and molecular perception to generate novel nanocarrier systems [21]. Self-assembles of supramolecular architecture can automatically form nanoparticles and currently used as the development of nanodrug carriers [22]. Method for self-assembling of nanoparticles significantly relies on the nature or character of the particles used and the medium which they are dispersed [23, 24]. The self-assembling technique denoted potential aspect to produce nanomaterials with tunable properties, or a device-like function has triggered a worldwide research attempt to develop bottom-side-up approaches using an extended set of nano-range building blocks [25–27].

Resorcinarene is an organic surfactant, a cyclic oligomer with a bowl-shaped head and several hydrogen-bonding tails based on the condensation reaction between resorcinol and aldehydes [28]. Resorcinarenes and cyclodextrins are the most widely investigated supramolecules for the development of SLNs [29, 30]. The cyclodextrins have been manifested to self-assemble as SLNs when suitably modified, but their intrinsic toxicity limits their application for biomedical purposes [31, 32]. SLNs based on resorcinarenes have been studied, and it has been demonstrated that in addition to their remarkable physicochemical properties, they exhibit no intrinsic toxicity [33, 34].

In addition, they have shown remarkable properties as controlling agents of a widely used UV absorber, which offers a new scope of applications as carriers for sunscreens [35]. Interestingly, resorcinarenes while derived from resorcinol and aldehyde neither show toxic effects nor provoke immune reactions [36]. Such molecules seem to be promising candidates for the expansion of supramolecular SLN systems and show their molecular acceptance properties with respect to biomolecules like carbohydrates, proteins, and amino acids [37, 38].

Precisely, bowl-shaped head of the resorcinarene molecules can enable them to adhere readily to the SLNs surface and permitted them to interreact with the substances outside the coating but restrained them from touching each other [39, 40]. This technique has a meaningful impact on the physical–functional properties and stability aspects of nanoparticles [40]. However, the definite capability of these supramolecular structures to entrapped various active components in both supramolecular cavity and the SLNs compartment might be an interesting substitutive to the conventional SLNs and potential for drug targeting, contrast agents in the biological system, and cosmetic additives. This chapter highlights the recent development in resorcinarene-based lipid nanocarriers for drug delivery and targeting.

2 Stereochemistry of Resorcinarenes

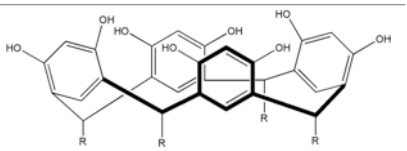
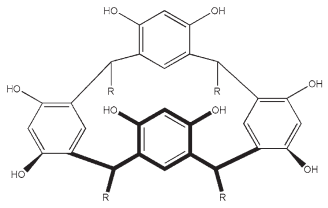
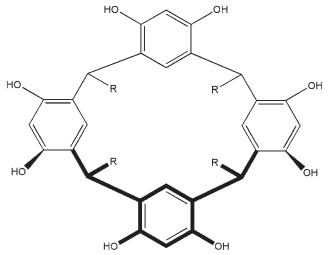
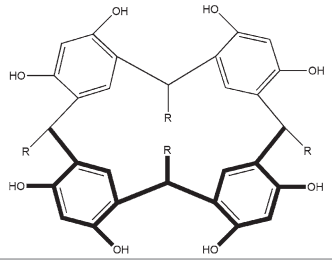
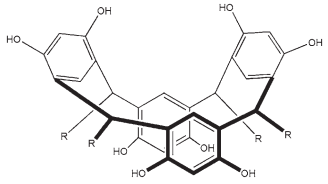
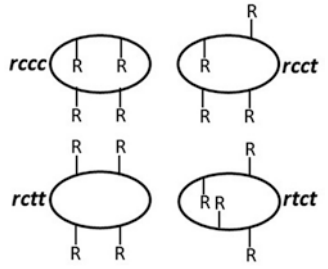
Resorcinarenes are nonpolar, 3D compounds and have generated a number of stereoisomers [41]. The stereochemistry of resorcinarenes is generally explained by a combination of the three subsequent stereochemical criteria [42]. The steric conformation of resorcinarenes is shown in Table 16.1.

The first criterion is the configuration of the macrocyclic ring. The macrocycle can adopt one of five highly symmetrical conformations, viz., Crown (C_{4v}), i.e., one macrocycle ring oriented in an upward position as like bowl shape, thus forming the crown conformer; Boat (C_{2v}), i.e., two opposite macrocycle rings can occupy an upward position with another two rings lying in perpendicular position to them, thus forming the boat conformer; Chair (C_{2h}), i.e., one upward ring oriented with two adjacent rings perpendicularly and the fourth ring in a downward position, thus forming chair isomer; Diamond (C_s), i.e., two adjacent rings oriented in upward and another two adjacent rings oriented in downward position; and Saddle (D_{2d}), i.e., two opposite rings facing upward and another two rings facing downward [43, 44].

The second criterion denotes the orientation of groups attached to carbon atoms at the benzylic position of resorcinarenes, results in four stereoisomers, viz., *cis (rccc)*, i.e., all attached groups facing the same direction in a *cis*-relationship to a reference group; *cis–cis–trans (rcct)*, i.e., one group on opposite orientation to the other three groups; *cis–trans–trans (rctt)*, i.e., two groups opposite to the pair containing the reference group; and *trans–cis–trans (rtct)*, i.e., reference group is in a *cis*-relationship with the substituent opposite to it and *trans*-relationship with those adjacent to it [44].

The last criterion is the configuration of individual substituents on the carbon atoms at the benzylic position of the resorcinarene macrocycle which may be either

Table 16.1 Resorcinarene stereoisomers and its structures

Sl. no.	Conformation	Structure
1	Crown (C_{4v})	
2	Boat (C_{2v})	
3	Chair (C_{2h})	
4	Diamond (C_s)	
5	Saddle (D_{2d})	
6	Configuration at methylene bridges	

axial or central position of the macrocycle C-symmetry [44]. The combination of all these three criteria results in a wide number of possible stereoisomers with several of which have been reported experimentally.

3 Synthesis of Resorcinarene Derivatives

3.1 Acid-Catalyzed Preparation of Resorcinarenes

Resorcinarenes are synthesized by a one-step condensation reaction procedure using an equal amount of resorcinol and aldehyde in the presence of a solvent and an acid catalyst [45, 46]. Methanol or ethanol is the most extensively used solvent in the resorcinarenes preparation [47]. The reaction mixtures are refluxed for a couple of hours, and the products formed are crystallized out from the solution on cooling [48]. An outline of the reaction scheme is shown in Fig. 16.1.

3.2 Novel Preparation of Variant Resorcinarenes

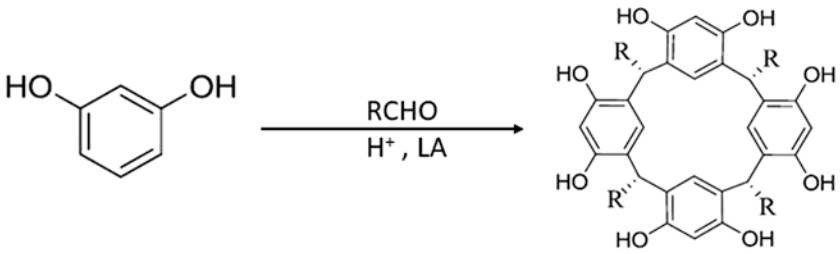
Functionalized or modified resorcinol is used as starting material in the preparation of functionalized resorcinarenes [49]. The resorcinols with electron donating groups at their 2-positions form any resorcinarenes under the acidic conditions, while under the basic conditions resorcinols with electron accepting groups at their 2-positions form novel resorcinarenes derivatives [49]. The synthesis scheme is shown in Fig. 16.1.

3.3 Preparation of Pyridine Derivatives of Resorcinarenes

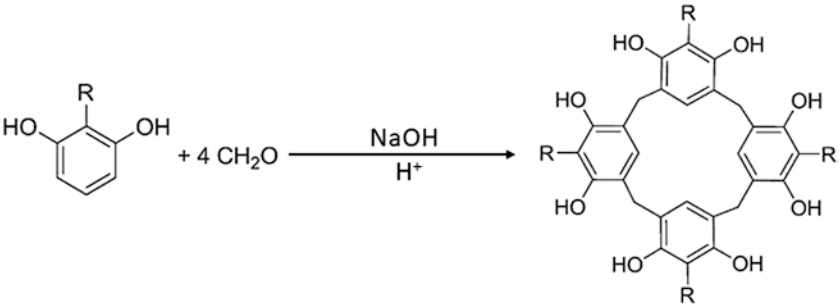
Pyridine derivatives of resorcinarenes, i.e., pyridine[4]arenes, have been prepared by cyclocondensation of 2,6-functionalized pyridines and aldehydes under similar reaction procedure as for the preparation of conventional resorcinarenes [50]. The synthesis scheme is shown in Fig. 16.1.

3.4 Synthesis of Heteroatom Containing Macrocyclic Cyclophanes or Heterocalixaromatics

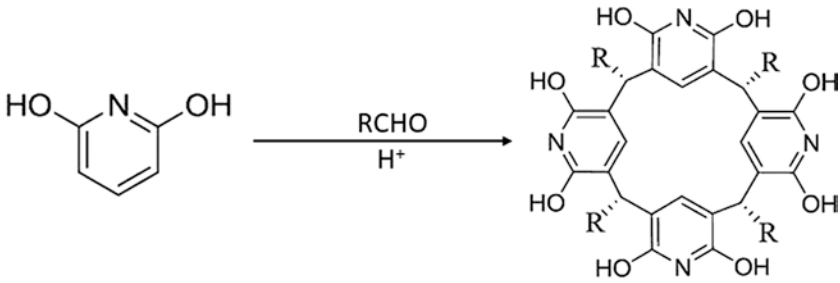
The addition of heteroatom bridges in place of methylene bridges i.e., nitrogen in a place of CH_2 at the benzylic position of the resorcinarenes is an exceptionally interesting modification conferring new class of resorcinarenes derivatives called heterocalixaromatics [51]. For instance, nitrogen can adopt sp^3 or sp^2 electronic configuration providing different conjugation system between the heteroatoms and



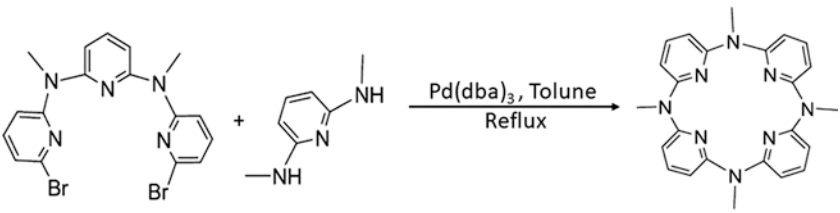
Scheme 1: Acid-catalyzed Preparation of Resorcinarenes



Scheme 2: Novel Preparation of Variant Resorcinarenes



Scheme 3: Preparation of Pyridine Derivatives of Resorcinarenes



Scheme 4: Synthesis of Heteroatom Containing Macrocyclic Cyclophanes

Fig. 16.1 Synthesis scheme of resorcinarene derivatives

adjoining aromatic rings [52]. Based on the configuration or conjugation, various *C–N* bond length and *C–N–C* bond angle are thus formed, and heteroatom linkages significantly affect the electron density of aromatic rings, and the electron features of macrocycle cavities may be regulated by heteroatoms [52]. Therefore, such compounds are synthesized using more complex procedures as compared to the conventional resorcinarenes. The synthesis scheme is shown in Fig. 16.1.

4 Functionalization of Resorcinarenes

Several types of methodologies have already been explored to define the functionalization of resorcinarenes over the last few years. These methodologies permit the preparation and modification of resorcinarenes that are mainly performed at the three functionalized positions, i.e., lower rim, upper rim, and *O*-alkylation or acylation (Fig. 16.2) [53].

4.1 Lower-Rim Functionalization

Functionalization at this position arises from the preferred type of aldehyde used in the preparation of resorcinarenes, since the group attached to the aldehyde functionality ends up composing lower rim [53, 54]. A vast variety of aldehydes including saturated alkyl aldehydes, unsaturated alkyl aldehydes, and phosphorous containing aldehydes are used in the resorcinarenes preparation [55]. These functionalized aldehydes lead to the construction of resorcinarenes bearing several lower-rim functionalities [56].

4.2 Upper-Rim Functionalization

Like any other aromatic compound, resorcinarenes can undergo electrophilic substitution reaction, at their upper-rim ortho positions to furnish tetrafunctionalized resorcinarenes under the suitable conditions [57]. The appearance of two

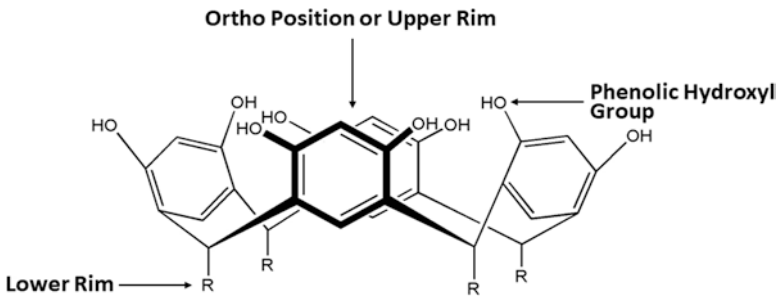


Fig. 16.2 Functionalizable positions of resorcinarenes

electron-donating hydroxy groups in the aromatic rings of the resorcinarenes is mainly susceptible to electrophilic substitution at ortho position [58]. The ortho substituent has the ability to form intramolecular H-bonds with the neighboring hydroxy groups of the resorcinarene units leading to the formation of C_4 spinning symmetry-based chiral derivatives [59].

4.3 O-*Alkylation*

O-Alkylation-based functionalization has been used for the synthesis of octafunctionalized resorcinarenes by reacting alkyl groups [60]. For example, reacting the 3-alkoxy-5-benzylbromidealkoxybenzyne with the phenolic hydroxyl group of resorcinarenes in the presence of potassium carbonate managed to furnish resorcinarene dendrimers via this type of functionalization [61, 62]. The phenolic hydroxyl group plays a major contribution in the equalization of resorcinarene conformation and allows for *O*-alkylation or acylation reactions which promote resorcinarenes chirality [63].

4.4 Selective Functionalization

Apart from the synthesis of functionalized resorcinarenes mentioned above, methodologies for the selective functionality of resorcinarene derivatives have been planned [64]. There are two methodologies, which can be adopted to access distal functionalized resorcinarenes, i.e., *lithium–halogen exchange* mainly generates nucleophilic organolithium intermediates from alkyl halides whose reaction with electrophiles leading to the formation of functionalized organic compounds [65, 66] and *selective acylation* depends mainly on the feature of both solvent and acylating agent leading to selective distal functionalization of resorcinarenes [67, 68]. For example, the reaction between octahydroxy resorcinarenes and *N*-bromosuccinimide resulted in the manufacturing of distally brominated resorcinarenes [69].

5 Resorcinarene-Based Self-Assembled SLNs

5.1 SLNs of Resorcinol-Dodecanal Cyclotetramer

Resorcinol-dodecanal cyclotetramer (RDC) was prepared by one-step synthesis procedure based on the acid-catalyzed reaction between resorcinol and dodecanal in ethanolic solution [70]. SLNs based on RDC were prepared by solvent displacement method [71]. These types of SLNs can be acquired by without surfactant and

comparison to other substitutive colloidal or nanocarrier system where a surfactant is essential in their formulation was of interest to consider the feasible impact of conjugated surfactant [72]. The result demonstrated that small but significantly larger size of the particle was detected when using Pluronic F68 (2% w/w) in the organic phase leads to the diameter of 180 nm [73]. Above this quantity, when the surfactant concentration is significantly raised up to 20% w/w, the size of the SLNs remained invariant. In general, a higher surfactant concentration lowers the surface tension and promotes the partition of the colloidal particle by decreasing the particle size which is joined to enlarge the superficial area [73]. The main variation in the nature of surfactant toward these SLN systems might be correlated with the response of the HLB value of each supramolecular structure which affects their self-emulsification characteristics [74, 75]. However, the particle size of the RDC-based SLNs remains stable or reliable in the pH range from 4 to 8, while vulnerable stability was noticed at pH range within 2–4. The impact of stirring or rotating speed, microwave irradiation, and ultrasonic and thermal treatment had no detectable impact on the drug stability as well as particle size [76].

5.2 *SLNs of Resorcinarene Bis-Crown Ethers*

Resorcinarene bis-crown ethers (*CNBC5*) was prepared by sequence beginning with a reaction between ethylene glycol and several tetramethoxy resorcinarenes [77]. SLNs based on *CNBC5* were prepared by solvent displacement method without surfactant and characterized by using photon correlation spectroscopy (PCS), which shows a hydrodynamic particle diameter of 220–320 nm [71–78]. The impact of alkyl chain length and concentration of *CNBC5* on the size of SLNs has been explored in two different ways, i.e., in the first case, with a fixed molar concentration of *CNBC5*, the size of particles significantly increases when the length of alkyl chain has been increasing, while in another case, the concentration was kept constant and the size of the particle was almost similar than that of the first case [78]. This result demonstrated that change in the *CNBC5* amount within the two cases did not produce any effect on the SLNs properties and the alteration of the particle size depended only on the length of the alkyl chain.

5.3 *SLNs of Tetrakis (*N*-Methylprolyl)Tetraundecylcalix[4] Resorcinarenes (*L*-RA-Pro)*

Amphiphilic tetrakis (*N*-methylprolyl)tetraundecylcalix[4]resorcinarenes (*L-RA-Pro*) was prepared by a Mannich-type reaction of L-proline, resorcinol-dodecanal cyclotetramer, and formaldehyde in ethanol [79]. SLNs based on *L-RA-Pro* was prepared by solvent displacement method and characterized by PCS, which shows a

hydrodynamic diameter around 195 ± 5 nm [71–80]. *L-RA-Pro*-based SLNs were chemically stabilized by *N*-hydroxysuccinimide and then reacted with bovine serum albumin (BSA) to obtain proteo-SLNs [80]. These results suggested that when L-RA-Pro based SLNs are reacted with BSA, forms a uniform surface monolayer due to a particular interaction between L-RA-Pro based SLNs and BSA. Photon correlation spectroscopy (PCS) analysis demonstrated that SLNs showed no indicative changes after the chemical modification in the case of particle size [80].

6 Surface Modification of Resorcinarene-Based Self-Assembled SLNs

The surface of the nanoparticle is modified with coupling agents after nanoparticle preparation. The polymer employed for the nanoparticles preparation must have one reactive group, which is observed on the nanoparticles surface for proper conjugation [81]. The coupling agents are dispersed in the external phase of a nanoparticles suspension, which activate the reactive group of the nanoparticles [82, 83]. These activated reactive groups of nanoparticles are then adjoined with targeting or functional material like ligand, drug, organic material, enzymes, etc. [84, 85]. Macrocyclic amphiphiles including cyclodextrins, resorcinarenes, and calixarenes are attractive materials for the nanoparticles preparation because they have outstanding self-organizing properties by forming host–guest complexes within their cavities [86]. Herein, very few information has been investigated regarding the surface modification of resorcinarene-based self-assembled SLNs. In 2007, Stefan Ehrler et al. reported that the synthesis of SLNs based on a prolyl bearing resorcinarene (*L-RA-Pro*) and their chemical modification with bovine serum albumin (BSA) and the interaction with surface-bound anti-albumin antibodies have been studied [80]. The prolyl bearing resorcinarene (*L-RA-Pro*)-based SLNs was prepared by solvent displacement method [71]. The amphiphilic properties of *L-RA-Pro* have been validated by Langmuir balance method, and it was demonstrated that these molecules at their air–water interface form monomolecular layers, with prolyl moieties immersed in the water sub-phase and able to contact with copper and to build up a ternary supramolecular enantioselective complex with phenylalanine [87]. The complex process of self-organization of amphiphiles to form SLNs is driven by amphiphilic self-assembly and it is expected that prolyl moieties of *L-RA-Pro* are partially operative at the SLNs surface [87]. For further modification of SLNs surface, it could be submitted to chemical activation using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) and subsequently reacted with BSA to develop proteo-SLNs [80] as shown in Fig. 16.3.

The evidence of the successful grafting of bovine serum albumin at the surface of the SLNs based on *L-RA-Pro* arises from their capability of interacting with surface-bound polyclonal specific antibodies, which form a fine layer at the surface of the SLNs [88].

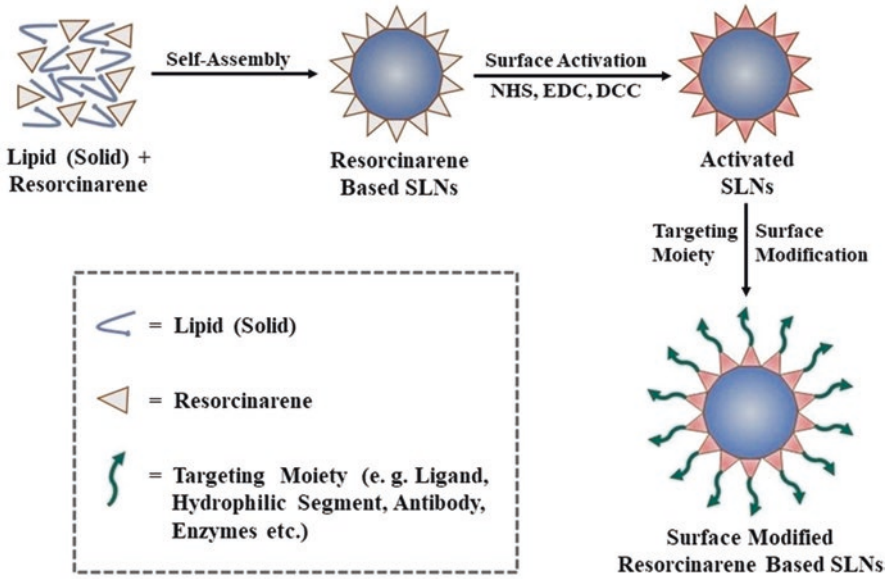


Fig. 16.3 Surface modification scheme of resorcinarene-based SLNs

7 Potential of Drug Targeting by Surface-Modified Resorcinarene-Based SLNs

The application of nanotechnology in drug targeting has driven to further advances in the preparation of novel nanoparticles system for diagnosis and treatment of many diseases [89]. As the conventional nanoparticulated system has some limitations, surface modification has enabled further growth and enhanced the capability of such nanoparticles [90, 91]. Generally, the nanoparticle surfaces are covered with the hydrophilic moiety or polymer to provide long circulation and are conjugated with enzymes or targeting ligands for site-specific delivery [92]. Solid lipid nanoparticles (SLNs) have gained attraction because of their outstanding properties such as biocompatibility, biodegradability, stability, superior drug-loading capacity, and ease of modification [93]. However, due to the hydrophobicity of SLNs, they must be stabilized to hinder aggregation and minimize the risk of clearance by the macrophage system [94]. Therefore, coating the SLNs surface by a highly hydrophilic moiety made of molecules such as polyethylene glycol or proteins such as albumin or supramolecules such as resorcinarenes leads to inhibit aggregation and nonspecific interaction with healthy tissues or cells as well as minimizing the harmful effects of toxic materials [95].

Supramolecular appeals have been engaged in the drug delivery system and fascinate much attention among the researchers [96]. The enormous research attempt has been assumed to enhance the therapeutic potency and reduce the side

effects of anticancer drugs, because they have demerits of affecting both cancer cells along with the healthy cells, with the concomitant secondary side effects such as cardiotoxicity, neurotoxicity, and cytotoxicity [97, 98].

Different generations of supramolecules or synthetic macrocycles such as resorcinarenes, calixarenes, cyclodextrins, and pillarenes have been hired for the construction of a new drug delivery system [99, 100]. Resorcinarenes are bowl-shaped synthetic supramolecular macrocycles composed of eight –OH groups that can engage in hydrogen bonding and capable of developing additional host-guest complexes through the self-associate process [101, 102]. Significantly, resorcinarenes and its water-soluble components show good biodegradability, biocompatibility, and nontoxicity, which are essential requirements for applications in any drug delivery carriers [103]. Therefore, the use of surface-modified resorcinarene-based SLNs may open new routes for drug targeting.

8 Surface Modification of Metal Nanoparticles with Resorcinarenes

Metal nanoparticles are one of the markedly studied nanomaterials with their potential significance for both basic and applied research [104]. Although such nanomaterials have magnificent physical, chemical, optical, or electronic properties, they do not hold compatible surface or interfacial properties for particular applications, so it may be urgent to modify such nanoparticles surface [105, 106]. The surface modification of metal nanoparticles is used to upgrade their stability, biocompatibility, surface energy, and so on [107]. The suitable modification of such nanoparticles surface differs the surface structure, composition, or morphology of materials and may result to controlled assembly or transferring of nanoparticles to the aqueous or organic media [108]. There are numerous ways for modification of the hydrophilicity or hydrophobicity on the surface of metal nanoparticles such as ligand exchange, ligand chemical modification, and/or supplementary layers of polymers that fix the particles in relevant phase [108, 109]. Therefore, modification of metal nanoparticle surfaces by the hydrophilic macrocycles, i.e., resorcinarene derivatives forming inclusion complexes on the surface of nanoparticles, resulted to the efficient delivery of metallic nanoparticles in aqueous or organic-phase count on the scientific demand [110, 111]. The surface modification of various metal nanoparticles by resorcinarenes and its derivatives are summarized in Table 16.2.

9 Conclusion and Future Prospects

The amphiphilic properties of macrocycles lead to the good stability of SLNs in an aqueous colloidal solution due to the arrangement of the macrocycles coat on the nanoparticles surface. The macrocycles layers are convenient for the self-associate

Table 16.2 Metal nanoparticles and surface-coating materials

Sl. no.	Nanoparticles	Surface coating	Particle size (nm)	References
1	Gold nanoparticles	Tetramethoxyresorcinarene tetraaminoamide	6–17	[112]
		Resorcinarenes 1–3 derivatives	3–20	[113]
		Resorcinarene 1, 2, 4–8 derivatives and tetraazaresorcinarene analog	9–35	[114]
		Tetrabenzylthiol resorcinarene	15–50	[115]
2	Silver nanoparticles	Tetrapentylcalix[4]resorcinarene	4–6	[116]
		Resorcinarene tetrahydrazide	15	[117]
		Ferrocene resorcinarene	60	[118]
3	Cobalt nanoparticles	C-undecylcalix[4]resorcinarene	15–100	[119]
4	Platinum nanoparticles	Amino resorcinarene	2–3	[120]

process leading to the partial association of SLNs in aqueous media while preventing their aggregation and also furnishing the preferences to the supramolecular modification of the SLNs surface through the intermolecular interaction with oppositely charged surfactants and organic molecules. The formation of resorcinarenes layered on the surface of SLNs has reinforced the receptor properties owing to the configuration of diverse aggregates with guest molecules via host-guest interrelation make them a preferred candidate for drug targeting. Although scientists have achieved remarkable success toward resorcinarene-stationed drug delivery system, so far, there are only a few researches that have been accomplished on a cellular level. The suspicious toxicity and the current unavailability of FDA approval to use these macrocycle supramolecules in medicine enable us to expect that such types of nanocarrier system deserve further clinical and preclinical medical testing in near future.

Declaration All figures and tables are original and self-made.

References

1. Mahapatro, A., & Singh, D. K. (2011). Biodegradable nanoparticles are excellent vehicle for site-directed in-vivo delivery of drugs and vaccines. *Journal of Nanobiotechnology, 9*(55), 1–11.
2. Yun, Y., Cho, Y. W., & Park, K. (2013). Nanoparticles for oral delivery: Targeted nanoparticles with peptidic ligands for oral protein delivery. *Advanced Drug Delivery Reviews, 65*(6), 822–832.
3. Soni, K., Kukereja, B. K., Kapur, M., & Kohli, K. (2015). Lipid nanoparticles: Future of oral drug delivery and their current trends and regulatory issues. *International Journal of Current Pharmaceutical Review and Research, 7*(1), 1–18.
4. Khan, A. K., Rashid, R., Murtaza, G., & Zahra, A. (2014). Gold nanoparticles: Synthesis and applications in drug delivery. *Tropical Journal of Pharmaceutical Research, 13*(7), 1169–1177.

5. Guo, D., Xie, G., & Luo, J. (2014). Mechanical properties of nanoparticles: Basics and applications. *Journal of Physics D: Applied Physics, 47*, 1–25.
6. Öztürk-Atar, K., Eroğlu, H., & Çalış, S. (2017). Novel advances in targeted drug delivery. *Journal of Drug Targeting, 23*, 1–10.
7. Fahmy, T. M., Fonga, P. M., Goyal, A., & Saltzman, W. M. (2005). Targeted for drug delivery. *Materials Today, 8*(8), 18–26.
8. Chowdhury, A., Kunjiappan, S., Panneerselvam, T., Somasundaram, B., & Bhattacharjee, C. (2017). Nanotechnology and nanocarrier-based approaches on treatment of degenerative diseases. *International Nano Letters, 7*, 91–122.
9. Singh, R., & Lillard, J. W., Jr. (2009). Nanoparticle-based targeted drug delivery. *Experimental and Molecular Pathology, 86*(3), 215–223.
10. Mukherjee, S., Ray, S., & Thakur, R. S. (2009). Solid lipid nanoparticles: A modern formulation approach in drug delivery system. *Indian Journal of Pharmaceutical Sciences, 71*(4), 349–358.
11. Yadav, N., Khatak, S., & Singh, U. V. S. (2013). Solid lipid nanoparticles: A review. *International Journal of Applied Pharmaceutics, 5*(2), 8–18.
12. Üner, M., & Yener, G. (2007). Importance of solid lipid nanoparticles (SLN) in various administration routes and future perspectives. *International Journal of Nanomedicine, 2*(3), 289–300.
13. Wissing, S. A., Kayser, O., & Muller, R. H. (2004). Solid lipid nanoparticles for parenteral drug delivery. *Advanced Drug Delivery Reviews, 56*(9), 1257–1272.
14. Pardeshi, C., Rajput, P., Belgamwar, V., Tekade, A., Patil, G., Chaudhary, K., & Sonje, A. (2012). Solid lipid based nanocarriers: An overview. *Acta Pharmaceutica, 62*, 433–472.
15. Müeller, R. H., Mäder, K., & Gohla, S. (2000). Solid lipid nanoparticles (SLN) for controlled drug delivery: A review of the state of the art. *European Journal of Pharmaceutics and Biopharmaceutics, 50*(1), 161–177.
16. Hu, L. D., Tang, X., & Cui, F. D. (2004). Solid lipid nanoparticles (SLNs) to improve oral bioavailability of poorly soluble drugs. *Journal of Pharmacy and Pharmacology, 56*, 1527–1535.
17. Geszke-Moritza, M., & Moritz, M. (2016). Solid lipid nanoparticles as attractive drug vehicles: Composition, properties and therapeutic strategies. *Materials Science and Engineering C, 68*, 982–994.
18. Nandini, P. T., Doijad, R. C., Shivakumar, H. N., & Dandagi, P. M. (2015). Formulation and evaluation of gemcitabine-loaded solid lipid nanoparticles. *Journal of Drug Delivery, 22*, 647–651.
19. Kalaycioglu, G. D., & Aydogan, N. (2016). Preparation and investigation of solid lipid nanoparticles for drug delivery. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 510*, 77–86.
20. Jie, K., Zhou, Y., Yao, Y., & Huang, F. (2015). Macrocyclic amphiphiles. *Chemical Society Reviews, 44*(11), 3568–3587.
21. Plachkova-Petrova, D., Petrova, P., Miloshev, S., & Novakov, C. (2012). Optimization of reaction conditions for synthesis C-tetramethylcalix[4]resorcinarene. *Bulgarian Chemical Communications, 3*, 208–215.
22. Yu, X., Trase, I., Ren, M., Duval, K., Guo, X., & Chen, Z. (2016). Design of nanoparticle-based carriers for targeted drug delivery. *Journal of Nanomaterials, 2016*, 1–15.
23. Motte, L., Billoudet, F., & Pileni, M. P. (1995). Self-assembled monolayer of nanosized particles differing by their sizes. *Journal of Physical Chemistry, 99*(44), 16425–16429.
24. Frankamp, B. L., Uzun, O., Ilhan, F., Boal, A. K., & Rotello, V. M. (2002). Recognition-mediated assembly of nanoparticles into micellar structures with diblock copolymers. *Journal of American Chemical Society, 124*(6), 892–893.
25. Pileni, M. P. (2001). Nanocrystal self-assemblies: Fabrication and collective properties. *Journal of Physical Chemistry-B, 105*(17), 3358–3371.
26. Collier, C. P., Vossmeyer, T., & Heath, J. R. (1998). Nanocrystal superlattices. *Annual Review of Physical Chemistry, 49*, 371–404.

27. Nicholas, A. K., & Paul, S. W. (2014). Self-assembly of nanoparticles: A snapshot. *ACS Nano, 8*(4), 3101–3103.
28. Martin Del Valle, E. M. (2003). Cyclodextrins and their uses: A review. *Process Biochemistry, 39*(9), 1033–1046.
29. Ryzhakov, A., Thi, T. D., Stappaerts, J., Bertoletti, L., Kimpe, K., Rodrigues, A. S. C., Saokham, P., Mooter, G. V., Augustijns, A., Somsen, G. W., Kurkov, S., Inghelbrecht, S., Arien, A., Jimidar, M. I., Schrijnemakers, K., & Loftsson, T. (2016). Self-assembly of cyclodextrins and their complexes in aqueous solutions. *Journal of Pharmaceutical Sciences, 105*, 2556–2569.
30. Hanumegowda, U. M., Wu, Y., & Adams, S. P. (2014). Potential impact of cyclodextrin containing formulations in toxicity evaluation of novel compounds in early drug discovery. *The Journal of Pharmacy and Pharmacology, 2*(1), 1–5.
31. Aburahma, M. H. (2016). Insights on novel particulate self-assembled drug delivery beads based on partial inclusion complexes between triglycerides and cyclodextrins. *Drug Delivery, 23*(7), 2205–2219.
32. Shahgaldian, P., Silva, E., & Coleman, A. W. (2003). A first approach to the study of calixarene solid lipid nanoparticle (SLN) toxicity. *Journal of Inclusion Phenomena and Macrocyclic Chemistry, 46*, 175–177.
33. Toma, H. E. (2000). Supramolecular chemistry and technology. *Anais da Academia Brasileira de Ciências, 72*(1), 5–25.
34. Busseron, E., Ruff, Y., Moulin, E., & Giuseppone, N. (2013). Supramolecular self-assemblies as functional nanomaterials. *Nanoscale, 5*, 7098–7140.
35. Ngurah, B. I. G. M., Jumina, J., Anwar, C., Sunardi, S., & Mustofa, M. (2017). Synthesis and in vitro evaluation of C-methylcalix[4]resorcinaryl octacinnamate and C-methylcalix[4] resorcinaryl octabenzoate as the sunscreen. *Indonesian Journal of Chemistry, 17*(1), 63–70.
36. Nicod, L., Chitry, F., Gaubert, F., & Lemaire, M. (1999). Application of water soluble resorcinarenes in nanofiltration-complexation with caesium and strontium as targets. *Journal of Inclusion Phenomena and Macrocyclic Chemistry, 34*, 141–151.
37. Pietraszkiewicz, M., Pietraszkiewicz, O., Uzig, E., Prus, P., Brzózka, Z., Woźniak, K., Bilewicz, R., Borowiak, T., & Mączyñski, M. (2000). Recent advances in calix[4]resorcinarene chemistry. *Journal Butlerov Communications, 3*, 55–65.
38. Hayashida, O., Mizuki, K., Akagi, K., Matsuo, A., Kanamori, T., Nakai, T., Sando, S., & Aoyama, Y. (2003). Macrocyclic glycoclusters. Self-aggregation and phosphate-induced agglutination behaviors of calix[4]resorcarene-based quadruple-chain amphiphiles with a huge oligosaccharide pool. *Journal of American Chemical Society, 125*(2), 594–601.
39. Durairaj, R. B. (2005). *Resorcinol: Chemistry, technology and applications* (p. 149). Germany: Springer.
40. Balasubramanian, R., Kim, B., Tripp, S. L., Wang, X., Lieberman, M., & Wei, A. (2002). Dispersion and stability studies of resorcinarene-encapsulated gold nanoparticles. *Langmuir, 18*, 3676–3681.
41. Hoegberg, A. G. S. (1980). Two stereoisomeric macrocyclic resorcinol-acetaldehyde condensation products. *Journal of Organic Chemistry, 45*(22), 4498–4500.
42. Tunstad, L. M., Tucker, J. A., Dalcanale, E., Weiser, J., Bryant, J. A., Sherman, J. C., Helgeson, R. C., Knobler, C. B., & Cram, D. J. (1989). Host-guest complexation. 48. Octol building blocks for cavitands and carcerands. *Journal of Organic Chemistry, 54*(6), 1305–1312.
43. Hoegberg, A. G. S. (1980). Stereoselective synthesis and DNMR study of two 1,8,15,22-T etraphenyl[1_4]metacyclophan-3,5,10,12,17,19,24,26-octols. *Journal of the American Chemical Society, 102*(19), 6046–6050.
44. Jain, V. K., & Kanaiya, P. H. (2011). Chemistry of calix[4]resorcinarenes. *Russian Chemical Reviews, 80*(1), 75–102.
45. Timmerman, P., Verboom, W., & Reinhoudt, D. N. (1996). Resorcinarenes. *Tetrahedron, 52*(8), 2663–2704.
46. Utomo, S. B., Jumina, J., Siswanta, D., Mustofa, M., & Kumar, N. (2011). Synthesis of thiomethylated calix[4]resorcinarene based on fennel oil via chloromethylation. *Indonesian Journal of Chemistry, 11*(1), 1–8.

47. Sardjonoa, R. E., Kadarohmana, A., & Mardhiyah, A. (2012). Green synthesis of some calix[4]resorcinarene under microwave irradiation. *Procedia Chemistry, 4*, 224–231.
48. Thoden van Velzen, E. U., Engbersen, J. F. J., De Lange, P. J., Mahy, J. W. G., & Reinhoudt, D. N. (1995). Self-assembled monolayers of resorcinarene tetrasulfides on gold. *Journal of the American Chemical Society, 117*, 6853–6862.
49. Bourgeois, J. M., & Stoeckli-Evans, H. (2005). Synthesis of new resorcinarenes under alkaline conditions. *Helvetica Chimica Acta, 88*(10), 2722–2730.
50. Gerkensmeier, T., Mattay, J., & Näther, C. (2001). A new type of calixarene: Octahydroxypyridine[4]arenes. *Chemistry A European Journal, 7*(2), 465–474.
51. Wang, M. (2008). Heterocalixaromatics, new generation macrocyclic host molecules in supramolecular chemistry. *Chemical Communication, 38*, 4541–4551.
52. Wang, M. (2012). Nitrogen and oxygen bridged calixaromatics: Synthesis, structure, functionalization and molecular recognition. *Accounts of Chemical Research, 45*(2), 182–195.
53. Iwanek, W., & Wzorek, A. (2009). Introduction to the chirality of resorcinarenes. *Mini-Reviews in Organic Chemistry, 6*, 398–411.
54. Hauke, F., Myles, A. J., & Rebek, J., Jr. (2005). Lower rim mono-functionalization of resorcinarenes. *Chemical Communication, 33*, 4164–4166.
55. Utomo, S. B., Saputro, A. N. C., & Rinanto, Y. (2016). Functionalization of C-4-methoxyphenylcalix[4]resorcinarene with several ammonium compounds. *IOP Science, 107*, 1–10.
56. Saito, S., Rudkevich, D. M., & Rebek, J., Jr. (1999). Lower rim functionalized resorcinarenes: Useful modules for supramolecular chemistry. *Organic Letters, 1*(8), 1241–1244.
57. Jayswal, K. P., Patel, J. R., Patel, V. B., & Patel, M. H. (2008). A new approach towards synthesis of some novel "upper rim" functionalized calix[4]resorcinarene Schiff-bases. *Acta Chimica Slovenica, 55*, 302–307.
58. Cram, D. J., Karbach, S., Kim, H., Knobler, C. B., Maverick, E. F., Ericson, J. L., & Helgeson, R. C. (1988). Host-guest complexation. 46. Cavitands as open molecular vessels form solvates. *Journal of the American Chemical Society, 110*(7), 2229–2237.
59. Osamu, M., Kazumichi, A., Tadahiko, N., & Seiji, S. (1990). Diazo-coupling with a resorcinol-based cyclophane: A new water-soluble host with a deep cleft. *Chemistry Letters, 19*(7), 1219–1222.
60. Fransen, J. R., & Dutton, P. J. (1995). Cation binding and conformation of octafunctionalized calix[4]resorcinarenes. *Canadian Journal of Chemistry, 73*, 2217–2223.
61. McIldowie, M. J., Mocerino, M., & Ogden, M. I. (2010). A brief review of C_n-symmetric calixarenes and resorcinarenes. *Supramolecular Chemistry, 22*(1), 13–39.
62. Castillo-Aguirre, A., Rivera-Monroy, Z., & Maldonado, M. (2017). Selective O-alkylation of the crown conformer of tetra(4-hydroxyphenyl)calix[4]resorcinarene to the corresponding tetraalkyl ether. *Molecules, 22*, 1–11.
63. Wiegmann, S., & Mattay, J. (2011). Inherently chiral resorcin[4]arenes: A new concept for improving the functionality. *Organic Letters, 13*(12), 3226–3228.
64. Boerrigter, H., Verboom, W., van Hummel, G. J., Harkema, S., & Reinhoudt, D. (1996). Selective functionalization of resorcinarene cavitands; single crystal X-ray structure of a distally functionalized cavitand. *Tetrahedron Letters, 37*(29), 5167–5170.
65. Irwin, J. L., & Sherburn, M. S. (2000). Optimized synthesis of cavitand phenol bowls. *Journal of Organic Chemistry, 65*, 5846–5848.
66. Barrett, E. S., Irwin, J. L., Turner, P., & Sherburn, M. S. (2001). Efficient distal-difunctionalization of cavitand bowls. *Journal of Organic Chemistry, 66*, 8227–8229.
67. Lukin, O. V., Pirozhenko, V. V., & Shivanyuk, A. N. (1995). Selective acylation of calixresorcinolarene. *Tetrahedron Letters, 36*(42), 7725–7728.
68. Shivanyuk, A., Paulus, E. F., Böhmer, V., & Vogt, W. (1998). Selective derivatizations of resorcarenes. 4. General methods for the synthesis of C_{2v}-symmetrical derivatives. *Journal of Organic Chemistry, 63*(19), 6448–6449.
69. Hisatoshi, K., Hidekazu, N., Hideki, N., Tsuyoshi, U., Kazuhiro, K., & Osamu, M. (1997). Regioselective distal-dibromination of calix[4]resorcinarene. *Chemistry Letters, 26*(2), 185–186.

70. Abis, L., Dalcanale, E., Du Vosel, A., & Spera, S. (1988). Structurally new macrocycles from the resorcinol-aldehyde condensation. Configurational and conformational analyses by means of dynamic NMR, NOE and T1 experiments. *Journal of Organic Chemistry, 53*, 5475–5479.
71. Shahgaldian, P., Da Silva, E., Coleman, A. W., Rather, B., & Zaworotko, M. J. (2003). Para-acyl-calixarene based solid lipid nanoparticles (SLNs): A detailed study of preparation and stability parameters. *International Journal of Pharmaceutics, 253*(1–2), 23–38.
72. Mora-Huertas, C. E., Garrigues, O., Fessi, H., & Elaissari, A. (2012). Nanocapsules prepared via nanoprecipitation and emulsification-diffusion methods: Comparative study. *European Journal of Pharmaceutics and Biopharmaceutics, 80*(1), 235–239.
73. Nik, A. M., Langmaid, S., & Wright, A. J. (2012). Nonionic surfactant and interfacial structure impact crystallinity and stability of β-carotene loaded lipid nanodispersions. *Journal of Agricultural and Food Chemistry, 60*(16), 4126–4135.
74. Gualbert, J., Shahgaldian, P., Lazar, A., & Coleman, A. W. (2004). Solid lipid nanoparticles (SLNs): Preparation and properties of calix[4]resorcinarene derived systems. *Journal of Inclusion Phenomena and Macrocyclic Chemistry, 48*(1–2), 37–44.
75. Siekmann, B., & Westesen, K. (1992). Submicron-sized parenteral carrier systems based on solid lipids. *Pharmaceutical and Pharmacological Letters, 1*, 123–126.
76. Mora-Huertas, C. E., Fessi, H., & Elaissari, A. (2011). Influence of process and formulation parameters on the formation of submicron particles by solvent displacement and emulsification-diffusion methods: Critical comparison. *Advances in Colloid and Interface Science, 163*(2), 90–122.
77. Salorinne, K., & Nissinen, M. (2006). Novel tetramethoxy resorcinarene bis-crown ethers. *Organic Letters, 8*(24), 5473–5476.
78. Helttmen, K., Salorinne, K., Barboza, T., Barbosa, H. C., Suhonen, A., & Nissinen, M. (2012). Cation binding resorcinarene bis-crowns: The effect of lower rim alkyl chain length on crystal packing and solid lipid nanoparticles. *New Journal of Chemistry, 36*(3), 789–795.
79. Iwanek, W., Urbaniak, M., Gawdzik, B., & Schurig, V. (2003). Synthesis of enantiomerically and diastereomerically pure oxazaborolo-benzoxazaborininone derivatives of resorcinarene from L-proline. *Tetrahedron: Asymmetry, 14*(18), 2787–2792.
80. Ehrler, S., Pieles, U., Wirth-Heller, A., & Shahgaldian, P. (2007). Surface modification of resorcinarene based self-assembled solid lipid nanoparticles for drug targeting. *Chemical Communication, 25*, 2605–2607.
81. Choi, S., Kim, W., & Kim, J. (2003). Surface modification of functional nanoparticles for controlled drug delivery. *Journal of Dispersion Science and Technology, 24*(3–4), 475–487.
82. Purcar, V., Somoghi, R., Nitu, S. G., Nicolae, C., Alexandrescu, E., Gîfu, I. C., Gabor, A. R., Stroescu, H., Ianchi, R., Căprărescu, S., & Cinteză, L. O. (2017). The effect of different coupling agents on nano-ZnO materials obtained via the sol–gel process. *Nanomaterials, 7*(439), 1–13.
83. Dalod, A. R. M., Henriksen, L., Grande, T., & Einarsrud, M. (2017). Functionalized TiO_2 nanoparticles by single-step hydrothermal synthesis: The role of the silane coupling agents. *Beilstein Journal of Nanotechnology, 8*, 304–312.
84. Wang, E. C., & Wang, A. Z. (2014). Nanoparticles and their applications in cell and molecular biology. *Integrative Biology, 6*(1), 9–26.
85. Sen, T., & Bruce, I. J. (2012). Surface engineering of nanoparticles in suspension for particle based bio-sensing. *Scientific Reports, 2*(564), 1–6.
86. Jie, K., Zhou, Y., Yaoa, Y., & Huang, F. (2015). Macrocyclic amphiphiles. *Chemical Society Reviews, 44*(11), 3568–3587.
87. Shahgaldian, P., Pieles, U., & Hegner, M. (2005). Enantioselective recognition of phenylalanine by a chiral amphiphilic macrocycle at the air-water interface: A copper-mediated mechanism. *Langmuir, 21*(14), 6503–6507.
88. Gualbert, J., Shahgaldian, P., & Coleman, A. W. (2003). Interactions of amphiphilic calix[4] arene-based solid lipid nanoparticles with bovine serum albumin. *International Journal of Pharmaceutics, 257*(1–2), 69–73.

89. Oyarzun-Ampuero, F., Kogan, M. J., Neira-Carrillo, A., & Morales, J. O. (2014). Surface-modified nanoparticles to improve drug delivery. In *Dekker encyclopedia of nanoscience and nanotechnology* (3rd ed., pp. 1–7). Abingdon: Taylor & Francis.
90. Holgado, M. A., Martin-Banderas, L., Alvarez-Fuentes, J., Fernandez-Arevalo, M. L., & Arias, J. (2012). Drug targeting to cancer by nanoparticles surface functionalized with special biomolecules. *Current Medicinal Chemistry, 19*(19), 3188–3195.
91. Ma, N., Ma, C., Li, C., Wang, T., Tang, Y., Wang, H., Mou, X., Chen, Z., & He, N. (2013). Influence of nanoparticle shape, size and surface functionalization on cellular uptake. *Journal of Nanoscience and Nanotechnology, 13*(10), 6485–6498.
92. Rizvi, S. A. A., & Saleh, A. M. (2018). Applications of nanoparticle systems in drug delivery technology. *Saudi Pharmaceutical Journal, 26*(1), 64–70.
93. Teixeira, M. C., Carbone, C., & Souto, E. B. (2017). Beyond liposomes: Recent advances on lipid based nanostructures for poorly soluble/poorly permeable drug delivery. *Progress in Lipid Research, 68*, 1–11.
94. Singhal, G. B., Patel, R. P., Prajapati, B. G., & Patel, N. A. (2011). Solid lipid nanoparticles and nano lipid carriers: A novel solid lipid based drug carrier. *International Research Journal of Pharmacy, 2*(2), 40–52.
95. Mariam, J., Sivakami, S., & Dongre, P. M. (2016). Albumin corona on nanoparticles: A strategic approach in drug delivery. *Drug Delivery, 23*, 2668–2676.
96. Guo, D. S., Wang, K., Wang, Y. X., & Liu, Y. (2012). Cholinesterase-responsive supramolecular vesicle. *Journal of the American Chemical Society, 134*(24), 10244–10250.
97. Wang, K., Guo, D. S., Wang, X., & Liu, Y. (2011). Multistimuli responsive supramolecular vesicles based on the recognition of p-sulfonatocalixarene and its controllable release of doxorubicin. *ACS Nano, 5*(4), 2880–2894.
98. Wang, A. Z., Langer, R., & Farokhzad, O. C. (2012). Nanoparticle delivery of cancer drugs. *Annual Review of Medicine, 63*(1), 185–198.
99. Duan, Q., Cao, Y., Li, Y., Hu, X., Xiao, T., Lin, C., Pan, Y., & Wang, L. (2013). pH-responsive supramolecular vesicles based on water-soluble pillar[6]arene and ferrocene derivative for drug delivery. *Journal of the American Chemical Society, 135*(28), 10542–10549.
100. Gallego-Yerga, L., Lomazzi, M., Sansone, F., Mellet, C. O., Casnatib, A., & Fernández, J. M. G. (2014). Glycoligand-targeted core-shell nanospheres with tunable drug release profiles from calixarene-cyclodextrin heterodimers. *Chemical Communication, 50*(56), 7440–7443.
101. Shivanyuk, A., & Rebek, J., Jr. (2001). Reversible encapsulation by self-assembling resorcinarene subunits. *PNAS, 98*(14), 7662–7665.
102. Liu, Z., Nalluri, S. K. M., & Stoddart, J. F. (2017). Surveying macrocyclic chemistry: From flexible crown ethers to rigid cyclophanes. *Chemical Society Reviews, 46*, 2459–2478.
103. Ma, X., & Zhao, Y. (2015). Biomedical applications of supramolecular systems based on host–guest interactions. *Chemical Reviews, 115*(15), 7794–7839.
104. Mody, V. V., Siwale, R., Singh, A., & Mody, H. R. (2010). Introduction to metallic nanoparticles. *Journal of Pharmacy and Bioallied Sciences, 2*(4), 282–289.
105. Ruckenstein, E., & Li, Z. F. (2005). Surface modification and functionalization through the self-assembled monolayer and graft polymerization. *Advances in Colloid and Interface Science, 113*(1), 43–63.
106. Pankhurst, Q. A., Thanh, N. T. K., Jones, S. K., & Dobson, J. (2009). Progress in applications of magnetic nanoparticles in biomedicine. *Journal of Physics D: Applied Physics, 42*(22), 1–15.
107. Ravindran, A., Chandran, P., & Khan, S. S. (2013). Biofunctionalized silver nanoparticles: Advances and prospects. *Colloids and Surfaces B: Biointerfaces, 105*, 342–352.
108. Sperling, R. A., & Parak, W. J. (2010). Surface modification, functionalization and bioconjugation of colloidal inorganic nanoparticles. *Philosophical Transactions Mathematical Physical & Engineering Sciences, 368*(1915), 1333–1383.
109. Yang, J., Lee, J. Y., & Ying, J. Y. (2011). Phase transfer and its applications in nanotechnology. *Chemical Society Reviews, 40*(3), 1672–1696.

110. Mayya, K. S., & Caruso, F. (2003). Phase transfer of surface-modified gold nanoparticles by hydrophobization with alkylamines. *Langmuir, 19*(17), 6987–6993.
111. Wei, Y., Yang, J., & Ying, J. Y. (2010). Reversible phase transfer of quantum dots and metal nanoparticles. *Chemical Communication, 46*(18), 3179–3181.
112. Yong, Y., Yan, S., Ying, H., & Chaoguo, Y. (2010). Preparation of resorcinarene-functionalized gold nanoparticles and their catalytic activities for reduction of aromatic nitro compounds. *Chinese Journal of Chemistry, 28*(5), 705–712.
113. Wei, A., Stavens, K. B., Pusztay, S. V., & Andres, R. P. (1999). Synthesis and characterization of resorcinarene-encapsulated nanoparticles. *MRS Proceedings, 581*, 59–63.
114. Kim, B., Balasubramanian, R., Pe'rez-Segarra, W., Wei, A., Decker, B., & Mattay, J. (2005). Self-assembly of resorcinarene-stabilized gold nanoparticles: Influence of the macrocyclic headgroup. *Supramolecular Chemistry, 17*(1–2), 173–180.
115. Hansen, M. N., Chang, L., & Wei, A. (2008). Resorcinarene-encapsulated gold nanorods: Solvatochromatism and magnetic nanoshell formation. *Supramolecular Chemistry, 20*(1–2), 35–40.
116. Ermakova, A. M., Morozova, J. E., Shalaeva, Y. V., Syakaev, V. V., Nizameev, I. R., Kadirov, M. K., Antipin, I. S., & Konovalov, A. I. (2017). The supramolecular approach to the phase transfer of carboxylic calixresorcinarene-capped silver nanoparticles. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 524*, 127–134.
117. Mishra, D., Kongor, A., Panchal, M., Modi, K., & Jain, V. (2018). Resorcinarene-embedded stable silver nanoparticles: A fluorescent nanoprobe for Pb(II) in water. *International Journal for Research in Applied Science & Engineering Technology, 6*(1), 1360–1370.
118. Sergeeva, T. Y., Aida, I., Samigullina, A. I., Gubaidullin, A. T., Irek, R., Nizameev, I. R., Kadirov, M. K., Mukhitova, R. K., Albina, Y., Ziganshina, A. Y., & Konovalov, A. I. (2016). Application of ferrocene-resorcinarene in silver nanoparticle synthesis. *RSC Advances, 6*(90), 87128–87133.
119. Tripp, S. L., Pusztay, S. V., Ribbe, A. E., & Wei, A. (2002). Self-assembly of cobalt nanoparticle rings. *Journal of the American Chemical Society, 124*(27), 7914–7915.
120. Zhou, J., Chen, M., & Diao, G. (2013). Assembling gold and platinum nanoparticles on resorcinarene modified graphene and their electrochemical applications. *Journal of Materials Chemistry A, 1*(6), 2278–2285.

Chapter 17
Targeted Delivery of Surface-Modified Nanoparticles: Modulation of Inflammation for Acute Lung Injury

Hiep X. Nguyen

Abstract Nanocarriers have been widely employed in the diagnosis and treatment of various diseases. The drug release kinetics and pharmacodynamics could be adjusted by changing the materials, designs, and physicochemical properties of the carriers. Furthermore, the carrier surface could be modified to minimize the particle clearance, increase the circulation duration, escape the biological protective mechanisms, penetrate through physical barriers, and prolong the residence of the drug at the target site. Among lung diseases, acute lung injury has been considered life-threatening with approximately 190,000 cases and 74,500 deaths per year in the USA. Numerous researches have reported the efficacy of drug-encapsulated nanoparticles in the treatment of acute lung injury. The use of nanoparticles could help minimize the effect of airway defenses in the lung, thus provides a prolonged retention, sustained drug release, and targeted delivery to the lung tissues. Meanwhile, the toxicity of nanoparticles in the lungs needs to be investigated thoroughly to alleviate the safety concerns. In this chapter, we discuss the targeted pulmonary delivery of surface-modified nanocarriers to efficiently treat acute lung injury.

Keywords Nanocarriers · Acute lung injury · Surface-modified nanoparticles Pulmonary delivery · Toxicity

H. X. Nguyen (✉)
Department of Pharmaceutical Sciences, College of Pharmacy, Mercer University, Atlanta, GA, USA
e-mail: Hiep.Xuan.Nguyen@live.mercer.edu

Y. V Pathak (ed.), *Surface Modification of Nanoparticles for Targeted Drug Delivery*, https://doi.org/10.1007/978-3-030-06115-9_17

1 Nanoparticles

1.1 Introduction of Nanoparticles

Targeted nanoparticles especially nano-sized drug delivery systems were first introduced into the field of medicine in the nineteenth century. In the 1960s, nanoparticles were developed for vaccination delivery [1]. From then on, nanotechnology has been furthered in numerous clinical trials and several products available on the market, particularly in the diagnosis and treatment of cancer [2, 3]. Also, a wide range of nanocarrier types has been fabricated and optimized as advanced drug delivery systems.

Nanoparticles have been fabricated from various macromolecular materials with the size ranging from 1 to 200 nm to drive therapeutic agents into the body tissue [4]. A wide range of diseases has been targeted with nanoparticles such as cancer [5] or tuberculosis [6]. Despite a long history of research and development, the number of marketed products with nanoparticles remains limited. The first commercial product was Abraxane® (available in 2005), which was formulated as an injectable albumin nanoparticle suspension with paclitaxel to treat cancer [7] (Table 17.1).

1.2 Properties of Nanoparticles

Nanoparticles have been commonly used as drug carriers where the active therapeutical ingredients could be dissolved, entrapped, encapsulated, adsorbed, or attached to the particles using various fabrication methods including solvent evaporation, nanoprecipitation, or multiple emulsions [7–9]. The drugs can be retained in the particles with covalent, electrostatic interactions or the like [10]. These solid colloidal nano-sized particles are constructed with biocompatible and biodegradable materials that decompose at a certain rate in the body. The degradation process of these materials could be adjusted to alter the drug release and the physicochemical properties of the nanoparticles [11].

The circulation, absorption, and elimination of nanoparticles in the human body vary depending on the properties of the particles and the targeted tissues. Blood–brain barrier allows nanoparticles with size less than 1 nm to efficiently pass through while nanoparticle of 6 nm dimension could penetrate the continuous capillaries in muscles, lungs, and skin tissue. With the size range from 40 to 60 nm, nanoparticles could escape the fenestrated capillaries in kidney, intestine, and endocrine or exocrine glands [12]. There has been found agglomeration of large nanoparticles (size of more than 600 nm) in liver, spleen, and bone marrow [13]. The electrostatic properties of nanoparticles could be employed to facilitate or inhibit the particle endocytosis. Positively charged nanoparticles could be attached rapidly to cells with the negatively charged surface [12]. Nanoparticles have long been used as imaging agents and drug carriers due to their numerous advantages such as (1) large

Table 17.1 List of approved nanopharmaceuticals

Brand name	Active ingredient	Properties	Indication	Provider	Approval
Liposomes					
AmBisome®	Amphotericin B	Amphotericin B encapsulated in liposomes for injection	Systemic fungal infections (IV)	Sigma-Tau Pharmaceuticals, Inc.	FDA 1997
DaunoXome®	Daunorubicin	Daunorubicin citrate-encapsulated liposome for injection	HIV-related Kaposi's sarcoma (IV)	Galen US Inc.	FDA 1996
DepoCyt®	Cytarabine	Cytarabine-encapsulated in multivesicular liposomes for injection	Lymphomatous malignant meningitis (IV)	Sigma-Tau Pharmaceuticals, Inc	FDA 1999/2007
DepoDur®	Morphine Sulfate	Morphine sulfate-encapsulated in multivesicular extended-release liposomes for injection	For the treatment of pain following major surgery	Endo Pharmaceuticals Inc.	FDA 2004
Doxil®	Doxorubicin hydrochloride	Doxorubicin hydrochloride encapsulated in Stealth® liposomes	AIDS-related Kaposi's sarcoma, multiple myeloma, ovarian cancer (IV)	ALZA Corporation	FDA 1995
Inflexal® V	Influenza vaccine	Influenza virus antigens on liposomes	Influenza vaccine	Crucell (former Berna Biotech Ltd.)	Switzerland 1997
Marqibo®	Vincristine sulfate	Vincristine sulfate encapsulated in liposomes	Acute lymphoid leukemia, Philadelphia chromosome-negative, relapsed or progressed (IV)	Talon Therapeutics, Inc.	FDA 2012
Mepact™	Mifamurtide	Mifamurtide incorporated into large multilamellar liposomes	Nonmetastasizing resectable osteosarcoma (IV)	Takeda France SAS	Europe 2009
Myocet®	Doxorubicin hydrochloride	Doxorubicin encapsulated in oligolamellar liposomes	The first line treatment of metastatic breast cancer in adult women (IV)	Teva UK Limited	Europe 2000
Visudyne®	Verteporfin	Verteporfin in liposomes for injection	Photodynamic therapy of wet age-related macular degeneration, pathological myopia, ocular histoplasmosis syndrome (IV)	Novartis AG	FDA 2000

(continued)

Table 17.1 (continued)

Brand name	Active ingredient	Properties	Indication	Provider	Approval
Nonliposomal lipid-based formulations					
Abelcet®	Amphotericin B	Amphotericin B complexed with two phospholipids (l-α-dimyristoyl phosphatidyl choline and l-α-dimyristoyl phosphatidyl glycerol)	The treatment of invasive fungal infections in patients who are refractory to or intolerant of conventional amphotericin B therapy	Exelead, Inc.	FDA 1995 and 1996
Amphotec®	Amphotericin B	Complex of amphotericin B and cholesteryl sulfate	The treatment of invasive aspergillosis	InterMune, Inc.	FDA 1996
PEGylated proteins, polypeptides, aptamers					
Adagen®	Pegademase bovine	PEGylated adenosine deaminase	Adenosine deaminase deficiency—severe combined immunodeficiency disease	Leadiant Biosciences, Inc.	FDA 1990
Cimzia®	Certolizumab pegol	PEGylated antibody	Crohn's disease, rheumatoid arthritis	UCB, Inc.	FDA 2008
Neulasta®	Pegfilgrastim	PEGylated filgrastim	Febrile neutropenia, In patients with nonmyeloid malignancies; prophylaxis (SC)	Amgen Inc	FDA 2002
Oncaspar®	Pegaspargase	PEGylated L-asparaginase	Acute lymphoblastic leukemia	Sigma-Tau Pharmaceuticals, Inc	FDA 1994
Pegasys®	Peginterferon Alfa-2a	PEGylated interferon alfa-2b	Hepatitis B and C	F. Hoffmann-La Roche AG	FDA 2002
PegIntron®	Peginterferon alfa-2b	PEGylated interferon alfa-2b	Hepatitis C	Schering-Plough Corporation	FDA 2001
Somavert®	Pegvisomant	PEGylated human growth hormone receptor antagonist	Acromegaly, second-line therapy	Pharmacia and Upjohn Company, LLC	FDA 2003
Macugen®	Pegaptanib Sodium	PEGylated anti-VEGF aptamer	Intravitreal Neovascular age-related macular degeneration	Valeant Pharmaceuticals International, Inc.	FDA 2004
Mircera®	Methoxy polyethylene glycol-epoetin beta	PEGylated epoetin beta	Anemia associated with chronic renal failure in adults	F. Hoffmann-La Roche AG	FDA 2007

Brand name	Active ingredient	Properties	Indication	Provider	Approval
Nanocrystals					
Emend®	Aprepitant	Aprepitant as nanocrystal	Emesis, antiemetic	Merck	FDA 2003
Megace ES®	Megestrol acetate	Megestrol acetate as nanocrystal	Anorexia, cachexia	Endo Pharmaceuticals	FDA 2005
Rapamune®	Sirolimus	Rapamycin (sirolimus) as nanocrystals formulated in tablets	Immunosuppressant	PF Prism C.V.	FDA 2002
Tricor®	Fenofibrate	Fenofibrate as nanocrystals	Hypercholesterolemia, hypertriglyceridemia	AbbVie Inc.	FDA 2004
Triglide®	Fenofibrate	Fenofibrate as insoluble drug-delivery microparticles	Hypercholesterolemia, hypertriglyceridemia	Skye Pharma AG	FDA 2005
Polymer-based nanoformulations					
Copaxone®	Glatiramer acetate	Polypeptide consist of four amino acids	Multiple sclerosis (SC)	Teva Pharmaceuticals USA	FDA 1996/2014
Eligard®	Leuprorelin acetate	Leuprolide acetate incorporated in nanoparticles	Advanced prostate cancer (SC)	Tolmar Therapeutics	FDA 2002
Genexol®	Paclitaxel	Paclitaxel incorporated in micelles	Metastatic breast cancer, pancreatic cancer (IV)	Samyang Biopharma	South Korea 2001
Opaxio®	Paclitaxel poliglumex	Paclitaxel covalently linked to solid nanoparticles	Glioblastoma	Cell Therapeutics, Inc.	FDA 2012
Renagel®	Sevelamer hydrochloride	Cross-linked poly allylamine hydrochloride	Hyperphosphatemia	Sanofi Genzyme	FDA 2000
Protein–drug conjugates					
Abraxane®	Paclitaxel	Paclitaxe-encapsulated nanoparticles	Metastatic breast cancer, non-small-cell lung cancer (IV)	Abraxis BioScience	FDA 2005
Kadcyla®	Ado-Trastuzumab Emtansine	Immunoconjugate	Metastatic breast cancer	Genentech	FDA 2013

(continued)

Table 17.1 (continued)

Brand name	Active ingredient	Properties	Indication	Provider	Approval
Ontak®	Denileukin diftitox	Recombinant fusion protein of fragment A of diphtheria toxin and subunit binding to interleukin-2 receptor	Primary cutaneous T-cell lymphoma, CD25-positive, persistent or recurrent disease	Eisai Inc.	FDA 1994/2006
Surfactant-based nanoformulations					
Fungizone®	Amphotericin B	Lyophilized powder of amphotericin B with added sodium deoxycholate	Systemic fungal infections (IV)	Bristol-Myers Squibb	FDA 1966
Diprivan®	Propofol	Oil-in-water emulsion of propofol	Sedative–hypnotic agent for induction and maintenance of anesthesia (IV)	Fresenius Kabi USA	FDA 1989
Estrasorb™	Estradiol hemihydrate	Emulsion of estradiol	Hormone replacement therapy during menopause	Exeltis USA, Inc.	FDA 2003
Metal-based nanoformulations					
Feridex®	Ferumoxides	Dextran-coated superparamagnetic iron oxide nanoparticles	Liver/spleen lesion magnetic resonance imaging (IV)	AMAG Pharmaceuticals, Inc.	FDA 1996
Feraheme™ (Ferumoxytol)	Ferumoxytol	Dextran-coated superparamagnetic iron oxide nanoparticles	Treatment of iron deficiency anemia in adults with chronic kidney disease	AMAG Pharmaceuticals, Inc.	FDA 2009
NanoTherm®	Iron oxide	Aminosilane-coated superparamagnetic iron oxide nanoparticles	Local ablation in glioblastoma, prostate, and pancreatic cancer	MagForce AG	Europe 2013
Virosomes					
Gendicine®	Gene therapy	A recombinant adenovirus engineered to express wild-typep53	Treat patients with tumors which have mutated p53 genes. Head and neck squamous cell carcinoma	Shenzhen SiBiono GeneTech	People's Republic of China 2003
Rexin-G®	Gene therapy	Gene inserted into the retroviral core of viral genes	For all solid tumors	Epeius Biotechnologies	Philippines 2007

FDA US Food and Drug Administration, *IV* intravenous, *SC* subcutaneous

surface-volume ratio, (2) biological mobility, (3) enhanced tissue penetration, (4) drug protection against degradation or loss, (5) sustained and controlled drug release, (6) reduction of dose frequency, (7) improved patient compliance, and (8) increased drug level at the target site [7, 14, 15].

1.3 Surface-Modified Nanoparticles

These days, nanoparticle surface and dimensions have been modified to minimize the particle clearance, increase the circulation time, escape the biological protective mechanisms, penetrate through physical barriers, and prolong the residence of the drug at the target site [7]. Thus, various moieties have been utilized for modification and functionalization of the nanoparticle surface in accord with different stimuli [12].

Nanoparticles could be stimulated by endogenous or exogenous factors. The endogenous stimulus includes redox, enzyme, and pH while light, ultrasound, and magnetic fields have been employed as exogenous factors to manipulate the behavior of nanoparticles [16]. Surface modification and polymeric coating of nanocarriers could allow altering the half-life, biocompatibility, biodistribution, circulation duration, stimuli reactivity, and therapeutic application [10, 17]. Furthermore, the hydrophilicity of nanoparticles was found to primarily determine the rate of particle binding on blood components. Hydrophobic nanoparticles without surface functionalization have been indicated to be eliminated rapidly whereas the circulation was significantly enhanced as these particles were coated with hydrophilic polymers or surfactant to increase the hydrophilicity [18] (Fig. 17.1).

Controlled and sustained drug release from nanoparticles is critical to the therapeutic efficacy. The drug release can be triggered by stimuli or occur in sustained mode over a certain period of time [10]. The drugs could diffuse out of the particles or the particles might slowly and gradually degrade to release the drug load. The

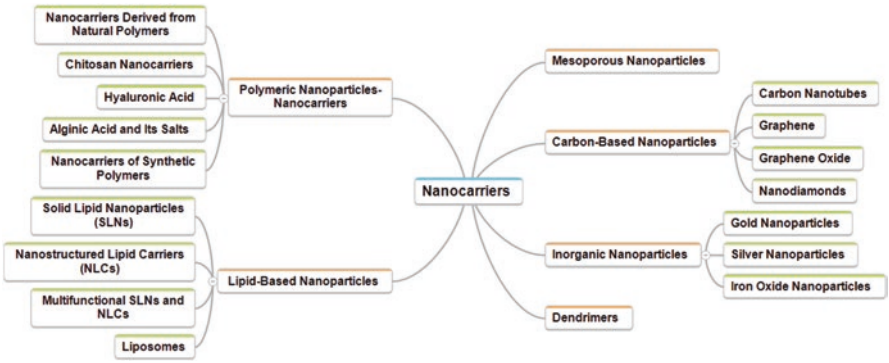


Fig. 17.1 Types of nanocarriers. *Source:* Author's representation

stimuli-responsive release of drug from nanoparticles may facilitate the drug accumulation in the targeted tissues. These stimuli could be generated by a modification in the biological environment including cell environment, pH alteration, and disease-related enzymes or external physical forces such as light, ultrasound, heat, electrical, and magnetic fields. Ultrasound-sensitive microbubbles have been employed to release therapeutic agent to the local targets [19]. Microbubbles fabricated from lung surfactants caused a significant enhancement in drug targeted deposition as compared to lipid-only microbubbles [20]. Interestingly, the application and control of magnetic fields could drive aerosol droplets encompassing superparamagnetic iron oxide to the targeted locations in the mice's lungs in vivo [21].

1.4 Potential Toxicity of Nanoparticles

A comprehensive understanding of biocompatibility, biodistribution, and degradation of nanoparticles is expected to properly utilize the particles [10]. The physical properties including geometry, dimensions, surface charge, and morphology have been found to alter the therapeutic effects of nanoparticles [22]. In particular, rod-shaped particles were more toxic than spherical particles [23]. Long fibers could less likely be captured by macrophages, thus minimize their elimination from the system and cause inflammation [24]. Surface functionalization of nanoparticles could change their biodistribution, effectiveness, and toxicity. Specifically, biopersistent carbon nanotubes could be modified and functionalized to enhance the hydrophilicity and rapid clearance via renal excretion [25]. A small change in the physicochemical properties of nanoparticles could be exaggerated into significant alteration in the biological response. Thus, the biological safety of nanoparticles needs to be evaluated and monitored with care [10].

1.5 Conclusion

Nano-sized carriers are a promising platform for controlled drug delivery due to their dimensions, permeability, and drug loading. Furthermore, surface functionalization could be used to enhance the targetability as well as to reduce the toxicity [12]. The nanomaterials could be coated or conjugated with various segments to prepare multifunctional and stimuli-responsive drug delivery system [12] which allows to modify drug release profile, specific targeting, compatibility, and several other advantages. More and more clinically effective nanocarriers are expected in the market in the future.

2 Acute Lung Injury

2.1 Pulmonary Drug Delivery

The lung offers several beneficial properties for rapid and efficient drug delivery due to the large alveolar surface area, a thin layer of the epithelial barrier, extensive blood circulation, avoidance of the first-pass hepatic metabolism, and low proteolytic activity in the alveolar space [26–29]. The metabolic activities in the lung are significantly lower than that in the gastrointestinal tract and the liver [10]. These properties not only enhance the systemic drug delivery but also improve the efficacy of treatment of lung diseases. Furthermore, drug administration via the lung has been preferred due to its noninvasiveness and the possibility for self-administration.

Despite the aforementioned advantages, there are only limited products on the market for lung delivery. The only inhalable product of therapeutic protein on the market is Pulmozyme® (dornase alfa, Genentech Inc., San Francisco, CA, USA). Exubera (Inhalable insulin, Pfizer, New York, NY, USA) was approved by US Food and Drug Administration in 2006, but later withdrawn from the US market in 2007 due to the deficiency of consumer demand.

The lung has an effective mechanism of clearing external agent, thus making it challenging to deposit drugs in a region in the lung. Inhalable particles could be eliminated from the lung by two mechanisms. Firstly, particles can be cleared by the moving patches of mucus layer in the conducting zone of the lung. The clearance would be more efficient in lung diseases with an increase in mucus production and thickness [10]. Secondly, in the deep lung, macrophages (on the air side of the alveolar cells) could engulf and digest to remove insoluble particles rapidly.

2.2 Introduction of Acute Lung Inflammation

An acute lung injury (ALI) is a disease in which the lungs could not sufficiently provide oxygen to the body, causing low levels of oxygen in the blood (hypoxemia). The leading causes of ALI have been found to be pneumonia, sepsis, lung trauma, burns, near drowning, and other condition related to inflammation or damage to the lungs. As first described in 1967, Acute lung injury (ALI) was diagnosed with acute respiratory distress, cyanosis refractory to oxygen therapy, decrease lung compliance, and diffuse infiltrates on chest radiography [30]. In 1988, this definition was expanded to encompass the level of positive end-expiratory pressure, the ratio of the partial pressure of arterial oxygen to the fraction of inspired oxygen, the static lung compliance, and the degree of infiltration evident on chest radiography [31]. Later, the lung injury score was included to evaluate the severity level of the disease in clinical trials [32]. American-European Consensus Conference Committee proposed a new definition that classifies the severity of lung injury and segregates the patients into two groups:acute lung injury (less severe hypoxemia) and acute respiratory distress syndrome (more severe hypoxemia) [33].

Recent surveys reveal that approximately 190,000 cases of ALI with 74,500 deaths per year in the US [34]. This life-threatening disease has been influencing millions of people every year over the world [35]. The in-hospital mortality rate of ALI was estimated to be 38.5% [34], which could be reduced to 31% using low-tidal volume ventilation [36, 37]. The primary cause of death was attributed to sepsis or multiple organ dysfunction syndromes.

2.3 Mechanism and Properties of Acute Lung Injury

ALI could occur due to either direct or indirect mechanical, toxic, infectious, or inflammatory challenges to the lung [38]. The most common cause of ALI was severe pulmonary sepsis, following by trauma, aspiration, multiple blood transfusion, acute pancreatitis, inhalation injury, and drug toxicity [39].

The interaction and communication between different cell types have been investigated to discover the pathology of ALI. Mechanism of ALI most likely relates to cell death in necrosis and apoptosis [35]. In lung injury, cell death could occur based on oncosis [40], cathepsin-dependent cell death, or autophagy. A wide range of cell types is associated with ALI such as alveolar epithelial and vascular endothelial cells (change in permeability to cause edema formation and alveolocapillary injury) and platelets and immune cells (inflammatory response) [41–44]. ALI is involved in a prothrombotic and antifibrinolytic shift that facilitate fibrin deposition, thus, advance the inflammation [45].

ALI and ARDS are represented by bilateral exudative chest infiltrate which could be diagnosed by roentgenograms [44, 46]. ALI can progress rapidly from an initial stage of a leaky edematous lung to a proliferative phase with deposition of fibrin and a reduction in respiratory compliance, and to a fibrotic phase with scarring lung. The hallmarks of ALI—protein extravasation and formation of lung edema—were caused by excessive inflammation, alteration in coagulation and fibrin deposition, and increase in permeability of the alveolocapillary barrier [38].

3 Nanoparticles to Treat Acute Lung Injury

3.1 Advantages of Nanoparticles for Lung Delivery

The large surface area of alveolar, a thin layer of epithelial barrier, and a dense network of blood vessels facilitate the delivery of various therapeutic agents [47]. The use of nanoparticles could help to minimize the effect of airway defenses in the lung. Once delivered to the lungs, nanoparticles could have a prolonged retention, sustained drug release, and targeted delivery to the lung tissues [10, 48]. Extensive studies have been conducted for the pulmonary delivery of polymeric nanoparticles for various compounds including asthmatic drugs [49, 50], antituberculosis drugs [51],

and anticancer drugs [52, 53]. Also, liposomal nanoformulations have been investigated and evaluated to be a promising platform for drug delivery. Currently, multiple liposomal formulations are FDA-approval products available on the market while several others are studied in clinical trials [54]. Liposome has a major advantage of being fabricated from compounds compatible and endogenous to the lung (such as lung surfactants), thus becoming a preferred pulmonary drug delivery system. Some marketed liposomal products for the treatment of acute respiratory distress syndrome are Exosurf® (Colfosceril Palmitate, GlaxoSmithKline, Brentford, UK) and Alveofact® (Bovactant, Lyomark Pharma, Oberhaching, Germany) [55]. Budesonide-encapsulated liposomes have been developed to deliver the drug at a controlled release rate to maintain the therapeutic concentrations in rat lungs in vivo. This liposomal formulation also helped to reduce the systemic exposure and toxicity [56]. Interestingly, multiple drugs could be combined and delivered to the lung simultaneously using nanocarrier systems. Nanoparticles offer several benefits as they could penetrate the lung more deeply and enter the alveolar areas [10]. Furthermore, nanoparticle-based systems evade macrophage clearance effectively and permeate the lung epithelium. Also, the surface of nanoparticles could be modified and functionalized to enhance the drug bioavailability, to improve the penetration into the mucus layer, and aid targeted delivery [10]. The deposition of nanocarriers could be prolonged by using mucoadhesive materials, for instance, biodegradable polysaccharide chitosan [7]. Yamamoto and colleagues fabricated peptide elcatonin-encapsulated PLGA nanoparticles whose surface was modified with chitosan. Once delivered to the lungs of guinea pigs, the particles caused a significant decrease in blood calcium levels as compared to the initial concentrations. Furthermore, this effect was prolonged and sustained up to 24 h (due to the slow elimination of chitosan-modified particles), which was markedly longer than unmodified particles [57].

3.2 Surface-Modified Nanoparticles for Lung Delivery

The use of surface-modified nanoparticles is beneficial for lung delivery. Brush-shaped nanoparticles have been formed with low molecular weight poly(ethylene glycol) chains (PEG) for a reduction of phagocytosis [58]. Furthermore, PEGylated nanocarriers were found to easily and readily penetrate the mucus layer in chronic obstructive lung diseases [59]. In contrary, chitosan could be used to modify the particle surface to enhance the mucoadhesion and circulation. Thus, chitosan-modified nanoparticles could reside for a longer period at the targeted site to improve the drug uptake, bioavailability, and therapeutic efficacy. This property is particularly desirable for the treatment of nonobstructive lung diseases including allergy and lung cancer [10]. Interestingly, biological fluids could modify the surface of particles to form protein corona whose properties are enhanced as compared to the original particles [60]. Lung surfactant phospholipids have been used to coat nanoparticles to alleviate toxicity as well as to improve cellular uptake [61]. Furthermore, the agglomeration of the particles helps create large agglomerates to be digested by macrophages [62] (Table 17.2).

Table 17.2 Surface-modified nanoparticles for acute lung injury

Nanoparticles	Effect	Mechanism	Study model	References
Polydopamine nanoparticles (similar to melanin)	Anti-inflammation therapeutic effect on acute inflammation-induced injury	Polydopamine with enriched phenol groups functioned as a radical scavenger to eliminate reactive oxygen species	Murine models	[63]
Lung-targeting functionalized nano-sterically stabilized unilamellar liposomes loaded with glucocorticoids methylprednisolone	Good particle size distribution, morphology, encapsulation efficiency, and high specificity to the lung.	Reduce the levels of TNF-α, IL-8, and TGF-β1 in rat bronchoalveolar lavage fluid and the expression of NK-κB in the lung tissues, thus alleviate lung injuries and enhance rat survival	Rat	[64]
Generation IV polyamidoamine dendrimers	The treatment of ischemia-reperfusion-induced acute lung injury	Dendrimers were taken up in epithelial cells and macrophages.	Ex vivo rabbit model	[65]
Dry powder formulations of inhalable apigenin-loaded bovine serum albumin nanoparticles	Good aerodynamic properties of the particles and antioxidant activity of encapsulated apigenin	Novel delivery system against lung injury with potential antioxidant activity		[66]
Two-component co-spray dried DMF:D-Man DPIs with high load of dimethyl fumarate	Capability to reach lower airways to treat inflammation in pulmonary diseases	Exhibit excellent aerosol dispersion performance with a human DPI device	In vitro predictive lung deposition modeling	[67]
Human amniotic fluid stem cells labeled with dual-polymer-coated UCNP-PEG-PEI nanoparticles	Display remarkable positive effects on ALI-damaged lung tissue repair.	Recover the integrity of the alveolar-capillary membrane, attenuate transepithelial leukocyte and neutrophil migration, and down-regulate proinflammatory cytokine and chemokine expression	A murine model of acute lung injury	[68]

(continued)

Table 17.2 (continued)

Nanoparticles	Effect	Mechanism	Study model	References
Poly-lactic-co-glycolic acid nanoparticles-facilitated cDNA delivery	Upregulate pulmonary EpoR expression and downstream signal transduction in rats for 21 days, Attenuate hyperoxia-induced damage in lung tissue based on apoptosis, oxidative damage of DNA, protein and lipid, tissue edema, and alveolar morphology	Targeted pulmonary EpoR upregulation mitigates acute oxidative lung damage	Rat lungs	[69]
Nanoparticles based on polyethyleneimine and DNA	When β2-Adrenergic Receptor (β2AR) was applied as the therapeutic gene, PEI/β2AR treatment significantly attenuated the severity of ALI. PEI/DNA nanoparticles could be an efficient agent in ALI treatment.	Attenuate alveolar fluid clearance, lung water content, histopathology, bronchioalveolar lavage cellularity, protein concentration, and inflammatory cytokines in mice with preexisting ALI	Mouse model of ALI induced by lipopolysaccharide	[70]
Shell cross-linked knedel-like polymer nanoparticles	The $K(d)$ values of the nanoparticle-attached PNAs were about an order of magnitude greater than the free PNAs	Recognize and selectively inhibit of mRNA sequences for inducible nitric oxide synthase (iNOS), which are overexpressed at sites of inflammation		[71]

3.3 Factors Affecting Nanoparticles Delivery to the Lung

The deposition and distribution of nanoparticles in the lungs vary depending on several factors such as breathing rate, lung volume, air flow, and particle size [10]. Multiple studies have suggested that particle size plays the most important role in manipulating the distribution and deposition of the particles in the lung. Small particles (size range from 1 to 5 μm) are deposited in the deep regions while inhaled particles (whose size is larger than 10 μm) are found primarily in the oropharyngeal region [72, 73]. A requirement for lung delivery is the proper design of the carrier systems [74]. Pulmonary delivery of inhaled particles is dominated by various factors such as particle size, particle density, and the mass median aerodynamic diameter in which the particle size could guarantee a maximum distribution and deposition of the particle in the deep lung [75]. The rate of clearance is primarily affected by the particle size in the alveolar region. Several studies have been performed to investigate the interaction between nanoparticles and macrophages. Large particle (aerodynamic diameter more than 6 μm) are exhaled without being phagocytosed [76], microparticles (aerodynamic diameter 1–5 μm) are effectively taken up by macrophages, while nanoparticles (aerodynamic diameter less than 200 nm) could penetrate the cellular barrier and the particle phagocytosis by alveolar macrophages can be reduced [48, 77, 78]. These indicate that nanoscale particles could avoid macrophage clearance while being deposited in the deep lung, especially the alveolar regions [10]. However, small particles require a high level of energy for fabrication and disaggregation. Inhaled particles could be deposited in the lung by inertial impaction, sedimentation, and diffusion. The particle deposition is analogous to the settling of spherical particles under gravity force through the air. Nanoparticles, which is not deposited in the lungs, is exhaled to result in a major loss of the delivered dose [79].

3.4 Pulmonary Delivery of Nanoparticles Using Dry Powder Carriers

Proper dry powder formulations and carrier systems have been developed and optimized to deposit nanoparticles to the alveolar regions to enhance the efficacy of their pulmonary delivery [80]. Kawashima and coworkers employed hydroxypropylmethyl cellulose phthalate (HPMCP) nanospheres (hydrophilic nanoparticles) to facilitate the inhalation properties of dry powder pranlukast hydrate [81]. The authors mixed the surface nanospheres and drug powder with lactose. Thus, in the in vitro inhalation test, the emission and dispersibility of surface-modified drug powder increased significantly and the powder was effectively delivered to the deep lung. Kawashima et al. fabricated insulin-loaded PLGA nanospheres. Then, an

ultrasonically assisted nebulizer was utilized to deliver the nanospheres to the trachea of guinea pigs. The authors reported a significant decrease in the blood glucose and a sustained hypoglycemic effect for 48 h. The results were attributed to the controlled release of insulin from the nanospheres and their deposition in multiple regions of the lung [82]. Tsapis and coworkers employed a spray drying method to manufacture large porous particles. Using 1,2-dipalmitoyl-sn-glycero-3-phosphocholine and 1,2-dimyristoyl-sn-glycero-3-phophoethanolamine (surfactants), and lactose, the authors could tailor the physicochemical properties of the spray-dried powder for pulmonary delivery [78]. Interestingly, nanoparticles could be loaded in a carrier matrix. Sham and colleagues dissolved lactose in nanoparticle suspension before spray drying to obtain nanoparticles-incorporated carrier powder. The authors observed a marked change in the particle size and reported the possibility of manipulating the delivery and release of nanoparticles [80]. In another study, Grenha et al. used lactose and mannitol together with a spray drying method to prepare microspheres, which contain insulin-incorporated nanoparticles for pulmonary drug delivery. The drug release from nanoparticles was found to be unaffected by the microencapsulation. Furthermore, this system allows to effectively deliver macromolecules via pulmonary administration [47]. The spray drying technique was also employed by Ely and colleagues to prepare effervescent carrier particles which incorporate ciprofloxacin-loaded nanoparticles. The authors could manage to alter the particle size to maximize the particle deposition in the deep lung. The use of effervescent carrier particles resulted in a marked enhancement in the drug release. Also, they observed an insignificant change in the nanoparticles dimension upon being released from the effervescent carrier particles [74]. Pulmonary administration is a promising platform to deliver nanoparticles carried in dry powders. The nebulization parameters of the matrix should be optimized to minimize particle aggregation and to facilitate the drug delivery into the deep lungs [83].

3.5 Pulmonary Delivery of Nanoparticle Suspensions Using Nebulization

Nanoparticles could be delivered by spraying or nebulizing the nanoparticle suspension. Dailey and coworkers formulated a surfactant-free nanoparticle suspension for pulmonary drug delivery. This system could provide a high encapsulation efficiency due to the electrostatic interactions between the drug molecules and the particles. The authors reported that the use of anionic diethylaminopropyl amine-poly(vinyl alcohol)-grafted-poly(lactide-co-glycolide)-contained formulation and an increase in the amount of carboxy methyl cellulose helped to minimize the particle aggregation [84]. Also, Yamamoto et al. prepared and modified the surface of PLGA nanospheres using chitosan to enhance the delivery efficacy of calcitonin to the lung. After the administration of chitosan-modified PLGA nanoparticles to the trachea of

guinea pigs, the blood calcium was reported to decrease by 80%. Furthermore, the therapeutic level of the drug was sustained for 24 h, which was markedly longer than the unmodified particles. This result could be explained by the mucoadhesion of the nanoparticles to the bronchial mucous and local tissue in the lung as well as the prolonged drug release from the particles. Moreover, chitosan could enhance the drug permeability by loosening the intercellular tight junctions [57]. In another study, itraconazole-loaded nanoparticles were fabricated, dispersed in aqueous media, and nebulized to the murine lung in vivo. This local delivery system led to a high drug concentration in the lung, and a decreased possibility of adverse effects [85]. Thus, nebulizing nanoparticle suspensions is a promising technique to deliver therapeutical agents to the lung. The physicochemical stability of the suspension needs to be maintained for clinical efficacy.

3.6 Local Delivery of Nanoparticles to the Lung

The local delivery of nanoparticles allows to enhance, sustain, and control the drug level at the target site to treat respiratory diseases [7]. Also, this targeted delivery could reduce the required dose, avoid the drug degradation in the gastrointestinal tract (oral administration), and minimize the systemic toxicity. Vaughn and colleagues delivered itraconazole-loaded nanoparticles to treat fungal infections of *Aspergillus fumigatus*. The pulmonary delivery of itraconazole nanoparticles to mice in vivo showed a significantly high and sustained drug concentration in the lung tissues while the drug level in serum was controlled to maximize the treatment efficacy as well as to reduce the possible systemic toxicity [86]. In addition to sustained drug concentrations in the lung, target delivery to certain cells or tissues has been found beneficial. For example, various therapeutic compounds including rifampicin, isoniazid, and pyrazinamide were formulated to target the drug delivery to alveolar macrophages to optimize the treatment of pulmonary tuberculosis [6]. Several studies fabricated and characterized drug-encapsulated nanoparticles for in vivo tests on guinea pigs. PLGA nanoparticles could be delivered to the lungs to maintain the therapeutic levels of the drugs in the plasma as well as in the lungs for a prolonged period of time [87]. Also, this approach allowed to decrease the overall dose and reduce the systemic exposure. Furthermore, the surface of PLGA nanoparticles has been functionalized and modified with wheat germ agglutinin whose bioadhesive properties facilitate its interaction with lectin receptors embedded in the alveolar epithelium to maintain the drug concentration in the lung tissues [88]. This system could result in a sustained drug level in the plasma for 14 days and in the lung for 15 days. Similarly, PLGA nanoparticles which were formulated with sodium alginate and chitosan provided a sustained drug delivery to the lungs of guinea pigs in vivo to eradicate tubercle bacilli from *M. tuberculosis*-infected guinea pigs [51].

3.7 Technical Issues of Nanoparticles to the Lung

Nanoparticles are usually formulated in colloidal solutions for nebulization [7]. However, the storage in an aqueous medium could induce polymer hydrolysis and degradation of drugs. Furthermore, the small dimensions and interactions between particles result in the agglomeration and settling of nanoparticles, thus, cause a reduced functionality of the nebulizer. To overcome these challenges, lyophilization has been employed to dry nanoparticles to obtain a stable storage form which can be later dispersed in an aqueous solution for administration [89, 90]. Stabilizers such as cryoprotectant sugars and surfactants are used to enhance the stability, maintain the characteristics of nanoparticles during lyophilization, and to facilitate resuspension of the dry particles. These stabilizers dissolve in the resuspended solution and are delivered together with the particles.

3.8 Toxicity of Nanoparticles to the Lung

Despite multiple advantages offered by nanoparticles, they still possess some safety concerns. Li et al. reported that polyamidoamine which is a promising material for nanocarriers could cause autophagic cell death in human lung carcinoma cell line (A549 cells) and acute lung injury in mice in vivo, especially the administration of polyamidoamine might lead to mortality [91]. Card and coworkers reviewed the applications of nanoparticles for imaging, diagnostic, and therapeutic use in the lung [92] and stated that several nanomaterials could cause inflammation and fibrosis in the lung. Toxicity of nanoparticles in the lungs has been evaluated in the environmental health field, especially “ultrafine” particles with the aerodynamic diameter less than 100 nm [7]. The nanoscale dimension of ultrafine particles, on the one hand, provide the therapeutic application, on the other hand, might result in toxicity and undesirable health effects. Ultrafine particles could penetrate epithelial and endothelial cells, be taken up efficiently by cells, and distributed in bone marrow, lymph nodes, spleen, liver, heart, the central nervous system, and ganglia [93–95]. The biological activity of nanoparticles could lead to inflammatory and oxidative stress reactions. Several authors have reviewed the effects of physicochemical properties of nanoparticles such as dimensions, surface charge, geometry, and lipophilicity on their efficacy in vivo [96–99]. The toxicity of nonbiodegradable nanoparticles could be markedly different from those biodegradables. There is an insignificant interaction between biodegradable materials and biological systems. Moreover, the speed of degradation of biodegradable nanoparticles leads to a certain variation in the toxic responses. In particular, biodegradable PLGA nanoparticles resulted in markedly lower inflammatory response than nonbiodegradable polystyrene nanoparticles [100]. Nanoparticles could translocate from the lung to other organs to cause adverse reactions in those organs. The toxicological potential of nanoparticles is negatively correlated with the particle size. Certain levels of

toxicity have been observed with inhalable single-wall carbon nanotubes (SWCNT) [85]. Warheit and colleagues investigated the toxicity of SWCNT on rats in vivo and reported that a high-dose pulmonary exposure led to mortality after 24 h. Pulmonary delivery of SWCNT caused multifocal granulomas, which indicated the nonuniform distribution and translocation of SWCNT-induced toxicity in rats [101]. Similarly, Shvedova et al. reported inflammatory responses in the lungs of mice after exposure to SWCNTs. The toxicity could progress to a reduction of pulmonary function as well as increased vulnerability to infection [102].

References

1. Kreuter, J. (2007). Nanoparticles—A historical perspective. *International Journal of Pharmaceutics, 331*(1), 1–10.
2. Estanqueiro, M., Amaral, M. H., Conceição, J., et al. (2015). Nanotechnological carriers for cancer chemotherapy: The state of the art. *Colloids and Surfaces. B, Biointerfaces, 126*, 631–648.
3. Poon, W., Zhang, X., & Nadeau, J. (2014). Nanoparticle drug formulations for cancer diagnosis and treatment. *Critical Reviews in Oncogenesis, 19*(3–4), 223–245.
4. Kreuter, J. (1991). Nanoparticle-based dmg delivery systems. *Journal of Controlled Release, 16*(1–2), 169–176.
5. Bannon-Peppas, L., & Blanchette, J. O. (2004). Nanoparticle and targeted system for cancer therapy. *Advanced Drug Delivery Reviews, 56*, 1649–1659.
6. Pandey, R., & Khuller, G. K. (2005). Antitubercular inhaled therapy: Opportunities, progress and challenges. *The Journal of Antimicrobial Chemotherapy, 55*(4), 430–435.
7. Sung, J. C., Pulliam, B. L., & Edwards, D. A. (2007). Nanoparticles for drug delivery to the lungs. *Trends in Biotechnology, 25*(12), 563–570.
8. Astete, C. E., & Sabliov, C. M. (2006). Synthesis and characterization of PLGA nanoparticles. *Journal of Biomaterials Science. Polymer Edition, 17*(3), 247–289.
9. Birnbaum, D. T., Kosmala, J. D., & Brannon-Peppas, L. (2000). Optimization of preparation techniques for poly (lactic acid-co-glycolic acid) nanoparticles. *Journal of Nanoparticle Research, 2*(2), 173–181.
10. van Rijt, S. H., Bein, T., & Meiners, S. (2014). Medical nanoparticles for next generation drug delivery to the lungs. *The European Respiratory Journal, 44*(3), 765–774.
11. Panyam, J., Dali, M. M., Sahoo, S. K., et al. (2003). Polymer degradation and in vitro release of a model protein from poly (D,L-lactide-co-glycolide) nano-and microparticles. *Journal of Controlled Release, 92*(1), 173–187.
12. Siafaka, P. I., Üstündağ Okur, N., Karavas, E., et al. (2016). Surface modified multifunctional and stimuli responsive nanoparticles for drug targeting: current status and uses. *International Journal of Molecular Sciences, 17*(9), 1440.
13. Arruebo, M., Fernández-Pacheco, R., Ibarra, M. R., et al. (2007). Magnetic nanoparticles for drug delivery. *Nano Today, 2*(3), 22–32.
14. Schütz, C. A., Juillerat-Jeanneret, L., Mueller, H., et al. (2013). Therapeutic nanoparticles in clinics and under clinical evaluation. *Nanomedicine, 8*(3), 449–467.
15. Siafaka, P., Betsiou, M., Tsolou, A., et al. (2015). Synthesis of folate-pegylated polyester nanoparticles encapsulating ixabepilone for targeting folate receptor overexpressing breast cancer cells. *Journal of Materials Science. Materials in Medicine, 26*(12), 275.
16. Karimi, M., Mirshekari, H., Aliakbari, M., et al. (2016). Smart mesoporous silica nanoparticles for controlled-release drug delivery. *Nanotechnology Reviews, 5*(2), 195–207.

17. Filippousi, M., Siafaka, P. I., Amanatiadou, E. P., et al. (2015). Modified chitosan coated mesoporous strontium hydroxyapatite nanorods as drug carriers. *Journal of Materials Chemistry B, 3*(29), 5991–6000.
18. Yoo, J.-W., Doshi, N., & Mitragotri, S. (2011). Adaptive micro and nanoparticles: temporal control over carrier properties to facilitate drug delivery. *Advanced Drug Delivery Reviews, 63*(14–15), 1247–1256.
19. Geers, B., Dewitte, H., De Smedt, S. C., et al. (2012). Crucial factors and emerging concepts in ultrasound-triggered drug delivery. *Journal of Controlled Release, 164*(3), 248–255.
20. Sirsi, S. R., Fung, C., Garg, S., et al. (2013). Lung surfactant microbubbles increase lipophilic drug payload for ultrasound-targeted delivery. *Theranostics, 3*(6), 409.
21. Hasenpusch, G., Geiger, J., Wagner, K., et al. (2012). Magnetized aerosols comprising superparamagnetic iron oxide nanoparticles improve targeted drug and gene delivery to the lung. *Pharmaceutical Research, 29*(5), 1308–1318.
22. Thorley, A. J., & Tetley, T. D. (2013). New perspectives in nanomedicine. *Pharmacology & Therapeutics, 140*(2), 176–185.
23. Sharifi, S., Behzadi, S., Laurent, S., et al. (2012). Toxicity of nanomaterials. *Chemical Society Reviews, 41*(6), 2323–2343.
24. Madani, S. Y., Mandel, A., & Seifalian, A. M. (2013). A concise review of carbon nanotube's toxicology. *Nano Reviews, 4*(1), 21521.
25. Singh, R., Pantarotto, D., Lacerda, L., et al. (2006). Tissue biodistribution and blood clearance rates of intravenously administered carbon nanotube radiotracers. *Proceedings of the National Academy of Sciences, 103*(9), 3357–3362.
26. Clark, A. (2002). Formulation of proteins and peptides for inhalation. *Drug Delivery System Sciences, 2*, 73–77.
27. Courrier, H. M., Butz, N., & Vandamme, T. F. (2002). Pulmonary drug delivery systems: Recent developments and prospects. *Critical Reviews in Therapeutic Drug Carrier Systems, 19*(4–5), 425–498.
28. Gill, S., Löbenberg, R., Ku, T., et al. (2007). Nanoparticles: Characteristics, mechanisms of action, and toxicity in pulmonary drug delivery—A review. *Journal of Biomedical Nanotechnology, 3*(2), 107–119.
29. Patton, J. S., & Platz, R. M. (1992). (D) Routes of delivery: Case studies: (2) Pulmonary delivery of peptides and proteins for systemic action. *Advanced Drug Delivery Reviews, 8*(2–3), 179–196.
30. Ashbaugh, D., Bigelow, D. B., Petty, T., et al. (1967). Acute respiratory distress in adults. *The Lancet, 290*(7511), 319–323.
31. Murray, J. F., Matthay, M. A., Luce, J. M., et al. (1988). An expanded definition of the adult respiratory distress syndrome. *The American Review of Respiratory Disease, 138*(3), 720–723.
32. Bernard, G. R., Artigas, A., Brigham, K. L., et al. (1994). The American-European Consensus Conference on ARDS. Definitions, mechanisms, relevant outcomes, and clinical trial coordination. *American Journal of Respiratory and Critical Care Medicine, 149*(3), 818–824.
33. Abraham, E., Matthay, M. A., Dinarello, C. A., et al. (2000). Consensus conference definitions for sepsis, septic shock, acute lung injury, and acute respiratory distress syndrome: Time for a reevaluation. *Critical Care Medicine, 28*(1), 232–235.
34. Rubenfeld, G. D., Caldwell, E., Peabody, E., et al. (2005). Incidence and outcomes of acute lung injury. *The New England Journal of Medicine, 353*(16), 1685–1693.
35. Liu, M., Zhang, H., & Slutsky, A. S. (2009). Acute lung injury: A yellow card for engineered nanoparticles? *Journal of Molecular Cell Biology, 1*(1), 6–7.
36. Abel, S. J. C., Finney, S. J., Brett, S. J., et al. (1998). Reduced mortality in association with the acute respiratory distress syndrome (ARDS). *Thorax, 53*(4), 292–294.
37. Milberg, J. A., & Steinberg, M. D. (1995). Respiratory Distress Syndrome (ARDS): 1983–1993. *Journal of the American Medical Association, 273*, 306–309.
38. McVey, M., Tabuchi, A., & Kuebler, W. M. (2012). Microparticles and acute lung injury. *American Journal of Physiology. Lung Cellular and Molecular Physiology, 303*(5), L364–L381.

39. Tsushima, K., King, L. S., Aggarwal, N. R., et al. (2009). Acute lung injury review. *Internal Medicine, 48*(9), 621–630.
40. Mura, M., Andrade, C. F., Han, B., et al. (2007). Intestinal ischemia-reperfusion-induced acute lung injury and oncotic cell death in multiple organs. *Shock, 28*(2), 227–238.
41. Maniatis, N. A., Kotanidou, A., Catravas, J. D., et al. (2008). Endothelial pathomechanisms in acute lung injury. *Vascular Pharmacology, 49*(4–6), 119–133.
42. Matthay, M. A., & Zimmerman, G. A. (2005). Acute lung injury and the acute respiratory distress syndrome: Four decades of inquiry into pathogenesis and rational management. *American Journal of Respiratory Cell and Molecular Biology, 33*(4), 319–327.
43. Ware, L. B. (2006). Pathophysiology of acute lung injury and the acute respiratory distress syndrome. *Seminars in Respiratory and Critical Care Medicine, 27*(4), 337–349.
44. Ware, L. B., & Matthay, M. A. (2000). The acute respiratory distress syndrome. *The New England Journal of Medicine, 342*(18), 1334–1349.
45. Bastarache, J. A., Ware, L. B., & Bernard, G. R. (2006). The role of the coagulation cascade in the continuum of sepsis and acute lung injury and acute respiratory distress syndrome. *Seminars in Respiratory and Critical Care Medicine, 27*(4), 365–376.
46. Schwarz, M. I., & Albert, R. K. (2004). "Imitators" of the ARDS*: Implications for diagnosis and treatment. *Chest, 125*(4), 1530.
47. Grenha, A., Seijo, B., & Remunán-López, C. (2005). Microencapsulated chitosan nanoparticles for lung protein delivery. *European Journal of Pharmaceutical Sciences, 25*(4–5), 427–437.
48. Edwards, D. A., Hanes, J., Caponetti, G., et al. (1997). Large porous particles for pulmonary drug delivery. *Science, 276*(5320), 1868–1872.
49. Oh, Y. J., Lee, J., Seo, J. Y., et al. (2011). Preparation of budesonide-loaded porous PLGA microparticles and their therapeutic efficacy in a murine asthma model. *Journal of Controlled Release, 150*(1), 56–62.
50. Wernig, K., Griesbacher, M., Andreae, F., et al. (2008). Depot formulation of vasoactive intestinal peptide by protamine-based biodegradable nanoparticles. *Journal of Controlled Release, 130*(2), 192–198.
51. Zahoor, A., Sharma, S., & Khuller, G. K. (2005). Inhalable alginate nanoparticles as antitubercular drug carriers against experimental tuberculosis. *International Journal of Antimicrobial Agents, 26*(4), 298–303.
52. Azarmi, S., Tao, X., Chen, H., et al. (2006). Formulation and cytotoxicity of doxorubicin nanoparticles carried by dry powder aerosol particles. *International Journal of Pharmaceutics, 319*(1–2), 155–161.
53. Karra, N., Nassar, T., Ripin, A. N., et al. (2013). Antibody conjugated PLGA nanoparticles for targeted delivery of paclitaxel palmitate: Efficacy and biofate in a lung cancer mouse model. *Small, 9*(24), 4221–4236.
54. Zhang, L., Gu, F. X., Chan, J. M., et al. (2008). Nanoparticles in medicine: Therapeutic applications and developments. *Clinical Pharmacology and Therapeutics, 83*(5), 761–769.
55. Müller, R. H., MaÈder, K., & Gohla, S. (2000). Solid lipid nanoparticles (SLN) for controlled drug delivery—A review of the state of the art. *European Journal of Pharmaceutics and Biopharmaceutics, 50*(1), 161–177.
56. Joshi, M., & Misra, A. (2001). Pulmonary disposition of budesonide from liposomal dry powder inhaler. *Methods and Findings in Experimental and Clinical Pharmacology, 23*(10), 531–536.
57. Yamamoto, H., Kuno, Y., Sugimoto, S., et al. (2005). Surface-modified PLGA nanosphere with chitosan improved pulmonary delivery of calcitonin by mucoadhesion and opening of the intercellular tight junctions. *Journal of Controlled Release, 102*(2), 373–381.
58. Amoozgar, Z., & Yeo, Y. (2012). Recent advances in stealth coating of nanoparticle drug delivery systems. *Wiley Interdisciplinary Reviews. Nanomedicine and Nanobiotechnology, 4*(2), 219–233.
59. Yuan, H., Chen, C.-Y., Chai, G., et al. (2013). Improved transport and absorption through gastrointestinal tract by PEGylated solid lipid nanoparticles. *Molecular Pharmaceutics, 10*(5), 1865–1873.

60. Monopoli MP, \AAberg C, Salvati A, et al. 2012. Biomolecular coronas provide the biological identity of nanosized materials. Nature Nanotechnology 7(12):779
61. Schleh, C., & Hohlfeld, J. M. (2009). Interaction of nanoparticles with the pulmonary surfactant system. *Inhalation Toxicology, 21*(sup1), 97–103.
62. Kendall, M., Ding, P., Mackay, R.-M., et al. (2013). Surfactant protein D (SP-D) alters cellular uptake of particles and nanoparticles. *Nanotoxicology, 7*(5), 963–973.
63. Zhao, H., Zeng, Z., Liu, L., et al. (2018). Polydopamine nanoparticles for the treatment of acute inflammation-induced injury. *Nanoscale, 10*(15), 6981–6991.
64. Li, N., Weng, D., Wang, S.-M., et al. (2017). Surfactant protein-A nanobody-conjugated liposomes loaded with methylprednisolone increase lung-targeting specificity and therapeutic effect for acute lung injury. *Drug Delivery, 24*(1), 1770–1781.
65. Grimm, J. C., Zhang, F., Magruder, J. T., et al. (2017). Accumulation and cellular localization of nanoparticles in an ex vivo model of acute lung injury. *The Journal of Surgical Research, 210*, 78–85.
66. Pápay, Z. E., Kósa, A., Böddi, B., et al. (2017). Study on the pulmonary delivery system of apigenin-loaded albumin nanocarriers with antioxidant activity. *Journal of Aerosol Medicine and Pulmonary Drug Delivery, 30*(4), 274–288.
67. Muralidharan, P., Hayes, D., Black, S. M., et al. (2016). Microparticulate/nanoparticulate powders of a novel Nrf2 activator and an aerosol performance enhancer for pulmonary delivery targeting the lung Nrf2/Keap-1 pathway. *Molecular Systems Design and Engineering, 1*(1), 48–65.
68. Xu, Y., Xiang, J., Zhao, H., et al. (2016). Human amniotic fluid stem cells labeled with upconversion nanoparticles for imaging-monitored repairing of acute lung injury. *Biomaterials, 100*, 91–100.
69. Ravikumar, P., Menon, J. U., Punnakitikashem, P., et al. (2016). Nanoparticle facilitated inhalational delivery of erythropoietin receptor cDNA protects against hyperoxic lung injury. *Nanomedicine: Nanotechnology, Biology and Medicine, 12*(3), 811–821.
70. Lin, E.-H., Chang, H.-Y., Yeh, S.-D., et al. (2013). Polyethyleneimine and DNA nanoparticles-based gene therapy for acute lung injury. *Nanomedicine: Nanotechnology, Biology and Medicine, 9*(8), 1293–1303.
71. Shrestha, R., Shen, Y., Pollack, K. A., et al. (2012). Dual peptide nucleic acid-and peptide-functionalized shell cross-linked nanoparticles designed to target mRNA toward the diagnosis and treatment of acute lung injury. *Bioconjugate Chemistry, 23*(3), 574–585.
72. Chow, A. H., Tong, H. H., Chattopadhyay, P., et al. (2007). Particle engineering for pulmonary drug delivery. *Pharmaceutical Research, 24*(3), 411–437.
73. Malcolmson, R. J., & Embleton, J. K. (1998). Dry powder formulations for pulmonary delivery. *Pharmaceutical Science & Technology Today, 1*(9), 394–398.
74. Ely, L., Roa, W., Finlay, W. H., et al. (2007). Effervescent dry powder for respiratory drug delivery. *European Journal of Pharmaceutics and Biopharmaceutics, 65*(3), 346–353.
75. Bosquillon, C., Lombry, C., Preat, V., et al. (2001). Influence of formulation excipients and physical characteristics of inhalation dry powders on their aerosolization performance. *Journal of Controlled Release, 70*(3), 329–339.
76. Carvalho, T. C., Peters, J. I., & Williams, R. O., III. (2011). Influence of particle size on regional lung deposition—What evidence is there? *International Journal of Pharmaceutics, 406*(1–2), 1–10.
77. Niven, R. W. (1995). Delivery of biotherapeutics by inhalation aerosol. *Critical Reviews in Therapeutic Drug Carrier Systems, 12*(2–3), 151–231.
78. Tsapis, N., Bennett, D., Jackson, B., et al. (2002). Trojan particles: Large porous carriers of nanoparticles for drug delivery. *Proceedings of the National Academy of Sciences, 99*(19), 12001–12005.
79. Heyder, J., Gebhart, J., Rudolf, G., et al. (1986). Deposition of particles in the human respiratory tract in the size range 0.005–15 μm. *Journal of Aerosol Science, 17*(5), 811–825.

80. Sham, J. O.-H., Zhang, Y., Finlay, W. H., et al. (2004). Formulation and characterization of spray-dried powders containing nanoparticles for aerosol delivery to the lung. *International Journal of Pharmaceutics, 269*(2), 457–467.
81. Kawashima, Y., Serigano, T., Hino, T., et al. (1998). A new powder design method to improve inhalation efficiency of pranlukast hydrate dry powder aerosols by surface modification with hydroxypropylmethylcellulose phthalate nanospheres. *Pharmaceutical Research, 15*(11), 1748–1752.
82. Kawashima, Y., Yamamoto, H., Takeuchi, H., et al. (1999). Pulmonary delivery of insulin with nebulized DL-lactide/glycolide copolymer (PLGA) nanospheres to prolong hypoglycemic effect. *Journal of Controlled Release, 62*(1–2), 279–287.
83. Azarmi, S., Roa, W. H., & Löbenberg, R. (2008). Targeted delivery of nanoparticles for the treatment of lung diseases. *Advanced Drug Delivery Reviews, 60*(8), 863–875.
84. Dailey, L. A., Kleemann, E., Wittmar, M., et al. (2003). Surfactant-free, biodegradable nanoparticles for aerosol therapy based on the branched polyesters, DEAPA-PVAL-g-PLGA. *Pharmaceutical Research, 20*(12), 2011–2020.
85. McConville, J. T., Overhoff, K. A., Sinswat, P., et al. (2006). Targeted high lung concentrations of itraconazole using nebulized dispersions in a murine model. *Pharmaceutical Research, 23*(5), 901–911.
86. Vaughn, J. M., McConville, J. T., Burgess, D., et al. (2006). Single dose and multiple dose studies of itraconazole nanoparticles. *European Journal of Pharmaceutics and Biopharmaceutics, 63*(2), 95–102.
87. Pandey, R., Sharma, A., Zahoor, A., et al. (2003). Poly (DL-lactide-co-glycolide) nanoparticle-based inhalable sustained drug delivery system for experimental tuberculosis. *The Journal of Antimicrobial Chemotherapy, 52*(6), 981–986.
88. Sharma, A., Sharma, S., & Khuller, G. K. (2004). Lectin-functionalized poly (lactide-co-glycolide) nanoparticles as oral/aerosolized antitubercular drug carriers for treatment of tuberculosis. *The Journal of Antimicrobial Chemotherapy, 54*(4), 761–766.
89. Saez, A., Guzman, M., Molpeceres, J., et al. (2000). Freeze-drying of polycaprolactone and poly (D,L-lactic-glycolic) nanoparticles induce minor particle size changes affecting the oral pharmacokinetics of loaded drugs. *European Journal of Pharmaceutics and Biopharmaceutics, 50*(3), 379–387.
90. Wendorf, J., Singh, M., Chesko, J., et al. (2006). A practical approach to the use of nanoparticles for vaccine delivery. *Journal of Pharmaceutical Sciences, 95*(12), 2738–2750.
91. Li, C., Liu, H., Sun, Y., et al. (2009). PAMAM nanoparticles promote acute lung injury by inducing autophagic cell death through the Akt-TSC2-mTOR signaling pathway. *Journal of Molecular Cell Biology, 1*(1), 37–45.
92. Card, J. W., Zeldin, D. C., Bonner, J. C., et al. (2008). Pulmonary applications and toxicity of engineered nanoparticles. *American Journal of Physiology. Lung Cellular and Molecular Physiology, 295*(3), L400–L411.
93. Elder, A., Gelein, R., Silva, V., et al. (2006). Translocation of inhaled ultrafine manganese oxide particles to the central nervous system. *Environmental Health Perspectives, 114*(8), 1172.
94. Kreyling, W. G., Semmler, M., Erbe, F., et al. (2002). Translocation of ultrafine insoluble iridium particles from lung epithelium to extrapulmonary organs is size dependent but very low. *Journal of Toxicology and Environmental Health. Part A, 65*(20), 1513–1530.
95. Oberdörster, G., Sharp, Z., Atudorei, V., et al. (2002). Extrapulmonary translocation of ultrafine carbon particles following whole-body inhalation exposure of rats. *Journal of Toxicology and Environmental Health. Part A, 65*(20), 1531–1543.
96. Borm, P. J., & Kreyling, W. (2004). Toxicological hazards of inhaled nanoparticles—Potential implications for drug delivery. *Journal of Nanoscience and Nanotechnology, 4*(5), 521–531.
97. Hoet, P. H., Brüske-Hohlfeld, I., & Salata, O. V. (2004). Nanoparticles – Known and unknown health risks. *Journal of Nanobiotechnology, 2*(1), 12.

98. Oberdörster, G., Oberdörster, E., & Oberdörster, J. (2005). Nanotoxicology: An emerging discipline evolving from studies of ultrafine particles. *Environmental Health Perspectives, 113*(7), 823.
99. Warheit, D. B., Webb, T. R., Reed, K. L., et al. (2007). Pulmonary toxicity study in rats with three forms of ultrafine-TiO_2 particles: Differential responses related to surface properties. *Toxicology, 230*(1), 90–104.
100. Dailey, L. A., Jekel, N., Fink, L., et al. (2006). Investigation of the proinflammatory potential of biodegradable nanoparticle drug delivery systems in the lung. *Toxicology and Applied Pharmacology, 215*(1), 100–108.
101. Warheit, D. B., Laurence, B. R., Reed, K. L., et al. (2004). Comparative pulmonary toxicity assessment of single-wall carbon nanotubes in rats. *Toxicological Sciences, 77*(1), 117–125.
102. Shvedova, A. A., Murray, A. R., Kisin, E. R., et al. (2003). Exposure to carbon nanotube material: Evidence of exposure-induced oxidant stress in human keratinocyte and bronchial epithelial cells.

Chapter 18
Surface Modification of Nanocarriers for Specific Cell Targeting for Better Therapeutic Effect

Doniya Milani, Umi Athiyah, Dewi Melani Hariyadi, and Yashwant V Pathak

Abstract Nanocarriers may potentially change the future of medicine. These nano-sized drug delivery vesicles have been employed to enhance the efficacy and safety of different therapeutic agents. Specifically designed to localize in target sites, nanocarriers have the ability to increase cellular uptake while decreasing toxicity. Surface-modified nanocarriers have the potential to overcome several biological barriers, thus further improving site specificity while also avoiding endosomal sequestration and degradation of the cargo. In this review, surface modifications aimed at cell targeting for better drug delivery are discussed.

Keywords Surface modifications · Nanomedicine · Nanocarriers · Cellular targeting · Drug targeting · Drug delivery · Organelle targeting

1 Introduction

The advancement of nanomedicine has subsequently led to the emergence and development of important, multipurpose tools, such as nanocarriers. These vesicles for drug delivery offer new and exciting approaches to the treatments of several different diseases. An increase in research on nanocarriers has unearthed new ways to increase the half-life, bioavailability, targeting, and safety of different therapeutic approaches in medicine [1]. Typical drug delivery poses the challenge of surpassing nondiseased organs when trying to get the drug where it is needed. This challenge

D. M. Hariyadi
College of Pharmacy, University of South Florida Health, Tampa, FL, USA

Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia

D. Milani · U. Athiyah
Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia

Y. V Pathak (✉)
College of Pharmacy, University of South Florida Health, Tampa, FL, USA

Adjunct professor at Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia
e-mail: ypathak1@health.usf.edu

Y. V Pathak (ed.), *Surface Modification of Nanoparticles for Targeted Drug Delivery*, https://doi.org/10.1007/978-3-030-06115-9_18

is of particular importance in cancer treatment where the tumor can be localized within various organs [2]. These issues are addressed through improved biodistribution, decreased toxicity, increased stability, and site-specific targeting [2]. Nanocarriers are created to specifically accrue at target sites [1, 2]. By developing methods of drug targeting of intracellular compartments, the efficacy of nanocarriers is improved tremendously [1]. The primary goal of increasing the utilization, understanding, and development of nanocarriers is to improve the efficacy of drug delivery while minimizing side effects and damage to the healthy surrounding cells.

The composition (organic, inorganic, or hybrid), size, and shape (sphere, rod, or cube) of a nanocarrier can be altered, thereby affecting its physiochemical properties [2]. These drug carrier systems are typically smaller than 500 nm, but have particularly high surface area to volume ratios that allow them to alter the basic properties and bioactivity of drugs [2]. Its surface properties, including characteristics such as surface charge, PEGylation, present functional groups, and attachment of targeting moieties, can also be altered [2]. This allows nanocarriers to be more fine-tuned drug delivery systems. However, it is not simply organ and cellular targeting that is of importance; how nanoparticles are eradicated from within the targeted cells is also relevant. Nanoparticles typically reach their fates intracellularly in endosomes or lysosomes for degradation [3]. Many drugs must be encapsulated to avoid intracellular endosomal sequestration and degradation in order to achieve optimal payload efficacy [1].

Nanocarriers are faced with several biological barriers that hinder their success. Many of these obstacles present themselves during transportation from the site of administration to the desired target [4]. These competent biological barriers, such as the body's clearance actions, physical obstructions via matrixes and membranes, cellular barriers, and enzymatic barriers, are some of the greatest impediments in the use of targeted therapies [5]. However, nanocarriers have been further refined to favor cell internalization and have shown improved interactions and responses to the biological environment of the body, thus maximizing their functioning [1]. Following internalization, nanocarriers must evade endosomal digestion in order to reach the cell cytoplasm [1]. This review discusses methods of endosomal sequestration and degradation evasion, how the cell cytoplasm is accessed to achieve organelle targeting, and examples of nanocarrier systems designed specifically to overcome the listed biological barriers to successfully reach the cytoplasm and targeted organelles.

2 Cell-Penetrating Peptides

Cell-penetrating peptides (CPPs) are relatively short (10–30 amino acids), cationic, or amphipathic peptides that are able to directly penetrate cellular membranes and deliver different therapeutic cargos directly to the cell cytoplasm [1, 6]. These drug delivery vectors are of particular importance in the field of nanomedicine due to their ability to safely and effectively penetrate the cell membrane at low

concentrations in vivo and in vitro [7]. CPPs can be amphipathic or polycationic [1, 6]. Amphipathic CPPs contain both hydrophobic and hydrophilic amino acid residues, while polycationic CPPs have an abundance of positively charged amino acids [1, 7]. Due to the existence of many arginine and lysine residues, CPPs have a net positive charge at physiological pH [1, 8]. The positive charge allows CPPs to interact favorably with the surfaces of cell membranes, whereas the hydrophobic residues, such as tryptophan, favor lipid structures [9]. Although there is no definite answer to the mechanism of CPP uptake, internalization of CPPs is most likely accomplished through both energy-independent processes and endocytosis [7]. However, the pathway used for CPP internalization is dependent on different variables, such as the concentration of CPPs [7].

Surface modification with CPPs can lead to increased cellular drug accumulation and cellular uptake, thereby amplifying the efficiency of certain therapeutic agents [10]. Transactivator of Transcription (TAT)-modified CPPs exhibit advantages such as greater tumor growth inhibition powers [10] and increased inhibition of multidrug resistance phenomena in vivo and in vitro [11]. These peptides are powerful tools for cytoplasmic drug delivery, as multiple studies have concluded that surface modification of nanocarriers through the covalent addition of cationic or amphipathic CPPs may successfully and sufficiently enhance cellular uptake [12].

3 Antimicrobial Peptides

Antimicrobial peptides (AMPs) are short, amphipathic molecules that have positive charges and have the ability to reach the cell cytoplasm [1, 13]. AMPs are produced by a variety of different life forms, from human beings to bacteria [13]. In addition to antimicrobial activity, AMPs also play a critical role in modulating innate immune responses in higher organisms [13, 14]. AMPs have recently been divided into four main groups through a study conducted by Vale and colleagues [15]. These four groups are α-helical peptides deprived of Cys residues, small peptides categorized by their multiple Arg and Lys residues and substantial hydrophobic portion; β-pleated peptides containing disulfide bridges, such as defensins, which is typically found in mammalian phagocytes; peptides rich in Pro, Gly, His, Arg, and Trp residues; and circular antimicrobial peptides [15]. As we shift into an era of antibiotic resistance, research into alternative therapies has intensified. AMPs preferentially bind to target membranes that are less anionic than mammalian cell membranes, allowing for specific targeting of microorganisms such as viruses and bacteria [7]. Because of these properties, AMPs are viable and useful candidates for modifying nanoparticles as a means of improving antibiotic effects [1, 7, 14].

There have been a couple of successful reports of modified nanocarriers conjugated with AMPs [16, 17]. The future development of AMPs offers significant potential for improved drug therapy. However, there are still unresolved issues

related to the clinical use of AMPs that require further research and clinical trials. Current research aims to improve the efficacy and safety of AMP usage through surface modifications that minimize toxicity of AMP to eukaryotic cells while still maintaining and further improving its antimicrobial properties [12].

4 Surface Modification to Enable Cytoplasmic Delivery

4.1 Endosomal Sequestration and Degradation

Although there have been numerous improvements in drug delivery, there are some limitations that must still be overcome. One of the biggest obstacles in targeted drug therapy is the endocytic pathway, which is the primary uptake mechanism of cells [18]. The endocytic pathway contains endosomes with an internal pH of approximately 5 [18]. Particles entering cells through the endocytic pathway, such as nanocarriers, become trapped in endosomes, eventually finding themselves in the lysosome, where they, and their payloads intended for delivery, are degraded [18]. Endosomal sequestration and subsequent degradation prevent therapeutic agents from reaching their intracellular targets.

4.2 pH-Buffering to Avoid Endosomal Sequestration and Degradation

Surface modifications of nanocarriers may be used as a method to escape endosomal sequestration. The "proton sponge effect" uses agents with high buffering capacities and flexibility during protonation [18]. Protonation induces osmotic lysis by prompting the inflow of ions and water into the endosome [18]. Tertiary amine groups containing hydrophobic chains that can be protonated are often used in this context due to their ability to accumulate in acidic endosomes, thereby disrupting the membrane [18]. Molecules with tertiary amine groups that may be protonated in low pH environments work by opposing the acidification of endolysosomal vesicles [19]. This acidification is accomplished through proton transmembrane pumps working in conjunction with chlorine ions [17]. By contrasting the acidification of the endolysosomal vesicles, these tertiary amine groups increase the chloride concentration [17, 19], thereby increasing the osmotic pressure and eventually leading to the rupture of the endosomal membrane [18, 19]. Once the endosomes rupture, their contents are released, allowing endosomal escape.

The addition of other moieties can further improve cytoplasmic delivery of agents through the pH-buffering effect. An example of this is histidine, which enhances the buffering effect upon protonation of its imidazole ring [1, 18, 20].

4.3 Conjugation with Fusogenic Peptides to Avoid Endosomal Sequestration

Another method of accomplishing endosomal escape is the destabilization of the endosomal membrane. Viruses, which contain specific fusogenic peptides, are able to penetrate the endosomal membrane through the fusion of the viral envelope and host endosomal membrane [21]. This allows the virus to transport its genome into the cytoplasm of the host cell [21]. An example of this is the influenza virus hemagglutinin protein, which has a hydrophobic fusion peptide domain that undergoes protonation during acidification of the endosome [21]. As a result of this protonation, the hydrophobic fusion peptide domain undergoes a conformational change that leads to the destabilization of the endosomal membrane [21, 22]. These peptides commonly acquire an amphipathic α-helical conformation that induces fusion [18, 21, 22]. By mimicking this approach, endosomal membrane destabilization can be achieved using conjugated nanocarriers that exhibit fusogenic properties, facilitating endosomal escape for nanocarriers.

4.4 Using pH-Responsive Lipids to Avoid Endosomal Sequestration

Lipids can also be used to create nanocarriers that are able to elude endosomal sequestration and successfully deliver payloads to cell cytoplasm [1]. Adding helper lipids to cationic liposomal formulations can increase their fusogenicity while simultaneously decreasing cytotoxicity [23]. Some of the first discovered helper lipids are DOPE and L-α-di-oleoyl phosphatidylcholine (DOPC) [24, 25].

Lipids containing histidine or imidazole moieties have also been used to improve the pH-responsiveness of nanocarriers [1]. Histidine's imidazole ring is protonated at a pH of 6.0, characterizing it as a weak base [23]. The use of histidine or imidazole moieties can provide nanocarriers with proton sponge tendencies, fusogenic properties, or a combination of the two [1, 23]. The use of these lipids enhances cytoplasmic delivery, subsequently improving the efficacy of a nanocarrier.

5 Organelle Targeting

5.1 The Importance of Organelle Targeting

An essential element in drug delivery is making sure the cargo is taken to its specific site of action. Without this target-specific affinity, results such as systemic toxicity and higher dosage requirements decrease the efficacy of a drug [26]. Targeted drug delivery is critical when treating diseases such as cancer, which poses the

complicated challenge of delivering chemotherapeutic agents specifically to proliferating tumor cells while avoiding the healthy cells [26]. A drug's therapeutic index is defined by its ability to successfully increase its toxicity to unhealthy cells while decreasing its toxicity to the normal cells [27]. To further enhance and increase the therapeutic index of a drug, methods of delivering the agents not just to the target tissues or cells, but more specifically into the targeted organelle, such as nuclei, lysosomes, mitochondria, and the Golgi/endoplasmic reticulum [26]. Accessing these organelles can potentially change the way organelle-associated diseases are combated. Thus, the development of nanoformulations with improved targeting selectivity and delivery efficacy are invaluable additions that can redefine the ways in which drug delivery is currently approached [26]. These nanoformulations involve drugs that are conjugated with selectively binding ligands or antibodies with affinities for a variety of receptors that are copiously expressed on target cell surfaces [26]. Using organelle targeting moieties to modify nanocarriers can increase overall drug efficacy, improve the tissue accumulation of different therapeutic agents, reduce the quantity of drug that is necessary to obtain a desired result, and reduce negative side effects [26].

5.2 Nuclear Targeting

The nucleus is an important organelle found in eukaryotic cells. The nucleus has several important roles, such as regulation of replication and transcription processes [1]. Additionally, the nucleus determines and controls the translocation of proteins between the cytoplasm and nucleus through its nuclear pore complex (NPC) [1, 28]. The nucleus is the final target for many drug therapies since it is responsible for all diseases that arise due to a gene mutation [26]. The nuclear pore complex is at the center of a eukaryotic cell's ability to control gene expression [29]. The nuclear envelope, which is a double membrane that separates the nucleus and cytoplasm, is perforated with large pores that make up the NPC, allowing proteins and RNA to undergo nucleocytoplasmic transfer [28, 29].

However, nanoparticle accumulation in the nuclei is somewhat rare. This is because the nuclear membrane's small pores prohibit the passage of larger molecules. Only molecules smaller than ~40 kDa may freely cross the nuclear membrane [29]. Molecules larger than this must be actively transported in order to cross the nuclear membrane, requiring involvement from nuclear localization signals (NLS) [1, 26, 29]. Thus, small molecules that cross the nuclear membrane via passive transport depend on the size of the nanocarriers, whereas the permeation of the membrane through active transport depends on the presence of NLS [30]. Nanoparticles can be functionalized with NLS, allowing cytoplasmic receptors, such as importins, to recognize them [1]. NLS sequences are typically stretches of basic amino acid residues [29]. NLS recognition is the first step in the passage of NLS-modified nanoparticles into the nucleus. Following this, the NLS peptide binds the NPC, and the cargo undergoes an energy-dependent translocation through the

pores of the NPC, eventually releasing the cargo into the nucleus [31]. An example of nuclear targeting through passive transport is the use of malonodiserinolamide fullerene C_{60} (C60-ser) nanoparticles [32]. This water-soluble, nonionic, and non-cytotoxic fullerene has been studied as a nanovector for the delivery of cancer drugs to treat liver cancer in vivo [32]. Due to its small size (2.8 kDa), C_{60}-serPF is able to infiltrate the NPC through passive diffusion, leading to preferential accumulation in the nuclei of liver cancer cells within 2 h of exposure, with increased concentrations in the cytoplasm [32]. It has also been shown that using surface-modified nanoparticles can aid in nuclear targeting. Surface-modified solid gold nanospheres with a cancer cell penetrating/proapoptotic peptide (RGD) and a NLS peptide integrated into a PEG coating (to increase specificity) were used in a study to target cancer cell nuclei [33]. The results showed that these modified nanocarriers inhibited cell division, thus prompting apoptosis and enhancing the cytotoxic effect of the drug on cancer cells [33].

5.3 Mitochondrial Targeting

Mitochondria, commonly referred to as the powerhouses of the cell, are an important organelle that provides the body with energy through oxidative phosphorylation, resulting in the production of adenosine triphosphate (ATP). Mitochondria also contribute to the metabolism of amino acids and lipids, cell signaling pathways, and eukaryotic apoptosis [34]. Because of the critical role of mitochondria in cell functioning, their dysfunction is the root of several diseases, such as muscular and neurodegenerative diseases, diabetes, cancer, and cardiovascular disease [34, 35].

Mitochondria have large membrane potentials, meaning their charge is negative inside [1]. Thus, positively charged molecules may be utilized for mitochondrial targeting. In a study conducted by Yoong and coworkers, multiwalled carbon nanotubes were functionalized with rhodamine, a lipophilic cation [36]. Their results showed improved liposome mitochondrial targeting. However, the most studied therapeutic agent for mitochondrial targeting is MitoQ, which was developed with a focus on mitochondrial oxidative damage [37]. MitoQ uses a quinone moiety (antioxidant Coenzyme Q10) conjugated with triphenylphosphonium (TPP) through a 10-carbon alkyl chain [38]. Lipophilic TPP cations readily pass through phospholipid bilayers, driven by the plasma membrane potential, leading to the accumulation of MitoQ in the cell's mitochondria [37]. MitoQ is being intensely studied for the treatment of diseases such as hepatitis C and Parkinson's disease [39, 40]. In the past, stearyl TPP-modified liposomes have been studied for their mitochondrial targeting, as well. However, these liposomes present nonspecific toxicity. To combat this, Biswas et al. synthesized a novel polyethylene glycol-phosphatidylethanolamine (PEG-PE) coating conjugated with the TPP group at the distal end of the PEG block (TPP-PEG-PE) [41]. TPP-PEG-PE can be used as a targeting ligand in the preparation of nontoxic liposomes with effective mitochondrial targeting [41].

Another strategy for mitochondrial targeting is the use of mitochondrial targeting signals (MTS), which are roughly 10–70 amino acids, to modify nanocarriers [1, 26]. The majority of mitochondrial proteins are translated in the cytosol and then directed into the mitochondria by the MTS [1, 26]. Using this information, restriction enzymes may target the mitochondria using MTS [26]. For example, the restriction endonuclease Sma1, which specifically digests mutant DNA, was fused to MTS, allowing targeted delivery of the restriction enzyme to the mitochondria [42]. As a result of this, the mutant mitochondrial DNA was successfully eliminated [42]. In addition, liposome-like vesicles have been used to deliver DNA to the mitochondria. Lipophilic dequalinium (DQA)-somes may be used as drug delivery tools due to their positive charge and unique properties which give them an affinity for the mitochondrial membrane [42, 43]. The dequalinium chloride that makes up these vesicles is a mitochondriotropic, delocalized cation that preferentially accumulates in the mitochondria of carcinoma cells [44]. Using this strategy, other mitochondriotropic molecules have been developed. Resveratrol liposomes surface-modified with dequalinium polyethylene glycol-distearoylphosphatidylethanolamine (DQA-PEG_{2000}-DSPE) have been shown to target mitochondria and overcome the intrinsic multidrug resistance of cancers that commonly hinders the success of chemotherapy [45]. These liposomes successfully targeted the mitochondria, leading to selective accumulation and increased cellular uptake, improving overall anticancer efficacy in vitro and in vivo [45]. Another example of an effective mitochondrial gene therapy is the development of MITO-Porter [46]. This liposome successfully introduces its cargo into the inner mitochondrial matrix using membrane fusion [46]. More recently, a dual function MITO-Porter was developed by integrating octaarginine (R8)-modified liposomes [47]. These liposomes, which contain double lipid shells, can pass through endosomal and mitochondrial membranes [47]. These effective drug delivery systems exhibit high fusogenic properties, low cytotoxicity, and the ability to evade endosomal sequestration [47]. Other surface-modified liposomes have been able to achieve endosomal escape and mitochondrial targeting in different ways. Liposomes modified with 1,5-dioctadecyl-1-glutamyl 2-histidyl-hexahydrobenzoic acid ($HHG2C_{18}$-L) and 1,5-distearyl *N*-(N-a-(4-mPEG2000) butanedione)-histidyl-L-glutamate (PEGHG2C18) were synthesized in a study conducted by Mo and coworkers [48]. These lipid moieties are pH-sensitive, particularly to the tumor microenvironmental pH and endosomal/lysosomal pH [48]. Because of their charge conversion capabilities, these pH-sensitive modified liposomes are able to change their surface charge in relation to the surrounding pH, leading to increased cellular uptake, successful mitochondrial targeting, and endosomal sequestration evasion [48].

5.4 Endoplasmic Reticulum Delivery

The endoplasmic reticulum (ER) is a membrane network containing tubules and sac-like structures called cisternae [1, 26]. The ER is the principal site for the folding of secretory and membrane proteins [49]. Protein delivery to the ER is mediated

by the KDEL sequence and its expressed receptor (KDEL-R), which is an ER localization signal that has been involved in the improvement of intracellular targeting [1]. Additional functions of the ER include lipid biosynthesis, the regulation of calcium homeostasis, apoptosis, and calcium signaling [49]. Instabilities or dysfunctions by numerous intracellular and extracellular stimuli typically lead to what is referred to as "ER stress" [49]. Under ER stress, protein misfolding may occur, leading to the aggregation of improper, erroneous proteins in the ER lumen [49]. These dysfunctions are often associated with many different disorders and diseases, emphasizing the importance of ER targeting in drug delivery.

In a recent study highlighting the use of an ER localization signal for intracellular targeting, the KDEL (Lys-Asp-Glu-Leu) peptide was utilized to conjugate gold nanoparticles and deliver siRNA to the ER to inhibit the expression of reduced nicotinamide adenine dinucleotide oxidase 4 (*Nox4*) [50]. The results of the study illustrated the enhanced cellular uptake, stability, and efficacy of the conjugated gold nanoparticles in the transfection of siRNA [50]. The results also suggested primary localization in the ER with marginal distribution within the Golgi bodies, corroborating the notion that the ER is a principal localization site for KDEL-modified gold nanoparticle delivery [50].

Some research has also indicated that ER targeting improves the vaccine formulations that are used in cancer immunotherapy [51]. Notably, antigens in antigen-presenting cells bind the major histocompatibility complex (MHC) class I presentation pathway located in the ER [52]. The efficiency of tumor antigen peptide delivery into the MHC plays a major role in the activation of antitumor cytotoxic T-lymphocytes (CTLs) [52]. An example of an approach to ER targeted peptide delivery is the use of an ER insertion signal sequence (Eriss) [52]. Combining fusogenic liposomes and the Eriss sequence results in a modified nanocarrier that is capable of inducing in vivo tumor immunity [52]. Not only were these fusogenic liposome-encapsulated Eriss peptides able to induce more potent tumor immunity, they also continued activity for a minimum of 140 h, in comparison to the 40–60 h of activity that the fusogenic liposomes without the Eriss sequence and the antigen presenting cells with free peptides exhibited [52]. Although further research and development is necessary, ER targeting can introduce new, more efficacious treatments for disorders such as cancer [53], diabetes [54], cardiovascular disease, [55], neurodegeneration [56], hypoparathyroidism [57], and Crigler–Najjar syndrome [57].

5.5 Golgi Apparatus Delivery

The Golgi apparatus is the central organelle of the cell secretory pathway [26], responsible for posttranslational modification of proteins by utilizing enzymes for acylation, methylation, glycosylation, and phosphorylation [58]. The Golgi apparatus is also implicated in the synthesis of proteoglycans and carbohydrates [58]. This organelle is involved in various neurodegenerative disorders, such as

Parkinson's and Alzheimer's disease [59], and a group of 15 congenital glycosylation-related disorders that are caused by deadly mutations in the genes responsible for encoding glycosylation enzymes or glycosylation-linked transport proteins [59]. The Golgi apparatus is also being studied as a potential target for anticancer therapy [60].

It is important to recognize and understand the interactions between the ER and the Golgi when considering targeted therapy. Both of these organelles are part of the cell secretory pathway, making up the ER-Golgi network [26, 60]. Thus, malfunctions in either organelle can prompt further dysfunction in the other. For example, in cancer, defects in the transport of misfolded proteins from the ER to the cytosol can result in ER stress due to the accumulation of misfolded proteins [26, 61].

6 Conclusion

Without the ability to reach certain subcellular targets, many drug treatments are rendered either useless or minimally effective. Thus, the development of modified nanocarriers that can breach the biological barriers thwarting targeted delivery can lead to advancements in the treatment of innumerable diseases. By combining different surface modifications (such as PEGylation, targeting moieties, pH-responsible polymers or peptides, and cell-penetrating peptides), nanocarriers capable of endosomal escape, prolonged circulation, increased cellular uptake, and subcellular targeting can be developed. The different approaches may be separated into three chief categories, based on the relevant definition of targeting; targeting defined by accumulation of the therapeutic agent in the relevant tissue, targeting defined by accumulation of the therapeutic agent in the cell of interest, and lastly, targeting defined by reaching explicit subcellular components [1]. The last category represents the most challenging, and most specific, aspect of drug delivery [1]. Without a deep, nuanced understanding of the microenvironment of a particular site of action, refined therapeutic systems cannot be developed. Nanocarriers have shown several benefits and advantages that are being further studied and even being used in clinical trials. However, there are unresolved questions that require more research and testing.

This review examined different strategies of cytoplasmic, nuclear, mitochondrial, ER, and Golgi apparatus targeting. Nanocarriers with the ability to escape endosomal sequestration and subsequent degradation can be formulated through the conjugation of pH-sensitive polymers or peptides onto their surfaces [1]. Organelle targeting can be accomplished through increased affinity of carriers toward the targeted compartments [1]. Through surface modification, nanocarriers can now address particular obstacles that are present in different stages of drug delivery. However, the ultimate goal is the development of integrated strategies that address all of the steps in the delivery process.

7 Future Trends

Tremendous effort, research, and dedication is still necessary in order to devise a single, integrated approach that can potentially overcome all of the obstacles facing nanocarriers during their journey from the site of administration to the site of action. Two significant goals in drug delivery are cytoplasmic delivery and organelle delivery/targeting. Although this may initially be costly, the development of these revolutionary drug delivery systems may lead to decreased overall healthcare costs due to the enhanced efficacy of drugs, decreased concentrations of drugs required for treatment, and improved outcomes for several diseases, including different types of cancers that affect millions of people worldwide. These techniques and modified drug delivery vesicles should be cost-effective, safe, and easily reproduced.

The applications of nanocarriers extend far beyond medicine. In the future, nanotechnology may help shape our approaches to a plethora of industries and issues facing our planet. Nanoparticles can potentially play critical roles in the agricultural industry, in the fight against climate change, and in the expansion of knowledge related to patterns and systems of health. They may also introduce unprecedented methods for the prevention and prediction of disease. Nanotechnology has the potential to expedite diagnostic techniques through early detection of chemical and biological changes in the body. The future of nanomedicine may even tackle complicated mental health issues through the targeted delivery of drugs to the brain, infiltrating the notorious blood–brain barrier.

The advancement of nanotechnology can profoundly impact human beings, animals, electronics, and the environment. With its rise in prominence and public awareness, the use of nanotechnology in medicine has the potential for advantageous and profitable job creation. The development of new fields aimed at improving and further developing nanocarriers for drug delivery, addressing relevant ethical issues, controlling costs of production and distribution, and ensuring public safety can boost our healthcare system and economy simultaneously.

References

1. Parodi, A., Corbo, C., Cevenini, A., et al. (2015). Enabling cytoplasmic delivery and organelle targeting by surface modification of nanocarriers. *Nanomedicine, 10*(12), 1923–1940. https://doi.org/10.2217/nnm.15.39.
2. Din, F. U., Aman, W., Ullah, I., et al. (2017). Effective use of nanocarriers as drug delivery systems for the treatment of selected tumors. *International Journal of Nanomedicine, 12*, 7291–7309. https://doi.org/10.2147/IJN.S146315.
3. De Jong, W. H., & Borm, P. J. (2008). Drug delivery and nanoparticles: Applications and hazards. *International Journal of Nanomedicine, 3*(2), 133–149.
4. Wang, J., Lu, Z., Wientjes, M. G., & Au, J. L. S. (2010). Delivery of siRNA therapeutics: Barriers and carriers. *The AAPS Journal, 12*(4), 492–503.
5. Hoffman, A. S. (2013). Stimuli-responsive polymers: Biomedical applications and challenges for clinical translation. *Advanced Drug Delivery Reviews, 65*(1), 10–16.

6. Hoffman, K., Milech, N., Juraja, S. M., et al. (2018). A platform for discovery of functional cell-penetrating peptides for efficient multi-cargo intracellular delivery. Scientific Reports Published 2018. Accessed August 25, 2018, from https://www.nature.com/articles/s41598-018-30790-2.pdf
7. Madani, F., Lindberg, S., Langel, Ü., Futaki, S., & Gräslund, A. (2011). Mechanisms of cellular uptake of cell-penetrating peptides. *Journal of Biophysics, 2011*, 414729. https://doi.org/10.1155/2011/414729.
8. Järver, P., & Langel, Ü. (2006). Cell-penetrating peptides—A brief introduction. *Biochimica et Biophysica Acta, 1758*(3), 260–263.
9. Sandgren, S., Cheng, F., & Belting, M. (2002). Nuclear targeting of macromolecular polyanions by an HIV-Tat derived peptide role for cell-surface proteoglycans. *The Journal of Biological Chemistry, 277*(41), 38877–38883.
10. Maniti, O., Blanchard, E., Trugnan, G., Lamazière, A., & Ayala-Sanmartin, J. (2012). Metabolic energy-independent mechanism of internalization for the cell penetrating peptide penetratin. *The International Journal of Biochemistry & Cell Biology, 44*(6), 869–875.
11. Liu, J., Zhao, Y., Guo, Q., et al. (2012). TAT-modified nanosilver for combating multidrug-resistant cancer. *Biomaterials, 33*(26), 6155–6161.
12. Jallouk, A. P., Palekar, R. U., Pan, H., Schlesinger, P. H., & Wickline, S. A. (2015). Modifications of natural peptides for nanoparticle and drug design. *Advances in Protein Chemistry and Structural Biology, 98*, 57–91. https://doi.org/10.1016/bs.apcsb.2014.12.001.
13. Mahlapuu, M., Håkansson, J., Ringstad, L., & Björn, C. (2016). Antimicrobial peptides: An emerging category of therapeutic agents. *Frontiers in Cellular and Infection Microbiology, 6*, 194. https://doi.org/10.3389/fcimb.2016.00194.
14. Pushpanathan, M., Gunasekaran, P., & Rajendhran, J. (2013). Antimicrobial peptides: Versatile biological properties. *International Journal of Peptides, 2013*, 675391. https://doi.org/10.1155/2013/675391.
15. Vale, N., Aguiar, L., & Gomes, P. (2014). Antimicrobial peptides: A new class of antimalarial drugs? *Frontiers in Pharmacology, 5*, 275. https://doi.org/10.3389/fphar.2014.00275.
16. Golubeva, O. Y., Shamova, O., Orlov, D., et al. (2011). Synthesis and study of antimicrobial activity of bioconjugates of silver nanoparticles and endogenous antibiotics. *Glass Physics and Chemistry, 37*(1), 78–84.
17. Yang, K., Gitter, B., Rüger, R., et al. (2011). Antimicrobial peptide-modified liposomes for bacteria targeted delivery of temoporfin in photodynamic antimicrobial chemotherapy. *Photochemical & Photobiological Sciences, 10*(10), 1593–1601.
18. Varkouhi, A. K., Scholte, M., Storm, G., & Haisma, H. J. (2011). Endosomal escape pathways for delivery of biologicals. *Journal of Controlled Release, 151*(3). https://doi.org/10.1016/j.conrel.2010.11.004.
19. Benjaminsen, R. V., Mattebjerg, M. A., Henriksen, J. R., Moghimi, S. M., & Andresen, T. L. (2013). The possible "proton sponge" effect of polyethylenimine (PEI) does not include change in lysosomal pH. *Molecular Therapy, 21*(1), 149–157. https://doi.org/10.1038/mt.2012.185.
20. Moreira, C., Oliveira, H., Pires, L. R., et al. (2009). Improving chitosan-mediated gene transfer by the introduction of intracellular buffering moieties into the chitosan backbone. *Acta Biomaterialia, 5*(8), 2995–3006. https://doi.org/10.1016/j.actbio.2009.04.021.
21. Oliveira, S., Rooy, I. V., Kranenburg, O., et al. (2007). Fusogenic peptides enhance endosomal escape improving siRNA-induced silencing of oncogenes. *International Journal of Pharmaceuticals, 331*(2), 211–214. https://doi.org/10.1016/j.ijpharm.2006.11.050.
22. Stegmann, T. (2001) Membrane fusion mechanisms: The influenza hemagglutinin paradigm and its implications for intracellular fusion. Wiley Online Access. https://doi.org/10.1034/j.1600-0854.2000.010803.x.
23. Midoux, P., Pichon, C., Yaouanc, J.-J., & Jaffrès, P.-A. (2009). Chemical vectors for gene delivery: A current review on polymers, peptides and lipids containing histidine or imidazole as nucleic acids carriers. *British Journal of Pharmacology, 157*(2), 166–178. https://doi.org/10.1111/j.1476-5381.2009.00288.x.

24. Gruner, S. M., Tate, M. W., Kirk, G. L., So, P. T., Turner, D. C., Keane, D. T., et al. (1988). X-ray diffraction study of the polymorphic behavior of N-methylated dioleoylphosphatidylethanolamine. *Biochemistry, 27*, 2853–2866.
25. Farhood, H., Serbina, N., & Huang, L. (1995). The role of dioleoyl phosphatidylethanolamine in cationic liposome mediated gene transfer. *Biochimica et Biophysica Acta, 1235*, 289–295.
26. Sakhrani, N. M., & Padh, H. (2013). Organelle targeting: Third level of drug targeting. *Drug Design, Development and Therapy, 7*, 585–599. https://doi.org/10.2147/DDDT.S45614.
27. Muller, P. Y., & Milton, M. N. (2012). The determination and interpretation of the therapeutic index in drug development. *Nature Reviews. Drug Discovery, 11*(10), 751–761.
28. Yasuhara, N., Takeda, E., Inoue, H., Kotera, I., & Yoneda, Y. (2004). Importin alpha/beta-mediated nuclear protein import is regulated in a cell cycle-dependent manner. *Experimental Cell Research, 297*(1), 285–293.
29. Kabachinski, G., & Schwartz, T. U. (2015). The nuclear pore complex – Structure and function at a glance. *Journal of Cell Science, 128*, 423–429. https://doi.org/10.1242/jcs.083246.
30. Kubitscheck, U., Grünwald, D., Hoekstra, A., et al. (2005). Nuclear transport of single molecules dwell times at the nuclear pore complex. *The Journal of Cell Biology, 168*(2), 233–243.
31. Cook, A., Bono, F., Jinek, M., & Conti, E. (2007). Structural biology of nucleocytoplasmic transport. *Annual Review of Biochemistry, 76*, 647–671.
32. Raoof, M., Mackeyev, Y., Cheney, M. A., Wilson, L. J., & Curley, S. A. (2012). Internalization of C60 fullerenes into cancer cells with accumulation in the nucleus via the nuclear pore complex. *Biomaterials, 33*(10), 2952–2960.
33. Mackey, M. A., Saira, F., Mahmoud, M. A., & El-Sayed, M. A. (2013). Inducing cancer cell death by targeting its nucleus: Solid gold nanospheres versus hollow gold nanocages. *Bioconjugate Chemistry, 24*(6), 897–906. https://doi.org/10.1021/bc300592d.
34. Fukasawa, Y., Tsuji, J., Fu, S.-C., Tomii, K., Horton, P., & Imai, K. (2015). MitoFates: Improved prediction of mitochondrial targeting sequences and their cleavage sites. *Molecular & Cellular Proteomics: MCP, 14*(4), 1113–1126. https://doi.org/10.1074/mcp.M114.043083.
35. Duchen, M. R., & Szabadkai, G. (2010). Roles of mitochondria in human disease. *Essays in Biochemistry, 47*, 115–137.
36. Yoong, S. L., Wong, B. S., Zhou, Q. L., et al. (2014). Enhanced cytotoxicity to cancer cells by mitochondria-targeting MWCNTs containing platinum (IV) prodrug of cisplatin. *Biomaterials, 35*(2), 748–759.
37. Smith, R. A. J., & Murphy, M. P. (2010). Animal and human studies with the mitochondria-targeted antioxidant MitoQ. *Annals of the New York Academy of Sciences, 1201*, 96–103.
38. Gruber, J., Fong, S., Chen, C.-B., Yoong, S., Pastorin, G., Schaffer, S., et al. (2013). Mitochondria-targeted antioxidants and metabolic modulators as pharmacological interventions to slow ageing. *Biotechnology Advances, 31*, 563–592.
39. Gane, E. J., Weilert, F., Orr, D. W., Keogh, G. F., Gibson, M., Lockhart, M. M., et al. (2010). The mitochondria-targeted anti-oxidant mitoquinone decreases liver damage in a phase II study of hepatitis C patients. *Liver International, 30*, 1019–1026.
40. Snow, B. J., Rolfe, F. L., Lockhart, M. M., Frampton, C. M., O'Sullivan, J. D., Fung, V., et al. (2010). A double-blind, placebo-controlled study to assess the mitochondria-targeted antioxidant MitoQ as a disease-modifying therapy in Parkinson's disease. *Movement Disorders, 25*, 1670–1674.
41. Biswas, S., Dodwadkar, N. S., Deshpande, P. P., & Torchilin, V. P. (2012). Liposomes loaded with paclitaxel and modified with novel triphenylphosphonium-PEG-PE conjugate possess low toxicity, target mitochondria and demonstrate enhanced antitumor effects *in vitro* and *in vivo*. *Journal of Controlled Release, 159*(3), 393–402. https://doi.org/10.1016/j.jconrel.2012.01.009.
42. Tanaka, M., Borgeld, H. J., Zhang, J., et al. (2002). Gene therapy for mitochondrial disease by delivering restriction endonuclease SmaI into mitochondria. *Journal of Biomedical Science, 9*(6 Pt 1), 534–541.

43. D'souza, G. G., Boddapati, S. V., & Weissig, V. (2005). Mitochondrial leader sequence-plasmid DNA conjugates delivered into mammalian cells by DQAsomes co-localize with mitochondria. *Mitochondrion, 5*(5), 352–358.
44. Weiss, M. J., Wong, J. R., Ha, C. S., et al. (1987). Dequalinium, a topical antimicrobial agent, displays anticarcinoma activity based on selective mitochondrial accumulation. *Proceedings of the National Academy of Sciences, 84*(15), 5444–5448.
45. Wang, X.-X., Li, Y.-B., Yao, H.-J., et al. (2011). The use of mitochondrial targeting resveratrol liposomes modified with a dequalinium polyethylene glycol-distearoylphosphatidyl ethanolamine conjugate to induce apoptosis in resistant lung cancer cells. *Biomaterials, 32*(24), 5673–5687.
46. Yasuzaki, Y., Yamada, Y., & Harashima, H. (2010). Mitochondrial matrix delivery using MITO-Porter, a liposome-based carrier that specifies fusion with mitochondrial membranes. *Biochemical and Biophysical Research Communications, 397*(2), 181–186.
47. Yamada, Y., Furukawa, R., Yasuzaki, Y., & Harashima, H. (2011). Dual function MITO-porter, a nano carrier integrating both efficient cytoplasmic delivery and mitochondrial macromolecule delivery. *Molecular Therapy, 19*(8), 1449–1456. https://doi.org/10.1038/mt.2011.99.
48. Mo, R., Sun, Q., Li, N., & Zhang, C. (2013). Intracellular delivery and antitumor effects of pH-sensitive liposomes based on zwitterionic oligopeptide lipids. *Biomaterials, 34*(11), 2773–2786.
49. Boelens, J., Lust, S., Offner, F., Bracke, M. E., & Vanhoecke, B. W. (2007). Review. The endoplasmic reticulum: A target for new anticancer drugs. *In Vivo, 21*(2), 215–226.
50. Acharya, S., & Hill, R. A. (2014). High efficacy gold-KDEL peptide-siRNA nanoconstruct-mediated transfection in C2C12 myoblasts and myotubes. *Nanomedicine, 10*(2), 329–337.
51. Rajendran, L., Knolker, H. J., & Simons, K. (2010). Subcellular targeting strategies for drug design and delivery. *Nature Reviews. Drug Discovery, 9*(1), 29–42.
52. Leifert, J. A., Rodriguez-Carreno, M. P., Rodriguez, F., & Whitton, J. L. (2004). Targeting plasmid-encoded proteins to the antigen presentation pathways. *Immunological Reviews, 199*(1), 40–53.
53. Bi, M. X., Naczki, C., Koritzinsky, M., et al. (2005). ER stress-regulated translation increases tolerance to extreme hypoxia and promotes tumor growth. *The EMBO Journal, 24*(19), 3470–3481.
54. Ozcan, U., Cao, Q., Yilmaz, E., et al. (2004). Endoplasmic reticulum stress links obesity, insulin action, and type 2 diabetes. *Science, 306*(5695), 457–461.
55. Glembotski, C. C. (2008). The role of the unfolded protein response in the heart. *Journal of Molecular and Cellular Cardiology, 44*(3), 453–459.
56. Unterberger, U., Hoftberger, R., Gelpi, E., Flicker, H., Budka, H., & Voigtlander, T. (2006). Endoplasmic reticulum stress features are prominent in Alzheimer disease but not in prion diseases *in vivo*. *Journal of Neuropathology and Experimental Neurology, 65*(4), 348–357.
57. Rutishauser, J., & Spiess, M. (2002). Endoplasmic reticulum storage diseases. *Swiss Medical Weekly, 132*(17–18), 211–222.
58. Alberts, B., Johnson, A., Lewis, J., Raff, M., Roberts, K., & Walter, P. (2002). Vesicular traffic in the secretory and endocytic pathways. In *Molecular biology of the cell* (4th ed., pp. 1065–1126). New York: Garland.
59. Aridor, M., & Hannan, L. A. (2000). Traffic jam: A compendium of human diseases that affect intracellular transport processes. *Traffic, 1*(11), 836–851.
60. Wlodkowic, D., Skommer, J., McGuinness, D., Hillier, C., & Darzynkiewicz, Z. (2009). ER-Golgi network – A future target for anti-cancer therapy. *Leukemia Research, 33*(11), 1440–1447.
61. McCracken, A. A., & Brodsky, J. L. (2003). Evolving questions and paradigm shifts in endoplasmic-reticulum-associated degradation (ERAD). *Bio Essays, 25*, 868–877.

Chapter 19
Polydopamine-Based Simple and Versatile Surface Modification of Polymeric Nano Drug Carriers

Malay K. Das, Anupam Sarma, and Trinayan Deka

Abstract The surface modification of polymeric nanoparticle (NP) with bioactive ligands and/or secondary polymeric layers is a common strategy to govern the interaction of NPs with cells, proteins, and other biomolecules. But such surface engineering is not always so simple when the surface is chemically nonreactive. Because of this, NP surface modification processes generally employ reactive connector or coupling agents or prefunctionalization of the polymer, which are very tricky and ineffective. However, prefunctionalization of polymers can reduce the ability of drug encapsulation efficiency if the inserted ligands hamper the chemical properties of the polymer. To solve this issue, scientists have discovered a method of dopamine polymerization as a way of NP surfaces functionalization. In brief, this method involves the incubation of raw NPs in a weak alkaline solution of dopamine and subsequent incubation with ligands. This reaction furnishes a universal coating of polydopamine for metals, polymers, and ceramics, irrespective of their physicochemical characteristics. Polydopamine-based surface modified nanomaterials emerge as novel nanocomposite and get the interests in the area of drug delivery and therapy because of their unique physicochemical features, such as multifaceted adhesive property, great chemical reactivity, exceptional biocompatibility and biodegradability, and strong photothermal conversion capacity. This chapter highlights the recent development of polydopamine-based surface modified polymeric nanoparticles for smart drug delivery and therapy.

Keywords Polymeric nanoparticle · Surface coating · Polydopamine · Drug targeting · Tumour targeting

M. K. Das (✉) · A. Sarma · T. Deka
Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh, India
e-mail: mkdps@dibru.ac.in

Y. V Pathak (ed.), *Surface Modification of Nanoparticles for Targeted Drug Delivery*, https://doi.org/10.1007/978-3-030-06115-9_19

1 Introduction

Over the past few decades, polymeric nanoparticles (NPs) have gained attention in relation to targeted drug delivery, which improves the drug distribution in the desired site and also reduces the toxic effects on other organs. To maximize drug delivery to the desired site, NPs should possess a long circulatory half-life until they reach the target site (stealth effect) and should bind and enter the target cells (internalization). The stealth behavior of NPs can be reached by trimming the NP surface with hydrophilic, electrically neutral polymers like polyethylene glycol (PEG) [1, 2]. The interaction of NPs with the target can be accelerated by coupling cell-specific ligands on the NPs surface, which enhance cell attachment and uptake of NPs. Hence, to achieve targeted and controlled drug delivery, NPs should be modified with various functional moieties, such as surface modifiers and targeting ligands [3, 4], and should possess triggered release property toward cellular stimuli, such as pH, redox reactions, and enzymes [5–7]. Moreover, surface functionalization of polymeric NPs is quite difficult if the surface is chemically nonreactive. In this case, it is necessary to activate the NPs surface with the help of reactive linkers [8, 9] or coupling agents [10, 11]. In another approach, NPs can be prepared using prefunctionalized polymers by conjugating functional ligands with polymers [12–14]. But, the process for the synthesis of the polymer–ligand conjugate is a bit lengthy and inefficient and different for each ligand. However, the ligand can change the chemical properties of the conjugate, hindering the drug encapsulation ability of the polymer. To overcome these issues, scientists have employed a dopamine polymerization-based simple and versatile surface functionalization strategy. Dopamine (3,4-dihydroxyphenylethylamine) is a catecholamine that acts as an important neurotransmitter in the nervous system [15]. On oxidation, dopamine undergoes self-polymerization and forms polydopamine (PDA), which is analogous to naturally occurring melanin (eumelanin) [16]. PDA shows identical physicochemical properties as melanins in optics, electricity, and paramagnetism, and also biocompatibility and biodegradation. Another important characteristic of PDA is that it is rich in reactive groups like catechol, amine, and imine. Owing to these versatile functional moieties, PDA employed as a versatile adhesive podium to bind desired materials, realizing a diversity of composites such as metals, oxides, ceramics, polymers and even Teflon with tunable structures and functions [17–19]. The PDA on NP surfaces binds with ligands via Michael addition and/or Schiff base reactions to link them over the surface [20, 21]. The only requirement is the availability of nucleophilic functional groups such as amine and thiol on the ligand molecules. As of its simplicity and versatility, this principle has widely been utilized for surface modification of different types of polymeric NPs [20]. The preparation process of PDA is simple, less laborious, and the physicochemical properties can be tailored by further chemical modification. Furthermore, PDA shows low cytotoxicity and excellent biocompatibility, which make it a versatile podium for drug delivery application. The aim of this chapter is to figure out the progress of PDA-based surface modified polymeric nanococarriers for drug delivery application.

2 Features of Polydopamine

A better knowledge of basic characteristic of PDA in details would help to gain more ideas from different sectors to promote the potential applications in future. PDA possesses the amino and catechol groups from the starting material dopamine. The basic chemical reactions involved that lead to the formation of PDA aggregates are oxidation, cyclization, reorganization, coupling, and oxidative degradation [22, 23]. The chemical heterogeneity of PDA is confirmed by the broad optical absorption of PDA from deep ultraviolet to near infrared [24, 25]. PDA also contains phenolic, amino, and pyrrole–carboxylic acid groups that have a charge at mild pH values. PDA does have an ampholytic character that can be utilized to control the transport of ions [26]. PDA particles formed from Tris buffer are 3D fractal objects [27]. The high-resolution transmission electron microscopy (TEM) has revealed that PDA is comprised of stacked aromatic structures. The interlayer spacing of these stacked structures is 0.35 nm [28]. PDA absorbs UV radiation exponentially toward the ultraviolet spectrum. Because of its strong absorbance in the ultraviolet wavelengths, PDA shows photoprotection effect. The radical scavenging ability of PDA has also been evaluated in vitro based on 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. At a dose level of 120 μg, PDA nanoparticles could scavenge 85% of DPPH organic free radicals. More interestingly, the radical scavenging activity of PDA nanoparticles had a trend of increasing as the nanoparticle size decreased, being comparable to the value of ascorbic acid, a universal free radical scavenging material [29]. One of the most important properties of PDA that particularly intrigues physicists and chemists is its robust and strong adhesion to virtually all types of surfaces, regardless of the surface chemistry [30–34].

3 Modification of Polydopamine Surfaces

There are two major features of PDA those make it suitable for surface functionalization. First, it has the ability to form a nanoscale, conformal, and durable coating to any type of surface. Second, PDA coated surfaces are capable of further modification as per the requirement.

3.1 Chemical Modification of Dopamine Monomer

The catechol and amine functional group make PDA-coated surfaces reactive to a range of chemicals. However, in some cases, dopamine has been remodeled prior to polymerization for further surface modification. For example, dopamine modified with bromoisobutyryl bromide when applied with unmodified dopamine at a molar ratio of 1:1, creates a PDA macro initiator coating which is suitable for further

surface initiation of radical polymerization [35, 36]. When dopamine is modified with 1,3-propane sultone, a sulfonate variant has been formed which creates a negatively charged coating surface to allow better dispersion of coated particles [37].

3.2 Chemical Modification of Polydopamine Surfaces

PDA can be used as an intermediary surface material to allow attachment of other materials or chemical species without requiring prefunctionalization. The reactivity of PDA surfaces toward nucleophiles is dependent on the catechol–quinone equilibrium and consequently, alkaline pH accelerates conjugation reactions [21, 38]. In this instance, the pK_a of the nucleophile is also relevant as it is deprotonated nucleophiles that react and so for neutral pH imidazole (p$K_a \approx 6$) reacts better than a primary amine (p$K_a \approx 10$), but this is reversed at higher pH [21, 39, 40]. The coupling between nucleophiles and PDA surfaces have been used for the attachment of small molecules, synthetic polymers, biomolecules, and the construction of metal-organic frameworks [21, 40–45]. Due to the importance of the quinone group to PDA reactivity, thermal oxidation [46] has been used to increase the concentration of quinone in PDA coatings, doubling the attachment of nucleophiles to the surface [47]. The presence of hydroxyl and amine groups at the surface of PDA coatings also allows for more functionalization methods based on the reactivity of these groups. For example, a random copolymer containing glycidyl groups was attached to PDA coatings through reaction to amines and hydroxyl groups [48]. Also, the free amine groups of PDA have been used as a surface initiator for the ring opening polymerization of lactide, generating a polylactic acid coating [49].

3.3 Physical Modification of Polydopamine

PDA coatings can also be physically modified by changing to the coating method. For example, multiple depositions have been used to increase surface coating thickness which is limited for most surfaces with single coatings. Also, coatings can be smoothed by sonication in Tris buffer after coating and roughened by rapid agitation (200–300 rpm) during coating [50–52]. Another physical method of inducing surface roughness in PDA coatings was made by applying the coating to a uniaxial prestrained polydimethylsiloxane substrate. Upon release of the substrate strain, a striped wrinkled pattern emerges in the PDA film [53]. At lower temperatures (<150 °C) the annealing process caused reorientation within the film structure, allowing unreacted amines to take part in cyclization and cross-linking reactions, stabilizing the film [54].

4 Method and Mechanism of Polydopamine-Based Surface Modification

The simple surface coat of PDA can be achieved by incubating raw NPs in 0.5 mg/mL solution of dopamine hydrochloride dissolved in 10 mM Tris buffer (pH 8.5) at room temperature for 3 h with stirring [55–60]. Alternatively, dopamine solution can also be added to the NPs suspension [61–66]. Due to the chemical reaction, the dopamine is polymerized and deposited over the NPs and forms a layer over the NPs. The coated particles are then collected by centrifugation (12,000 rpm for 20 min) at 4 °C [67–71]. In some cases, the undeposited PDA and PDA coated NPs are separated by dialysis method in deionized water [72].

The versatile surface modification of polymeric NPs involves the second component during the deposition of dopamine over nanoparticles, including polymers, bio-macromolecules, small organic molecules, nanomaterials, and inorganic precursors. For surface functionalization, PDA coated NPs are resuspended in Tris buffer (10 mM, pH 8.5), which contained different ligands, incubated at room temperature for 30 min with rotation. The ligands are conjugated with the dopamine precoat by different physicochemical interaction. The functionalized particles are then washed with deionized water after collection by centrifugation [55, 57, 59, 69].

Lee et al. had proposed at first about the mussel-inspired chemistry for surface functionalization. In that study, they reported that a well-known neurotransmitter, dopamine, can form PDA coating on various materials after self-polymerization in the weak alkaline solution under the open air [20]. The mechanism of PDA coating involves two major ways: oxypolymerization and surface adhesion.

On oxidation, Dopamine is transformed to dopaminequinone and subsequently, it converted to 5,6 dihydroxyindole (DHI) through the intermolecular cyclization and then to polydopamine (Fig. 19.1) [19]. Scientists showed three different views on the polymerization mechanism of dopamine. PDA can form by dopamine molecules through noncovalent bonds including hydrogen bonding and π–π stacking [73], PDA may also form by the formation of dimers and trimmers via oxidation, coupling and then congregated to the PDA [74] and Liebscher et al. reported that some oligomers are produced in the solution of dopamine [75]. PDA is widely considered as a supramolecular aggregate rather than a covalent polymer with high molecular weight and the noncovalent bonds play a crucial role during the dopamine coating.

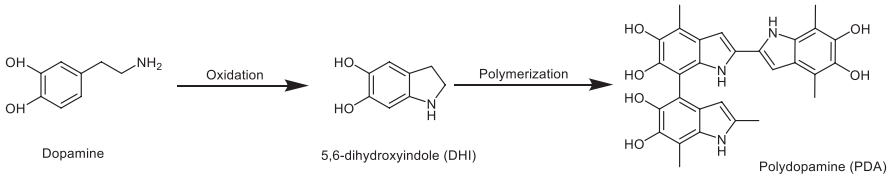


Fig. 19.1 Schematic illustration of Polydopamine (PDA) formation

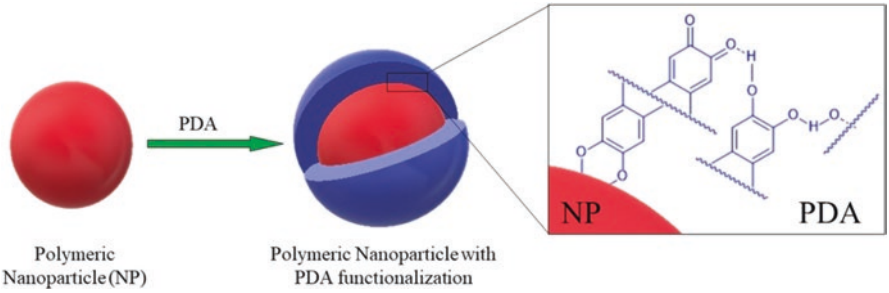


Fig. 19.2 Schematic illustration of PDA functionalization over polymeric nanoparticle

Another critical factor is the adhesion mechanism of PDA over nanoparticles (Fig. 19.2). The amino and catechol groups of PDA could interact with the surface of nanoparticles [76]. The aromatic structure present on PDA could interact with hydrophobic surfaces of nanoparticles through hydrophobic interaction and π–π stacking [77]. Whereas both amino and catechol functional groups of PDA are hydrogen donor, so it can able to form hydrogen bonds with the hydrogen acceptor group possessing polymers like PVP [78]. The electrostatic interactions are also observed in the case of charged surfaces because the amino groups are positively charged while the catechol groups are negatively charged [79]. In summary, the mechanism of PDA deposition combines the formation of PDA assemblies through single or multiple covalent/noncovalent interactions. The cross-linked structure in PDA also contributes to the adhesion.

The dopamine-assisted versatile surface modification of NPs is very vital as the interactions between the cocomponent and the PDA determines the deposition behavior and the final functionalization properties. There are various groups present in the dopamine molecule and PDA aggregate, like amino, phenolic hydroxyl, and an aromatic ring. Therefore, dopamine/PDA can interact with cocomponents via noncovalent interactions including hydrogen bond, hydrophobic force, and electrostatic attraction. For example, Zhang et al. investigated the interactions of several nonionic polymers like poly(ethylene glycol) (PEG), poly(*N*-vinyl pyrrolidone) (PVP), and poly(vinyl alcohol) (PVA) as cocomponents with PDA in the coating assembly, where the hydrogen bonds play a significant role [78]. But it was observed that the deposition amount of PDA/cocomponent decreases as compared to that of the neat PDA, especially in the case of PDA/PVP. The scientist postulated that PVP could hinder the formation of PDA aggregates by interrupting the noncovalent interactions of hydrogen bonding between PVP and DHI.

PDA is also able to interact with cocomponents via the electrostatic interactions. The amine group of codeposits can react with dopamine via Michael addition and Schiff-base reactions, whereas the quaternary amine can interact with PDA via the electrostatic interactions because PDA is always negatively charged in the deposition condition (generally in a pH range of 7–9). By contrast, polyanions can inhibit not only PDA aggregation but also PDA deposition. It has been found that PDA can be codeposited with poly(sulfobetaine methacrylate) (PSBMA), a typical

zwitterionic polymer, onto polypropylene membrane and steel mesh surfaces [80, 81]. The main mechanism of interaction is postulated as the local electrostatic attractions between the deprotonated phenol group and the quaternary ammonium rather than the hydrogen bonding [82]. But the hydrogen bonds may also form between PDA and zwitterionic polymer. For example, Chang et al. coated Si wafers by PDA with poly(methacryloyloxyethyl phosphorylcholine) (PMPC) through hydrogen bonding and the cation–π interaction [83].

As compared to the noncovalent versatile surface modification, the covalent versatile surface modification provides a stable coating. For example, PDA and low-molecular-weight polyethyleneimine (PEI) can be codeposited onto the surfaces of microporous polypropylene membrane [84, 85]. Due to the interaction between PDA and PEI accelerates the deposition process. Although PDA may react with PEI via different routes, Zhao et al. found that PEI molecules coupled with PDA mainly via the Michael addition reaction [86]. Individually PDA generally creates rough coating due to the stacking of large PDA aggregated particles. While the codeposited coating becomes uniform because the incorporation of PEI prevents the noncovalent interactions in PDA and reduces the particle size. Last but most important, the stability of the codeposited coating is greatly improved, especially in acidic and alkaline environments due to the formation of covalent bonds. Quan et al. reported the versatile surface modification by dopamine and poly(ethylene glycol) diglycidyl ether through reactions between the epoxy group and the hydroxyl or amino groups in dopamine [87].

5 Factors Affecting the Polymerization of Dopamine

The coating of NPs by PDA is generally held at room temperature in Tris–HCl buffer of pH 8.5. Numerous studies have been performed to find out the crucial parameters that can affect dopamine polymerization (i.e., duration of coating, dopamine coating solution concentration, pH of Tris–HCl, and temperature). Since dopamine is polymerized by oxidation hence some oxidizing agents are generally involved in the reaction. Normally, the dissolved oxygen that already in the coating solution is sufficient to progress the polymerization process [88]. But to maintain uniform oxygen concentration in the mixture, it must be kept on agitating during the reaction time. Otherwise, the fine PDA coat is formed at the air-water interface that increases in thickness as compared to submerged surfaces with reaction time over 24 h [89]. Usually for surface coating by PDA involves dopamine at a concentration of 2 mg/mL in 10 mM Tris buffer of pH 8.5 and applied to the surface with agitation under ambient temperature and pressure [20]. Under these circumstances, the thickness of the PDA coating depends upon coating time, remarkably 10 nm in 3 h and achieved approximately 40–50 nm thickness within 24 h [20, 21, 50]. Dopamine concentration also has an influence on PDA coating rate and thickness. It has been seen that coating rate increases with dopamine concentration up to 7.6 mg/mL [90]. Likewise, increasing the dopamine concentration within 0.5–10 mg/mL has been found to

Table 19.1 Factors affecting the polydopamine coating

Factors	Effects on the polydopamine coating
Rate of oxidation	Coating efficiency increases with increase in oxidation rate
Concentration of dopamine	Coating efficiency increases with increase in dopamine concentration
Coating duration	Coating efficiency increases with increase in coating time
Buffer pH	At pH $\leq$ 4.5 poor coating happens At pH 7 relatively improved coating happens At pH 8.5 highest coating efficiency observed At pH $\geq$ 11 an unstable coating forms
Temperature	Coating efficiency increases with rise in temperature

increase maximum film thickness, but with an unwanted increase in surface roughness [51, 91, 92]. Generally, temperature has shown proportionately minimal effects on coating kinetics compared to other universally accepted factors (i.e., 2 mg/mL, pH 8.5) [93]. A report suggested that higher temperature with agitation could increase coating kinetics, and the resultant PDA coat exhibit the same type of reactivity that had been modified at standard conditions [94]. Under UV light dopamine undergoes quick polymerization and form PDA coat more rapidly, resulting in 80 nm thick coating after 10 h as compared to 20 nm for coating conducted in the dark environment. The UV irradiation accelerates the intramolecular cyclization of oxidized quinone, generating a larger quantity of reactive monomers, which speeds up the polymerization [95]. Also, UV irradiation stabilizes catechol or nitrogen centered radicals for continuing polymerization and has been used in surface initiated free radical polymerization for modification of PDA surfaces. The Table 19.1 describes the different factors that affect the PDA coating.

6 Stability of Polydopamine Coating

Defining stability for PDA coatings can be difficult, particularly in the mild base in which monomers and poorly bound oligomers can be released without necessarily completely destroying the structure of the coating [39]. This release can occur because the stability in the mild base is in part mediated by the charge of the dopamine monomers. Under acidic and neutral conditions, the amine in dopamine is protonated and the charge is positive, while the overall charge of the aggregate is negative leading to opportunities for electrostatic interactions [96]. However, as the pH increases, deprotonation of the first hydroxyl ($pK_a \approx 9$) followed by the ammonium ($pK_a \approx 10.5$) and the second hydroxyl group ($pK_a \approx 12$) [76] changes the charge on the unreacted dopamine to negative [97]. This breaks the electrostatic interactions between dopamine monomers and the aggregate, releasing the dopamine into the solution. Similarly, relatively nonpolar DHI monomers will gain negative charge with deprotonation and become more soluble in polar solution [25]. As the dopamine and DHI monomers are part of the overall PDA supramolecular

structure, their release, along with the changing charge profile of the aggregate, can lead to changes to both the coating structure as well as to the supramolecular associations of the coating with the surface [98].

7 The Recent Development of Polydopamine-Based Surface-Modified Polymeric Nanoparticles for Drug Delivery

In many clinical situations, the most of the therapeutic agents are nonspecific and unable to reach the desired site in the body in adequate quantity due to various reasons which lead to systemic side effects and improper treatment. To reduce these inadequacies, the development of functional nanoparticles as drug delivery systems came across, which enable selective delivery of therapeutic agents at desired target sites of the body in the appropriate way. Polydopamine has been recently proposed as a good material for drug delivery, not only because of their excellent chemical versatility, high water solubility, excellent biocompatibility and biodegradation but also because of their cavities as well as their surfaces that allow for high payloads of drug molecules.

Now a day functionalization of the surface of a polymeric nanoparticle (NP) with cell targeting ligands or secondary polymeric layers is a trend to control the interaction between NPs and cells/proteins. However, this is not so easy when the surface is chemically nonreactive. To overcome this problem, generally, prefunctionalization of the polymer with reactive linkers or coupling agents is employed. Moreover, in this process, the polymers may lose the ability to encapsulate a drug. To solve this issue, Park et al. prepared a poly(lactic-co-glycolic acid) (PLGA) NPs, surface-modified with dopamine by incubating the preformed NPs in a weak alkaline dopamine solution. The PDA-derived NPs were then incubated with three different surface modifiers folate, Arg-Gly-Asp peptide, and polymer [poly(carboxybetaine methacrylate)] which act as a ligand and form the particle of size 100 nm. The cell uptake study on KB cells and HUVEC cells illustrates the effective internalization of the functionalized NPs as shown by confocal microscopy and flow cytometer [59]. Gastric cancer retains the third place in cancer-related mortality worldwide. Although gradually its occurrence is decreased, but still it poses a major challenge due to poor prognosis and limited treatments. Barbaloin (BBL) is the major bioactive composition of aloe vera possessing antioxidant, anti-inflammatory, and antitumor activities. The surface modification of polymeric NPs by Polydopamine (pD) is easy with the ability to conjugate ligands and additional polymeric layers. Wang et al. developed BBL-loaded pD-functionalized NPs, which were prepared by polylactide-TPGS (PLA-TPGS) (pD-PLA-TPGS/NPs). And galactosamine (Gal) was conjugated as a ligand on the NPs (Gal-pD-PLA-TPGS/NPs) for targeting the gastric cancer cells. The particle size of the prepared NPs was found 204.8 nm with a zeta potential of −13.8 mV. The cellular uptake study revealed that BBL-loaded Gal-pD-PLA-TPGS/NPs were efficiently taken up by gastric cancer cells

(SGC-7901) and significantly reduced the gastric cancer cell viability. In vivo study on mice showed that Gal-pD-PLA-TPGS/NPs were specifically targeted to tumor site as confirmed by the low tumor volume and tumor weight. And there was no significant difference was observed in body and liver weight, as well as the histological changes in major organs isolated from each group of mice which indicated the nontoxic behaviour of the formulation [55]. It is universally accepted that bone comprises of collagen and nanohydroxyapatite (nano-HA) in needle-like shapes. Because of its excellent bone-binding capacity and biological stability, HA material has great application in the clinic as artificial bone. However, it was also mentioned that nano-HA can hinder the growth of osteoblasts depending upon concentration. So to use HA as implantation candidate in bone graft substitutes, nano-HA need to be further modified. Mussel-inspired polydopamine (pD) coatings have several unique characteristics such as durability, versatility, and robustness. Sun et al. successfully prepared a novel bone forming peptide BMP-7 decorated, mussels inspired adhesive proteins dopamine coated nano-HA. Here nano-HA was prepared and coated by pH favoured dopamine polymerization, subsequently, the BMP-7 peptide was affixed onto polydopamine (pDA)-coated nano-HA (HA-pDA). The cell line study demonstrated that the BMP-7 conjugated nano-HA crystals could prompt the adhesion and proliferation of MG-63 cells. Moreover, the surface engineered nano-HA showed high alkaline phosphatase activity which signifies that the grafted peptide could maintain its bioactivity after conjugation with HA-pDA. These functionalized nano-HA crystals have the immense potential as biologically active materials in bone repairing and bone regeneration coating applications [72]. Sunoqrot et al. have designed and developed pD-coated methoxypolyethylene glycol-b-poly(ε-caprolactone) NPs (mPEG-PCL@pD) for gastro-retentive drug delivery (GRDD) with particle size 55.4 ± 3.7 nm and zeta potential −0.1 ± 0.6 mV. The mucoadhesive property of pD-coated NPs was studied in vitro using mucin under simulated condition mimicking the stomach lumen environment. The Mucin and NPs interactions at ratios of 1:1, 1:2, and 1:4 w/w were observed by dynamic light scattering, and an altered particle size was noticed. The increased turbidity of mucin/NPs was observed, which implies the development of bulky mucin-NP conjugation. They also concluded that there were no electrostatic interactions between mucin-pD-coated NPs as confirmed by zeta potential. The ex vivo wash-off experiment showed 78% attachment of pD-coated NPs on sheep stomach mucosa after incubation of 8 h, as compared to 33% of uncoated NPs. And a similar in vitro controlled release profile of rifampicin was observed between pD-coated and uncoated NPs [61]. Incorporating imaging and targeted moieties in multifunctional nanomaterials of biocompatible constituents provides great possibilities in cancer theranostic applications. Ao et al. showed a combination approach for surface modification of PDA based nanocomposites with magnetic and stimuli-controlled drug release property for clinical cancer theranostics. Here the iron oxide nanolayer was sandwiched in between PDA nanoparticles and surface coating PDA layer of the nanocomposite with increased near-infrared (NIR) photothermal conversion as well as great superparamagnetic property. Moreover, due to the high reactive property of PDA, it allowed facile linkage with doxorubicin and polyethylene

glycol chains for in vivo chemotherapy of cancer. The particle size of the nanocomposite was found 267 nm and a partially neutralized zeta potential of −15.6 mV. With the application of magnetic resonance imaging/photoacoustic imaging, the dual-modal tumor imaging and active magnetic tumor targeting of the nanocomposite for the successful tumor abolition were attained as confirmed by confocal imaging of 4 T1 cells [62]. Stability of nanoformulation in vitro as well as in vivo plays a vital role in nanotherapeutics. Amoozgar et al. prepared a protein-based doxorubicin loaded PLGA nanotherapeutic formulations to enhance the stability and therapeutic efficiency. In the study, proteins were embedded in the surface of PDA functionalized nanoparticles (NPs) to enhance protein stability and enzymatic activity. The NPs formed showed the particle size of <180 nm and surface charges −10 mV. The surface-coated protein provided a barrier, preventing the burst release of encapsulated doxorubicin. So the sustained delivery of doxorubicin reduced drug resistance in a breast tumor cell line, 4 T1 [63]. Nowadays, demand for chemically active polymeric layers of functionalized nanoparticles has arrived, which is a very complicated task as it may lead to the loss of ability to encapsulate the drug in sufficient amount. Bi et al. developed a pH-sensitive platform for functionalizing the surface of PLGA nanoparticles with PDA. Doxorubicin was successfully conjugated (DOX)-PDA-(PLGA) NPs with two targeting moieties folate (FA) and a peptide (Arg-Gly-Asp, RGD). The particle size came out 162.9 nm with negative zeta potential. The particles were quite stable in different physiological solutions and showed pH-dependent drug release property. In comparison to DOX-NPs, the targeting nanoparticles have tremendous targeting capability in HeLa cells. Moreover, the in vivo study explains 70% tumor inhibition by targeting nanoparticles with decreased DOX related side effects and improve drug accumulation in tumor site [67]. Metronomic chemotherapy hinders the development of drug resistance. But to achieve sustained tumor-specific chemotherapy remains difficult. Amoozgar et al. prepared paclitaxel-loaded PLGA nanoparticles functionalized with PDA and a successive layer of poly(ethylene glycol) (PEG). The particle size of the NPs was found 161.53 ± 1.42 nm and zeta potential −4.50 ± 8.92 mV. These particles attained a 3.8-fold higher loading compared to PLGA−PEG copolymer based nanoparticle. In vitro drug release kinetics and in vivo drug distribution profiles exhibited sustained release of paclitaxel. Moreover, administration of prepared nanoparticles intraperitoneally to drug-resistant ovarian tumor-bearing mice showed significant survival benefits without any systemic toxic effect [65]. Nie et al. developed a novel drug delivery system for the treatment of breast cancer using a PDA-based surface modification of NPs. The docetaxel (DTX)-loaded star-shaped copolymer cholic acid-PLGA nanoparticles (CA-PLGA@PDA/NPs) were coated with PDA and were conjugated with amino-poly(ethylene glycol)-folic acid (NH_2-PEG-FA) and bortezomib (BTZ) to form the targeting nanocomposite. The particle size of the nanocomposite was found 168.7 ± 4.2 nm and zeta potential −11.20 ± 3.6 mV. The in vitro cell uptake study on MCF-7, a breast cancer cell line, showed active targeting of the NPs. Moreover, BTZ release from the NPs was pH dependent on the tumor acidic environment for synergistic action with DTX [66]. Han et al. developed a nanoparticulate drug carrier system that interacts with tumor cells of the

mildly acidic microenvironment. The prepared polymeric nanoparticles were coated with PDA and modified with amidated TAT peptide. The treatment of cis-aconitic anhydride (CA) and succinic anhydride (SA) with the TAT-conjugated nanocomposite, transformed the amine groups of lysine in TAT peptide into β-carboxylic amides, by inserting carboxylic groups that go through pH-dependent protonation and deprotonation. The nanoparticles conjugated with amide derived TAT peptide (NLpT-CA and NPpT-CA) withstand the interactions with colon cancer cell line LS174T and macrophages cell line J774A.1 at pH 7.4, but showed the interaction with LS174T cell line at pH 6.5, and delivered paclitaxel effectively to the cells in a short contact time. In a mice model, NPpT-CA exhibited less localization in the lung compared to NPpT, indicating the shielding effect of amidation with minimal tumor accumulation of NPpT and NPpT-CA [68]. The main limitations of cancer chemotherapy include the ineffective strategy for targeted chemotherapeutic drug delivery and the difficulty to obtain significant efficacy from a single treatment. Kong et al. reported a synergistic strategy of chemophotothermal therapy for cancer. They have prepared docetaxel-loaded CA-(PCL-ran-PLA) based nanoparticles functionalized with polydopamine (pD) and conjugated with aptamer (Apt) for effective targeting and enhanced therapeutic effect. The particle size of the final NPs was 124.6 ± 5.1 nm and zeta potential −19.2 ± 5.2 mV with entrapment efficiency of 94.18 ± 2.76%. The cell uptake study on MCF-7 cells showed excellent internalization and increased drug concentration in tumor sites in vivo [57]. Xiong et al. synthesized block copolymer methoxy poly(ethylene glycol)-b-poly(ε-caprolactone) by ring-opening polymerization method and developed paclitaxel (PTX)-loaded MPEG-b-PCL nanoparticles, surfaces coated with polydopamine (PTX-loaded MPEG-b-PCL NPs@PDA) for malignant melanoma therapy. The modified nanoprecipitation technique was utilized for NPs preparation. The particle size was found as 141.8 ± 5.8 nm and zeta potential of 10.9 ± 1.5 mV. The cell uptake study showed effective internalization of coumarin-6-loaded NPs@PDA in A875 cells lines. The PTX-loaded NPs@PDA significantly suppress tumor growth as compared to Taxol® and pristine PTX-loaded NPs in nude mice model [99]. Zhu et al. synthesized DTX-loaded NPs using D-a-tocopherol polyethylene glycol 1000 succinate-poly(lactide) (pD-TPGS-PLA/NPs) and surface modified with polydopamine. Galactosamine was linked over prepared NPs (Gal-pD-TPGS-PLA/NPs) to increase the targeting efficiency in hepatocarcinoma cells, via ligand-driven endocytosis. The size of Gal-pD-TPGS-PLA/NPs was found 209.4 ± 5.1 nm and zeta potential of −13.7 ± 2.1 mV. The coumarin 6-loaded Gal-pD-TPGS-PLA/NPs exhibited effective cellular uptake in HepG2 cell line, as confirmed by confocal microscopy and flow cytometry. DTX-loaded Gal-pD-TPGS-PLA/NPs suppress the development of HepG2 cells in a greater extent than a clinically available DTX formulation (Taxotere). The in vivo anticancer efficacy study showed the decreased tumor size on hepatocarcinoma-bearing nude mice model [58]. In an another study Tao et al. prepared a DTX-loaded CA-PLGA-b-TPGS NPs (DTX/NPs) with polydopamine (pD)-based surface modification subsequently conjugated with aptamer (Apt-pD-DTX/NPs) for enhanced therapeutic effects of breast cancer. The size of the particles was found 112.1 ± 5.3 nm and zeta potential of −14.3 ± 3.9 mV. The aptamer conjugated NPs

showed great in vitro internalization in MCF-7 cells and MDA-MB-231 cells barely changed the characters of NPs. The in vivo animal studies in the rat model explains that the Apt-pD-DTX/NPs have the significant targeting ability and increased therapeutic response as compared to clinical Taxotere [69]. The Nanoparticle-based drug delivery to cancer is hampered by the heterogeneity of the enhanced permeability and retention (EPR) effect in cancer cells and release of drug during circulation before reaching the tumor site. To overcome this challenge Park et al. prepared a magnetophoretic strategy to NP delivery to cancer cells. They prepared polymer–iron oxide nanocomposites (PINCs) from PLGA and colloidal Fe_3O_4 and coated with PDA. The *Z*-average of PINCs was found 218 nm with a zeta potential of −12 mV. The PINCs were stable in serum-containing medium and gives a quick response to magnetic field gradients over 1 kG/cm. Under the field gradients, PINCs were rapidly get entered into SKOV3 cells as confirmed by cell uptake study. The In vivo study showed accumulation of PINCs in poorly vascularized subcutaneous SKOV3 xenografts without EPR effect [70]. Low molecular weight chitosan (LMWC) is a potential polymer for surface engineering of nanoparticles (NPs), which can perform stealth effect and electrostatic interaction with tumors at mild acidic pH. Abouelmagd et al. prepared paclitaxel loaded PLGA NPs coated with PDA and conjugated with LMWC (PLGA-pD-LMWC NPs). The size of the particles were found 209 nm and zeta potential measurement showed positive value when dispersed in MES buffer pH 6.2 and a negative value when dispersed in phosphate buffer pH 7.4. So, PLGA-pD-LMWC NPs had a pH-dependent surface charge and acid-specific NP-cell interaction and increased drug delivery to weakly acidic cell environment as showed by cell uptake study on SKOV-3 cells. PLGA-pD-LMWC NPs also showed less uptake by phagocyte as described by J774A.1 macrophages uptake study [71]. Gullotti et al. prepared PTX loaded PLGA NPs coated with PDA and further modified with TAT peptide (PLGA-pDA-TAT NPs) or dual modified with TAT peptide and hybrid of PEG and MMP-substrate peptide (peritumorally activatable NPs, PANPs). The particle size of the formulation was found 291.8 nm with a zeta potential of +0.3 mV. PLGA-pDA-TAT NPs and MMP-2-pretreated PANPs exhibited better cellular uptake in SKOV-3 cells [60]. Some of the important polydopamine based surface modified nano drug carriers are listed below (Table 19.2).

8 Conclusion and Future Prospects

In this chapter, an overview of the recent progress in research on dopamine-based surface modified polymeric nanoparticles in drug therapy is presented. Since the discovery of self-polymerization of dopamine by oxidation for fabrication of PDA derived materials, the focus has been on the preparation and applications of PDA-based nanocomposites. As time passed researchers starts to give attention to the interaction and cofabrication of dopamine with other materials to form a functional component. Dopamine derived materials possess many interesting physicochemical

Table 19.2 Polydopamine-based surface modified polymeric nanoparticle and its application

Polymer	Drug	Ligand	Cell line	Application	Ref.
PLGA	–	Folate, Arg-Gly-Asp, and poly(carboxybetaine methacrylate)	KB cells or HUVEC	Cancer	[59]
Polylactide-TPGS	Barbaloin	Galactosamine	SGC-7901	Gastric cancer	[55]
Hydroxyapatite	BMP-7	–	MG-63	Bone regeneration	[72]
Methoxypolyethylene glycol-b-poly(ε-caprolactone)	Rifampicin	–	–	GRDD	[61]
Polydopamine	Doxorubicin	–	4 T1	Brest cancer	[62]
PLGA	Doxorubicin	Lysozyme, DNase, collagenase I, or E-selectin antibody	4 T1	Brest cancer	[63]
PLGA	Doxorubicin	Folate and RGD	HeLa	Cervical cancer	[67]
PLGA	Paclitaxel	–	BR5FVB1-Akt	Ovarian cancer	[65]
Cholic acid-poly(lactide-co-glycolide)	Docetaxel, Bortezomib	Amino-poly(ethylene glycol)-folic acid	MCF-7	Breast cancer	[66]
PLGA	Paclitaxel	TAT peptide	LS174T or J774A	Colon cancer	[68]
CA-(PCL-ran-PLA) copolymer	Docetaxel	AS1411 Aptamer	MCF-7	Breast cancer	[57]
Methoxy poly(ethylene glycol)-b-poly(ε-caprolactone)	Paclitaxel	–	A875	Malignant melanoma	[99]
D-a-tocopherol polyethylene glycol 1000 succinate-poly(lactide)	Docetaxel	Galactosamine	HepG2	Liver cancer	[58]
CA-PLGA-b-TPGS	Docetaxel	AS1411 aptamer	MCF-7 or LNCaP	Breast cancer	[69]
PLGA	–	–	SKOV3	Ovarian cancer	[70]
PLGA	Paclitaxel	–	SKOV-3	Ovarian cancer	[71]
PLGA	Paclitaxel	TAT peptide	SKOV-3	Ovarian cancer	[60]

properties, including versatility in adhesion to the surface of any type and shape, great chemical reactivity with thiol and amino terminated materials and metal ions, and superb biocompatibility and biodegradability. These exciting features make dopamine-based nanomaterials very enticing for fabrication of functional

nanomedicine, which has been widely utilized in the treatment of various diseases, from diagnosis of a tumor, bio imaging, targeted or site-specific, controlled drug delivery, photo thermal therapy to combination therapy and imaging-guided therapies.

Although dopamine-based materials possess impressive advances in the preparation, functionalization, and drug delivery applications, there are still many challenges that should be sorted out in the near future to transform these materials from research into clinical applications. One of these is the lack of proper understanding of the mechanism of polymerization and the detailed structures of PDA. For a better understanding of the adhesive properties of the materials, such knowledge is essential. As dopamine itself is a drug, it is an immediate requirement for the toxicity evaluation of dopamine-based materials. Furthermore, more efforts must be directed to dopamine-based nanoplatform in targeted drug delivery and controlled release, which is a development direction for biomedicine in future.

In summary, due to its unique features and the simplicity of derivatization dopamine-based materials have been greatly utilized only in anticancer research till date. But equally, it has the potential for site-specific drug delivery including to brain as well. Although various obstacles exist in their clinical applications, it can be expected that dopamine-based materials will be available in the near future, creating a new solution in drug therapy.

Declaration All figures and tables are original and self-made.

References

1. Hong, R., Huang, C., & Tseng, Y. (1999, November). Direct comparison of liposomal doxorubicin with or without polyethylene glycol coating in C-26 tumor-bearing mice: Is surface coating with polyethylene glycol beneficial? Direct comparison of liposomal doxorubicin with or without polyethylene glycol coa. *Clinical Cancer Research, 5*, 3645–3652.
2. Hatakeyama, H., Akita, H., & Harashima, H. (2011). A multifunctional envelope type nano device (MEND) for gene delivery to tumours based on the EPR effect: A strategy for overcoming the PEG dilemma. *Advanced Drug Delivery Reviews, 63*(3), 152–160.
3. Kim, D., Kim, E., Kim, J., Park, K. M., Baek, K., Jung, M., Ko, Y. H., Sung, W., Kim, H. S., Suh, J. H., Park, C. G., Na, O. S., Lee, D. K., Lee, K. E., Han, S. S., & Kim, K. (2007). Direct synthesis of polymer nanocapsules with a noncovalently tailorable surface. *Angewandte Chemie, International Edition, 46*(19), 3471–3474.
4. Kim, E., Kim, D., Jung, H., Lee, J., Paul, S., Selvapalam, N., Yang, Y., Lim, N., Park, C. G., & Kim, K. (2010). Facile, template-free synthesis of stimuli-responsive polymer nanocapsules for targeted drug delivery. *Angewandte Chemie, International Edition, 49*(26), 4405–4408.
5. Liang, K., Such, G. K., Zhu, Z., Yan, Y., Lomas, H., & Caruso, F. (2011). Charge-shifting click capsules with dual-responsive cargo release mechanisms. *Advanced Materials, 23*(36), 273–277.
6. Yan, Y., Wang, Y., Heath, J. K., Nice, E. C., & Caruso, F. (2011). Cellular association and cargo release of redox-responsive polymer capsules mediated by exofacial thiols. *Advanced Materials, 23*(34), 3916–3921.

7. Ochs, C. J., Such, G. K., Yan, Y., Van Koeverden, M. P., & Caruso, F. (2010). Biodegradable click capsules with engineered drug-loaded multilayers. *ACS Nano, 4*(3), 1653–1663.
8. Sahoo, S. K., & Labhasetwar, V. (2005). Enhanced antiproliferative activity of transferrin-conjugated paclitaxel-loaded nanoparticles is mediated via sustained intracellular drug retention. *Molecular Pharmaceutics, 2*(5), 373–383.
9. Rao, K. S., Reddy, M. K., Horning, J. L., & Labhasetwar, V. (2008). TAT-conjugated nanoparticles for the CNS delivery of anti-HIV drugs. *Biomaterials, 29*(33), 4429–4438.
10. Narayanan, S., Binulal, N. S., Mony, U., Manzoor, K., Nair, S., & Menon, D. (2010). Folate targeted polymeric 'green' nanotherapy for cancer. *Nanotechnology, 21*(28), 1–13.
11. Mo, Y., & Lim, L. Y. (2005). Paclitaxel-loaded PLGA nanoparticles: Potentiation of anticancer activity by surface conjugation with wheat germ agglutinin. *Journal of Controlled Release, 108*(2–3), 244–262.
12. Gu, F., Zhang, L., Teply, B. A., Mann, N., Wang, A., Radovic-Moreno, A. F., Langer, R., & Farokhzad, O. C. (2008). Precise engineering of targeted nanoparticles by using self-assembled biointegrated block copolymers. *Proceedings of the National Academy of Sciences, 105*(7), 2586–2591.
13. Hrkach, J., Von Hoff, D., Ali, M. M., Andrianova, E., Auer, J., Campbell, T., De Witt, D., Figa, M., Figueiredo, M., Horhota, A., Low, S., McDonnell, K., Peeke, E., Retnarajan, B., Sabnis, A., Schnipper, E., Song, J. J., Song, Y. H., Summa, J., Tompsett, D., Troiano, G., Hoven, T. V. G., Wright, J., LoRusso, P., Kantoff, P. W., Bander, N. H., Sweeney, C., Farokhzad, O. C., Langer, R., & Zale, S. (2012). Preclinical development and clinical translation of a PSMA-targeted docetaxel nanoparticle with a differentiated pharmacological profile. *Science Translational Medicine, 4*(128), 1–11.
14. Tosi, G., Costantino, L., Rivasi, F., Ruozi, B., Leo, E., Vergoni, A. V., Tacchi, R., Bertolini, A., Vandelli, M. A., & Forni, F. (2007). Targeting the central nervous system: In vivo experiments with peptide-derivatized nanoparticles loaded with Loperamide and Rhodamine-123. *Journal of Controlled Release, 122*(1), 1–9.
15. Bibb, J. A., Snyder, G. L., Nishi, A., Yan, Z., Meijer, L., Flenberg, A. A., Tsai, L. H., Kwon, Y. T., Girault, J. A., Czernik, A. J., Huganir, R. L., Hemmings, H. C., Nairn, A. C., & Greengard, P. (1999). Phosphorylation of DARPP-32 by Cdk5 modulates dopamine signalling in neurons. *Nature, 402*(6762), 669–671.
16. d'Ischia, M., Napolitano, A., Ball, V., Chen, C.-T., & Buehler, M. J. (2014). Polydopamine and eumelanin: From structure-property relationships to a unified tailoring strategy. *Accounts of Chemical Research, 47*(12), 3541–3550.
17. Cui, X., Yin, Y., Ma, Z., Yin, Y., Guan, Y., Rong, S., Gao, J., Niu, Y., & Li, M. (2015). Polydopamine used as hollow capsule and core–shell structures for multiple applications. *Nano, 10*(05), 1530003-1–1530003-23.
18. Lynge, M. E., Van Der Westen, R., Postma, A., & Städler, B. (2011). Polydopamine – A nature-inspired polymer coating for biomedical science. *Nanoscale, 3*(12), 4916–4928.
19. Liu, Y., Ai, K., & Lu, L. (2014). Polydopamine and its derivative materials: Synthesis and promising applications in energy, environmental, and biomedical fields. *Chemical Reviews, 114*(9), 5057–5115.
20. Lee, H., Dellatore, S. M., Miller, W. M., & Messersmith, P. B. (2007). Mussel-inspired surface chemistry for multifunctional coatings haeshin. *Science, 318*(5849), 426–430.
21. Lee, H., Rho, J., & Messersmith, P. B. (2009). Facile conjugation of biomolecules onto surfaces via mussel adhesive protein inspired coatings. *Advanced Materials, 21*(4), 431–434.
22. Dreyer, D. R., Miller, D. J., Freeman, B. D., Paul, D. R., & Bielawski, C. W. (2013). Perspectives on poly(dopamine). *Chemical Science, 4*(10), 3796–3802.
23. D'Ischia, M., Napolitano, A., Ball, V., Chen, C. T., & Buehler, M. J. (2014). Polydopamine and eumelanin: From structure-property relationships to a unified tailoring strategy. *Accounts of Chemical Research, 47*(12), 3541–3550.
24. Wu, T. F., & Hong, J. D. (2016). Synthesis of water-soluble dopamine-melanin for ultrasensitive and ultrafast humidity sensor. *Sensors Actuators, B Chemical, 224*, 178–184.

25. Watt, A. A. R., Bothma, J. P., & Meredith, P. (2009). The supramolecular structure of melanin. *Soft Matter, 5*(19), 3754–3760.
26. Yu, B., Liu, J., Liu, S., & Zhou, F. (2010). Pdop layer exhibiting zwitterionicity: A simple electrochemical interface for governing ion permeability. *Chemical Communications, 46*(32), 5900–5902.
27. Fyodor, N., Vecchia, D., Luchini, A., Napolitano, A., Errico, G. D., Vitiello, G., Szekely, N., Ischia, M., Paduano, L., Ii, F., & Tecchio, P. V. (2014). Tris buffer modulates polydopamine growth, aggregation, and paramagnetic properties. *Langmuir, 30*(32), 9811–9818.
28. Zheng, W., Fan, H., Wang, L., & Jin, Z. (2015). Oxidative self-polymerization of dopamine in an acidic environment. *Langmuir, 31*(42), 11671–11677.
29. Barreto, W. J., Ponzoni, S., & Sassi, P. (1998). A Raman and UV-Vis study of catecholamines oxidized with Mn(III). *Spectrochimica Acta, Part A, 55*(1), 65–72.
30. Tsai, W. B., Chien, C. Y., Thissen, H., & Lai, J. Y. (2011). Dopamine-assisted immobilization of poly(ethylene imine) based polymers for control of cell-surface interactions. *Acta Biomaterialia, 7*(6), 2518–2525.
31. Coyne, K. J., Qin, X. X., & Waite, J. H. (1997). Extensible collagen in mussel byssus: A natural block copolymer. *Science, 277*(5333), 1830–1832.
32. Akemi Ooka, A., & Garrell, R. L. (2000). Surface-enhanced Raman spectroscopy of DOPA-containing peptides related to adhesive protein of marine mussel, *Mytilus edulis*. *Biopolymers, 57*(2), 92–102.
33. Lee, B. P., Dalsin, J. L., & Messersmith, P. B. (2002). Synthesis and gelation of DOPA-modified poly(ethylene glycol) hydrogels. *Biomacromolecules, 3*(5), 1038–1047.
34. Lee, B. P., Chao, C. Y., Nunalee, F. N., Motan, E., Shull, K. R., & Messersmith, P. B. (2006). Rapid gel formation and adhesion in photocurable and biodegradable block copolymers with high DOPA content. *Macromolecules, 39*(5), 1740–1748.
35. Zhu, B., & Edmondson, S. (2011). Polydopamine-melanin initiators for surface-initiated ATRP. *Polymer, 52*(10), 2141–2149.
36. Ye, G., Lee, J., Perreault, F., & Elimelech, M. (2015). Controlled architecture of dual-functional block copolymer brushes on thin-film composite membranes for integrated 'defending' and 'attacking' strategies against biofouling. *ACS Applied Materials & Interfaces, 7*(41), 23069–23079.
37. Chung, Y. C., & Huang, J. Y. (2014). Water-borne composite coatings using nanoparticles modified with dopamine derivatives. *Thin Solid Films, 570*(Part B), 376–382.
38. Mostert, A. B., Powell, B. J., Pratt, F. L., Hanson, G. R., Sarna, T., Gentle, I. R., & Meredith, P. (2012). Role of semiconductivity and ion transport in the electrical conduction of melanin. *Proceedings of the National Academy of Sciences, 109*(23), 8943–8947.
39. Ball, V. (2014). Physicochemical perspective on 'polydopamine' and 'poly(catecholamine)' films for their applications in biomaterial coatings. *Biointerphases, 9*(3), 030801-1–030801-10.
40. Yeroslavsky, G., Girshevitz, O., Foster-Frey, J., Donovan, D. M., & Rahimipour, S. (2015). Antibacterial and antibiofilm surfaces through polydopamine-assisted immobilization of lysostaphin as an antibacterial enzyme. *Langmuir, 31*(3), 1064–1073.
41. Cai, T., Li, X., Wan, C., & Chung, T. S. (2016, September). Zwitterionic polymers grafted poly(ether sulfone) hollow fiber membranes and their antifouling behaviors for osmotic power generation. *Journal of Membrane Science, 497*, 142–152.
42. Schaubroeck, D., Mader, L., Dubruel, P., & Vanfleteren, J. (2015). Surface modification of an epoxy resin with polyamines and polydopamine: Adhesion toward electroless deposited copper. *Applied Surface Science, 353*, 238–244.
43. Wang, N., Liu, Y., Qiao, Z., Diestel, L., Zhou, J., Huang, A., & Caro, J. (2015). Polydopamine-based synthesis of a zeolite imidazolate framework ZIF-100 membrane with high H_2/CO_2 selectivity. *Journal of Materials Chemistry A, 3*(8), 4722–4728.
44. Fu, L., Shi, Y., Wang, K., Zhou, P., Liu, M., Wan, Q., Tao, L., Zhang, X., & Wei, Y. (2015). Biomimic modification of graphene oxide. *New Journal of Chemistry, 39*(10), 8172–8178.

45. Vaish, A., Vanderah, D. J., Richter, L. J., Dimitriou, M., Steffens, K. L., & Walker, M. L. (2015). Dithiol-based modification of poly(dopamine): Enabling protein resistance via short-chain ethylene oxide oligomers. *Chemical Communications, 51*(30), 6591–6594.
46. Luo, R., Tang, L., Wang, J., Zhao, Y., Tu, Q., Weng, Y., Shen, R., & Huang, N. (2013, June). Improved immobilization of biomolecules to quinone-rich polydopamine for efficient surface functionalization. *Colloids Surfaces B Biointerfaces, 106*, 66–73.
47. Martín, M., Orive, A. G., Lorenzo-Luis, P., Creus, A. H., González-Mora, J. L., & Salazar, P. (2014). Quinone-rich poly(dopamine) magnetic nanoparticles for biosensor applications. *Chemphyschem, 15*(17), 3742–3752.
48. Yang, W. J., Neoh, K.-G., Kang, E.-T., Lay-Ming Teo, S., & Rittschof, D. (2013). Stainless steel surfaces with thiol-terminated hyperbranched polymers for functionalization via thiol-based chemistry. *Polymer Chemistry, 4*(10), 3105–3115.
49. Mrówczyński, R., Nan, A., Turcu, R., Leistner, J., & Liebscher, J. (2015). Polydopamine – A versatile coating for surface-initiated ring-opening polymerization of lactide to polylactide. *Macromolecular Chemistry and Physics, 216*(2), 211–217.
50. Bernsmann, F., Ponche, A., Ringwald, C., Hemmerlé, J., Raya, J., Bechinger, B., Voegel, J., Schaaf, P., & Ball, V. (2009). Characterization of dopamine−melanin growth on silicon oxide. *Journal of Physical Chemistry C, 113*(19), 8234–8242.
51. Cho, J. H., Katsumata, R., Zhou, S. X., Bin Kim, C., Dulaney, A. R., Janes, D. W., & Ellison, C. J. (2016). Ultrasmooth polydopamine modified surfaces for block copolymer nanopatterning on flexible substrates. *ACS Applied Materials & Interfaces, 8*(11), 7456–7463.
52. Su, L., Yu, Y., Zhao, Y., Liang, F., & Zhang, X. (2016). Strong antibacterial polydopamine coatings prepared by a shaking-assisted method. *Scientific Reports, 6*(24420), 1–8.
53. Meng, J., Xie, J., Han, X., & Lu, C. (2016, May). Surface wrinkling on polydopamine film. *Applied Surface Science, 371*, 96–101.
54. Proks, V., Brus, J., Pop-Georgievski, O., Večerníková, E., Wisniewski, W., Kotek, J., Urbanová, M., & Rypáček, F. (2013). Thermal-induced transformation of polydopamine structures: An efficient route for the stabilization of the polydopamine surfaces. *Macromolecular Chemistry and Physics, 214*(4), 499–507.
55. Wang, Y. R., Yang, S. Y., Chen, G. X., & Wei, P. (2018). Barbaloin loaded polydopamine-polylactide-TPGS (PLA-TPGS) nanoparticles against gastric cancer as a targeted drug delivery system: Studies in vitro and in vivo. *Biochemical and Biophysical Research Communications, 499*(1), 8–16.
56. Wang, H., Zhu, W., Huang, Y., Li, Z., Jiang, Y., & Xie, Q. (2017, October). Facile encapsulation of hydroxycamptothecin nanocrystals into zein-based nanocomplexes for active targeting in drug delivery and cell imaging. *Acta Biomaterialia, 61*, 88–100.
57. Kong, N., Deng, M., Sun, X. N., Chen, Y. D., & Sui, X. B. (2018). Polydopamine-functionalized CA-(PCL-ran-PLA) nanoparticles for target delivery of docetaxel and chemo-photothermal therapy of breast cancer. *Frontiers in Pharmacology, 9*(125), 1–14.
58. Zhu, D., Tao, W., Zhang, H., Liu, G., Wang, T., Zhang, L., Zeng, X., & Mei, L. (2016, January). Docetaxel (DTX)-loaded polydopamine-modified TPGS-PLA nanoparticles as a targeted drug delivery system for the treatment of liver cancer. *Acta Biomaterialia, 30*, 144–154.
59. Park, J., Brust, T. F., Lee, H. J., Lee, S. C., Watts, V. J., & Yeo, Y. (2014). Polydopamine-based simple and versatile surface modification of polymeric nano drug carriers. *ACS Nano, 8*(4), 3347–3356.
60. Gullotti, E., Park, J., & Yeo, Y. (2013). Polydopamine-based surface modification for the development of peritumorally activatable nanoparticles. *Pharmaceutical Research, 30*(8), 1956–1967.
61. Sunoqrot, S., Hasan, L., Alsadi, A., Hamed, R., & Tarawneh, O. (2017, August). Interactions of mussel-inspired polymeric nanoparticles with gastric mucin: Implications for gastro-retentive drug delivery. *Colloids Surfaces B Biointerfaces, 156*, 1–8.

62. Ao, L., Wu, C., Liu, K., Wang, W., Fang, L., Huang, L., & Su, W. (2018). Polydopamine-derivated hierarchical nanoplatforms for efficient dual-modal imaging-guided combination in vivo cancer therapy. *ACS Applied Materials & Interfaces, 10*(15), 12544–12552.
63. Amoozgar, Z., & Goldberg, M. S. (2017). Surface modulation of polymeric nanocarriers enhances the stability and delivery of proteins and small molecules. *Nanomedicine, 12*(7), 729–743.
64. Cui, J., Yan, Y., Such, G. K., Liang, K., Ochs, C. J., Postma, A., & Caruso, F. (2012). Immobilization and intracellular delivery of an anticancer drug using mussel-inspired polydopamine capsules. *Biomacromolecules, 13*(8), 2225–2228.
65. Amoozgar, Z., Wang, L., Brandstoetter, T., Wallis, S. S., Wilson, E. M., & Goldberg, M. S. (2014). Dual-layer surface coating of PLGA-based nanoparticles provides slow-release drug delivery to achieve metronomic therapy in a paclitaxel-resistant murine ovarian cancer model. *Biomacromolecules, 15*(11), 4187–4194.
66. Nie, J., Cheng, W., Peng, Y., Liu, G., Chen, Y., Wang, X., Liang, C., Tao, W., Wei, Y., Zeng, X., & Mei, L. (2017). Co-delivery of docetaxel and bortezomib based on a targeting nanoplatform for enhancing cancer chemotherapy effects. *Drug Delivery, 24*(1), 1124–1138.
67. Bi, D., Zhao, L., Yu, R., Li, H., Guo, Y., Wang, X., & Han, M. (2018). Surface modification of doxorubicin-loaded nanoparticles based on polydopamine with pH-sensitive property for tumor targeting therapy. *Drug Delivery, 25*(1), 564–575.
68. Han, N., Pang, L., Xu, J., Hyun, H., Park, J., & Yeo, Y. (2017). Development of surface-variable polymeric nanoparticles for drug delivery to tumors. *Molecular Pharmaceutics, 14*(5), 1538–1547.
69. Tao, W., Zeng, X., Wu, J., Zhu, X., Yu, X., Zhang, X., Zhang, J., Liu, G., & Mei, L. (2016). Polydopamine-based surface modification of novel nanoparticle-aptamer bioconjugates for in vivo breast cancer targeting and enhanced therapeutic effects. *Theranostics, 6*(4), 470–484.
70. Park, J., Kadasala, N. R., Abouelmagd, S. A., Castanares, M. A., David, S., Wei, A., Yeo, Y., Pharmacy, P., Lafayette, W., Lilly, E., Lafayette, W., & Lafayette, W. (2016, September). Polymer–iron oxide composite nanoparticles for EPR-independent drug delivery. *Biomaterials, 101*, 285–295.
71. Abouelmagd, S. A., Ku, Y. J., Yeo, Y., Pharmacy, P., Lafayette, W., & Lafayette, W. (2015). Low molecular weight chitosan-coated polymeric nanoparticles for sustained and pH-sensitive delivery of paclitaxel. *Journal of Drug Targeting, 23*(7–8), 725–735.
72. Sun, Y., Deng, Y., Ye, Z., Liang, S., Tang, Z., & Wei, S. (2013, November). Peptide decorated nano-hydroxyapatite with enhanced bioactivity and osteogenic differentiation via polydopamine coating. *Colloids Surfaces B Biointerfaces, 111*, 107–116.
73. Dreyer, D. R., Miller, D. J., Freeman, B. D., Paul, D. R., & Bielawski, C. W. (2012). Elucidating the structure of poly(dopamine). *Langmuir, 28*(15), 6428–6435.
74. Hong, S., Na, Y. S., Choi, S., Song, I. T., Kim, W. Y., & Lee, H. (2012). Non-covalent self-assembly and covalent polymerization co-contribute to polydopamine formation. *Advanced Functional Materials, 22*(22), 4711–4717.
75. Liebscher, J., Mrówczyński, R., Scheidt, H. A., Filip, C., Haìdade, N. D., Turcu, R., Bende, A., & Beck, S. (2013). Structure of polydopamine: A never-ending story? *Langmuir, 29*(33), 10539–10548.
76. Ye, Q., Zhou, F., & Liu, W. (2011). Bioinspired catecholic chemistry for surface modification. *Chemical Society Reviews, 40*(7), 4244–4258.
77. Leng, C., Liu, Y., Jenkins, C., Meredith, H., Wilker, J. J., & Chen, Z. (2013). Interfacial structure of a DOPA-inspired adhesive polymer studied by sum frequency generation vibrational spectroscopy. *Langmuir, 29*(22), 6659–6664.
78. Zhang, Y., Thingholm, B., Goldie, K. N., Ogaki, R., & Städler, B. (2012). Assembly of poly(dopamine) films mixed with a nonionic polymer. *Langmuir, 28*(51), 17585–17592.
79. Lee, H., Scherer, N. F., & Messersmith, P. B. (2006). Single-molecule mechanics of mussel adhesion. *Proceedings of the National Academy of Sciences, 103*(35), 12999–13003.

80. Zhou, R., Ren, P. F., Yang, H. C., & Xu, Z. K. (2014, September). Fabrication of antifouling membrane surface by poly(sulfobetaine methacrylate)/polydopamine co-deposition. *Journal of Membrane Science, 466*, 18–25.
81. Ren, P. F., Yang, H. C., Jin, Y. N., Liang, H. Q., Wan, L. S., & Xu, Z. K. (2015). Underwater superoleophobic meshes fabricated by poly(sulfobetaine)/polydopamine co-deposition. *RSC Advances, 5*(59), 47592–47598.
82. Ren, P. F., Yang, H. C., Liang, H. Q., Xu, X. L., Wan, L. S., & Xu, Z. K. (2015). Highly stable, protein-resistant surfaces via the layer-by-layer assembly of poly(sulfobetaine methacrylate) and tannic acid. *Langmuir, 31*(21), 5851–5858.
83. Chang, C. C., Kolewe, K. W., Li, Y., Kosif, I., Freeman, B. D., Carter, K. R., Schiffman, J. D., & Emrick, T. (2016). Underwater superoleophobic surfaces prepared from polymer zwitterion/dopamine composite coatings. *Advanced Materials Interfaces, 3*(6), 1–9.
84. Yang, H. C., Liao, K. J., Huang, H., Wu, Q. Y., Wan, L. S., & Xu, Z. K. (2014). Mussel-inspired modification of a polymer membrane for ultra-high water permeability and oil-in-water emulsion separation. *Journal of Materials Chemistry A, 2*(26), 10225–10230.
85. Yang, H. C., Xu, W., Du, Y., Wu, J., & Xu, Z. K. (2014). Composite free-standing films of polydopamine/polyethyleneimine grown at the air/water interface. *RSC Advances, 4*(85), 45415–45418.
86. Zhao, C., Zuo, F., Liao, Z., Qin, Z., Du, S., & Zhao, Z. (2015). Mussel-inspired one-pot synthesis of a fluorescent and water-soluble polydopamine-polyethyleneimine copolymer. *Macromolecular Rapid Communications, 36*(10), 909–915.
87. Quan, S., Li, S., Wang, Z., Yan, X., Guo, Z., & Shao, L. (2015). A bio-inspired CO_2-philic network membrane for enhanced sustainable gas separation. *Journal of Materials Chemistry A, 3*(26), 13758–13766.
88. Karkhanechi, H., Takagi, R., & Matsuyama, H. (2014). Enhanced antibiofouling of RO membranes via polydopamine coating and polyzwitterion immobilization. *Desalination, 337*(1), 23–30.
89. Wu, T. F., & Hong, J. D. (2015). Dopamine-melanin nanofilms for biomimetic structural coloration. *Biomacromolecules, 16*(2), 660–666.
90. Zhang, C., Lv, Y., Qiu, W. Z., He, A., & Xu, Z. K. (2017). Polydopamine coatings with nanopores for versatile molecular separation. *ACS Applied Materials & Interfaces, 9*(16), 14437–14444.
91. Zhang, L., Shi, J., Jiang, Z., Jiang, Y., Qiao, S., Li, J., Wang, R., Meng, R., Zhu, Y., & Zheng, Y. (2011). Bioinspired preparation of polydopamine microcapsule for multienzyme system construction. *Green Chemistry, 13*(2), 300–306.
92. Ball, V., Del Frari, D., Toniazzo, V., & Ruch, D. (2012). Kinetics of polydopamine film deposition as a function of pH and dopamine concentration: Insights in the polydopamine deposition mechanism. *Journal of Colloid and Interface Science, 386*(1), 366–372.
93. Shin, Y. M., Jun, I., Lim, Y. M., Rhim, T., & Shin, H. (2013). Bio-inspired immobilization of cell-adhesive ligands on electrospun nanofibrous patches for cell delivery. *Macromolecular Materials and Engineering, 298*(5), 555–564.
94. Zhou, P., Deng, Y., Lyu, B., Zhang, R., Zhang, H., Ma, H., Lyu, Y., & Wei, S. (2014). Rapidly-deposited polydopamine coating via high temperature and vigorous stirring: Formation, characterization and biofunctional evaluation. *PLoS One, 9*(11), 1–10.
95. Sheng, W., Li, B., Wang, X., Dai, B., Yu, B., Jia, X., & Zhou, F. (2015). Brushing up from 'anywhere' under sunlight: A universal surface-initiated polymerization from polydopamine-coated surfaces. *Chemical Science, 6*(3), 2068–2073.
96. Ball, V. (2010). Impedance spectroscopy and zeta potential titration of dopa-melanin films produced by oxidation of dopamine. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 363*(1–3), 92–97.
97. Mack, F., & Bönisch, H. (1979). Dissociation constants and lipophilicity of catecholamines and related compounds. *Naunyn-Schmiedeberg's Archives of Pharmacology, 310*(1), 1–9.

98. Klosterman, L., Riley, J. K., & Bettinger, C. J. (2015). Control of heterogeneous nucleation and growth kinetics of dopamine-melanin by altering substrate chemistry. *Langmuir, 31*(11), 3451–3458.
99. Xiong, W., Peng, L., Chen, H., & Li, Q. (2015). Surface modification of MPEG-b-PCL-based nanoparticles via oxidative self-polymerization of dopamine for malignant melanoma therapy. *International Journal of Nanomedicine, 10*, 2985–2996.

Chapter 20
Surface Modification of Gold Nanoparticles for Targeted Drug Delivery

Benson Peter Mugaka, Yihui Hu, Yu Ma, and Ya Ding

Abstract Gold nanoparticles (AuNPs) are the most widely studied and used inorganic nanoparticles in biomedical researches and applications, due to their controllable shape and size, biological inertia, and optical and photothermal therapeutic properties. Besides the shape and size, surface property is also a critical factor that influences the performance of AuNPs in vivo, especially for targeted drug delivery using AuNPs as the carriers. Two approaches, noncovalent and covalent interactions, are commonly employed to modify the surface of AuNPs. Each of them has advantages and disadvantages. In this chapter, we focus on these two kinds of surface modification methods and their applications in regulating the properties and performance of AuNP-based nanosystems for targeted drug delivery. It provides valuable reference and guide to implement modification of the surface for nanomaterials and realize the unique functions in diagnosis and treatment.

Keywords Gold nanoparticles · Surface modification · Drug delivery · Specific targeting

1 Introduction

The advances in nanotechnology accelerate the development of biomedicine, especially the nanoparticle (NP)-based targeted delivery systems (DDs) and their precise therapy [1, 2]. Gold nanoparticles (AuNPs) are the most widely studied and considered the most promising nanomaterials for biomedical applications due to the finely controlled shape, size, and surface chemistry [3–5]. Besides the biological inert rooted in the nature of zero valent gold, the desirable physicochemical and biological properties of AuNPs, including the solubility, dispersity, and biocompatibility, are critically influenced by the surface chemistry [6]. The surface chemistry not only decide the recognition between biomolecules and active drugs loaded but also determine the destination of the nanoparticles [7–9].

B. P. Mugaka · Y. Hu · Y. Ma · Y. Ding (✉)
Department of Pharmaceutical Analysis, China Pharmaceutical University, Nanjing, China
e-mail: dingya@cpu.edu.cn

Y. V Pathak (ed.), *Surface Modification of Nanoparticles for Targeted Drug Delivery*, https://doi.org/10.1007/978-3-030-06115-9_20

The surface modification of AuNPs provides a flexible platform for biological assemblies and is helpful to control the physical and chemical bounding of drugs [10–12]. Through rationally designing the chemical structure of surface ligand or choose naturally generated modifier, the surface functional groups (such as $-COO^-$, $-NH_3^+$, and $-$ SH) [6, 13], charges (e.g., positive, negative, and zwitterionic), and density of surface modifier on AuNPs can be finely adjusted [14, 15]. Furthermore, the attachment of function groups allow the conjugation of a variety of agents (e.g., antibodies, peptides/amino acids, target ligands to coordinate and bind with receptors, desired drugs, and intelligent genes) to build multifunctional nanoparticles for research and application in biosensors [9, 16], bioimaging [17–19], and biomedicine such as that for cancers [20–24].

Using AuNPs as drug carriers started with the conjugation of tumor necrosis factor (TNF) on the surface of gold through the formation of covalent bonds [25]. Up to date, several surface modification strategies have been developed, involving noncovalent and covalent methods. The relative research has been reported in our previous literatures [26, 27]. Recently, the specificity and selectivity response to a cellular or tumor microenvironments become more important in the research of precise treatment. The design of nanoparticles that integrates each unit of whole system and plays the possible synergistic effect attracts more attentions than before. In this chapter, we focus on the targeted drug delivery based on the surface modification of AuNPs via smart fabrication.

2 Surface Modifications of AuNPs by Noncovalent Interaction

A noncovalent interaction is a simple surface modification method. Different from a covalent bond, it does not involve the sharing of electrons. Although showing weaker chemical energy than covalent bond, noncovalent interactions are critical in maintaining the three-dimensional structure of large molecules and offer the reversible bonding and release between molecules. It can be classified into different categories, such as electrostatic, π–π effects, van der Waals forces, and hydrophobic effects.

2.1 Surface Modification by Electrostatic Interaction

Electrostatic phenomena arise from the forces that electric charges exert on each other. Using citrate as the protector, AuNP preparation in the presence of citrate salt is the most common method. Taking advantages of the passive charge of citrate, electrostatic interaction become the simplest method to modified gold surface. Many artificial and naturally produced molecules having negative or positive charges have been modified on the surface of AuNPs via directly electrostatic effect or layer-by-layer (L-b-L) method.

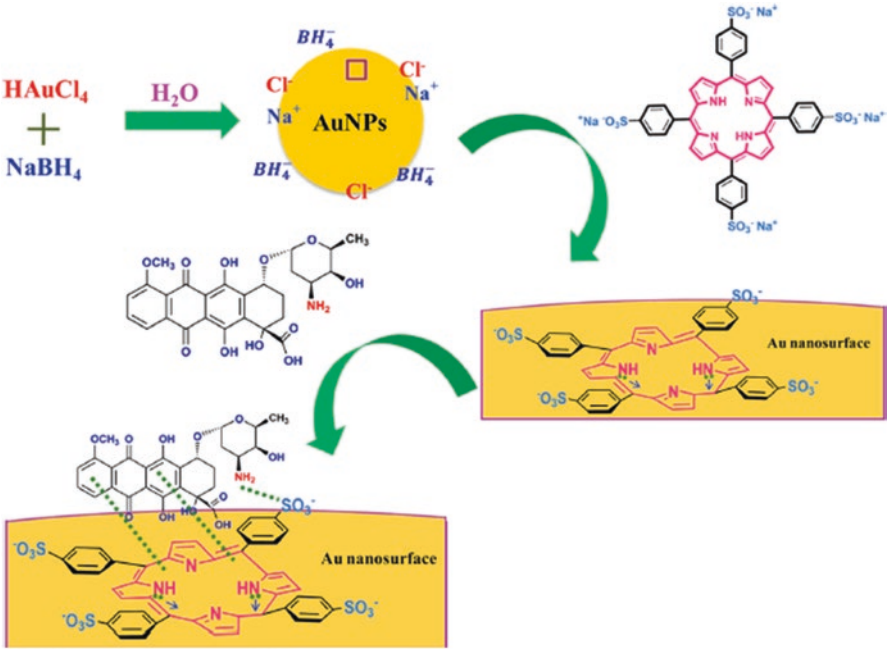


Fig. 20.1 Schematic representation of the possible mechanism of the attachment of TPPS on the surface of AuNPs and further loading of DOX. Reproduced from Bera et al. [28] with permission from ACS Publications

To overcome multidrug resistance, AuNPs were prepared by $NaBH_4$. Tetrasodium salt of meso-tetrakis-(4-sulfonatophenyl)porphyrin (TPPS) was armored on the surface of AuNPs via the polyamino groups in the chemical structure of TPPS. The antitumor drug doxorubicin (DOX) further interacted with TPPS and achieved the internalization by tumor cells with the help of this gold nanocarrier [28]. The as-prepared DOX-loaded nanocomposite (DOX@TPPS-AuNPs) demonstrated the DOX could be released by acidic pH and particularly to the nucleus of diseased cells (Fig. 20.1).

Through the lysozyme (Lyz)-induced aggregation of AuNPs, a versatile nanocarrier has been fabricated [29]. Both hydrophilic (DOX) and hydrophobic (pyrene) molecules can be loaded on the surface of Lyz-aggregated AuNPs via the noncovalent effect. To stabilize the carrier and enhance its uptake by cancer cells, an albumin layer was decorated in the outermost layer of the system. This AuNP–protein agglomerate worked as an efficient nanocarrier of DOX and improved the inhibition ability in a human cervical cancer HeLa cell line.

Besides the direct absorption between AuNPs and molecules with opposite charges, the L-b-L method has also been employed in the AuNP-based drug delivery system. For example, charge-reversal AuNPs prepared by L-b-L technique were employed to deliver small interfering RNA (siRNA) and plasmid DNA into cancer

cells [30]. Using this system, Lamin A/C, an important nuclear envelope protein, was effectively silenced by lamin A/C-siRNA, which showed better knockdown efficiency than that of commercial Lipofectamine 2000. Moreover, the engineering AuNPs by L-b-L method were also employed for immune modulation [31]. Polyethylene glycol (PEG), polyvinyl alcohol (PVA) or a mixture of both with either positive or negative surface charge were coated on the surface of AuNPs. The uptake and cell response in monocyte-derived dendritic cells (MDDCs) were investigated. It has been revealed that MDDCs can take up AuNPs efficiently and difference in surface modification influences the state of MDDCs. Although limitedly internalized by MDDCs, PEG-COOH AuNPs caused a significant increase in tumor necrosis factor-alpha. On the other hand, the readily internalized (PEG + PVA)-NH_2 and PVA-NH_2 AuNPs caused the secretion of interleukin-1beta.

Moreover, changing the proteins absorbed on the AuNP surface would alternate the biodistribution of the particles [32]. Comparing the AuNPs (15 and 80 nm in gold core size) conjugated either with human serum albumin (alb-AuNP) or apolipoprotein E (apoE-AuNP) with citrate-protected AuNPs, protein conjugation massively reduced liver retention (alb-AuNP: 52%, apoE-AuNP: 72%, cit-AuNP: >95%, at 19 h and 48 h). The study suggested that the modulation of artificial protein corona could be a promising method to control the targeting and destiny of NP-based drug delivery systems.

2.2 Surface Modification by Complementation of Base Pairs

With the development of nanomedicine, various biological macromolecules (e.g., nucleic acids and proteins) are not only delivered as the treatment agents but also participate in the construction of nanocarriers. Antitumor drugs acting on nucleic acids specifically adsorbed or embedded on designed double stranded nucleic acids that conjugated on the surface of AuNPs, which can improve the cellular uptake of drugs significantly. For example, folic acid (FA) was attached at one terminal of single-stranded DNA via the reaction of the isolated FA N-hydroxysuccinimide ester with the amino-DNA and conjugating the complementary DNA strand on the surface of AuNPs, after a simple mixture process, FA-target moiety was easily labeled on the gold surface [33]. DOX was loaded on the complementary DNA strands and led to a high cytotoxicity of vehicles having both FA and DOX. When the structure and ratio of DNA was designed and adjusted properly, the formation of AuNP dimers was through the click chemistry of the alkyne and azide groups between two thiol-modified oligonucleotides [34]. Two different oligonucleotides modified on the surface of these AuNP dimers allowed to intercalate two different drug (DOX and mitoxantrone, MTX) loadings [35]. At the high level of two mRNA targets in tumor cells (2.5-fold higher than normal cells), these dimers simultaneously or independently deliver one or two DNA-intercalating anticancer drugs in several tumor cells, including 16 HBE, MRC 5, and A 549 cells. This is a multidrug delivery system corresponding to the high mRNA stimulation in tumor microenvironment (Fig. 20.2).

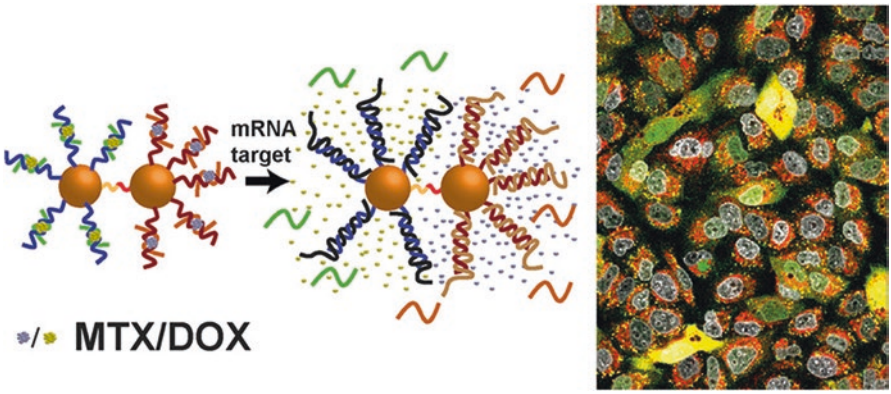


Fig. 20.2 Schematic Illustration of the multiplexed nanoparticle dimer used in drug release and the image of mRNA detection taken by laser scanning confocal microscopy (LSCM). Reproduced from Kyriazi et al. [35] with permission from ACS Publications

3 Surface Modification by Covalent Methods

Covalent modification means connecting molecules on the surface of AuNPs via chemical bonds. S-Au covalent bond is the most important interaction that makes the surface chemistry of AuNPs simpler and more versatile. It was considered as covalent bond because the strong metal–ligand interaction between sulfur and gold, although the interaction is partially covalent (~35%) and mostly electrostatic (~65%) [36]. In general, the "S–Au covalent bond" is a widely accepted denotation and it is the first step in the modification process of AuNPs. Various targeting and therapeutic ligands can be anchored on gold surface via direct S-Au interaction, and a short spacer with terminated thiol group is also attached on the gold surface and allowing the further conjugation of drug molecules through chemical reactions.

3.1 Surface Modification by Ligand Exchange

Ligand exchange for surface modification of AuNPs is an effective and important strategy to prepare functionalized nanoparticles for biomedical and nanomedical applications. Ligand exchange reactions can be compared with the ligand substitution reactions, which involve displacement of original ligands by thiol-containing biomolecules, drugs, and prodrugs [37]. In most cases, the incoming ligand biomolecules have stronger interactions with the inorganic nanoparticles' surface than the leaving ligands [38], by which aptamer, peptide, polymer, and chemical drugs has been modified and offered their functions to AuNP-based nanosystems.

Attached target ligands with the ability of specific recognition by the receptor located on the surface of tumor membrane will increase the selectivity and uptake of modified AuNPs in tumor cells. Small interleukin-6 receptor (IL-6R) specific aptamer [39] molecules (AIR-3A) were modified on the surface of AuNPs via a thiol-Au bond. AIR-3A in different amounts to the particles was adjusted to investigate the specificity of the delivery to IL-6R-carrying cells. AuNPs of diameters from 2 nm to larger 7 nm and 36 nm have been employed. The results demonstrated that the AIR-3A-modification can significantly improve the recognition by IL-6R-carrying cells, and that polyethylene glycol (PEG), particle size, concentration, aptamer surface coverage, incubation time, and temperature also showed relative influence on the internalization by target cells. Using the same method, peptide-modified 13-nm AuNPs can be introduced into subcellular organelles such as lysosomes [40].

With the aim of improving brain visualization and drug permeability across the blood–brain barrier (BBB), several glucose-modified AuNPs (ca. 2 nm) carrying BBB-permeable neuropeptides have been prepared. The position emitter ^{68}Ga was also chelated on the gold surface via a NOTA ligand for in vivo position emission tomography (PET) tracking. All glucose, neuropeptide, and ^{68}Ga were conjugated on AuNPs via the thiol-Au covalent bond [41]. The results showed that the accumulation of radioactivity in different organs after intravenous administration was dependent on the modified ligands. AuNPs anchoring a Leu-enkephalin peptide (Enk) improve BBB crossing (0.020 ± 0.0050% ID/g) nearly threefold compared to nontargeted GNPs (0.0073 ± 0.0024%ID/g). To improve the biocompatibility of AuNPs and facilitate the further surface modification of AuNPs, cysteine-functionalized alginate was coated on the gold surface via the thiol group of cysteine [20]. Similar to the poly(ethylene) glycol (PEG) modification, alginate-derived polymers provided colloidal stability to AuNPs and avoided recognition and sequestration by the body's defense system, demonstrating a promising potential in constructing sustained drug release systems.

In addition, chemical drugs can be also conjugated on the surface of AuNPs to achieve specific target and selective release at tumor sites. Several chemical drugs and their derivatives including Tiopronin (TPN) [42], paclitaxel (PTX) [43, 44], and DOX [45, 46] have been connected on the gold surface via a simple ligand exchange method. For instance, loading DOX on the surface of AuNPs via a hydrazone, an acid-responsive linker, and angiopep-2, a specific ligand of low-density lipoprotein receptor-related protein-1 (LRP1) was also functionalized as a target ligand for glioma [47] (Fig. 20.3). This system, 39.9 nm in size, −19.3 mV in zeta potential, and 9.7% DOX loading capacity, showed a BBB penetration and targetability for glioma cells. Upon the low pH sensitive drug release in tumor cells, glioma-bearing mice treated with An-PEG-DOX-AuNPs displayed the longest median survival time, which was 2.89-fold longer than that of saline.

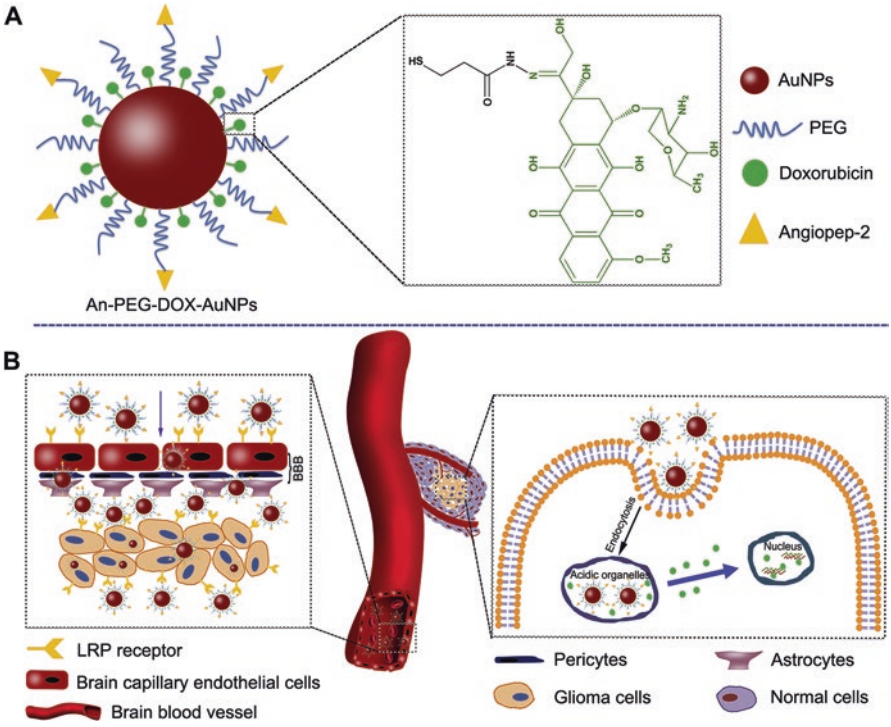


Fig. 20.3 Schematic illustration of the structure of An-PEG-DOX-AuNPs and their delivering procedure to penetrate through BBB and target to glioma cells. Reproduced from Ruan et al. [47] with permission from Science Direct Publications

3.2 Surface Modification by Chemical Reactions

The ligand exchange method to modify the gold surface required a high synthesis technology. Each product needs to be purified and characterized. It ensures the exact structure of the subject system but is not so economical when different and more ligands are expected to be connected on the gold surface. Therefore, modified AuNPs using a short spacer firstly, and then conjugated other ligand on this spacer via a chemical reaction have been also employed in surface modification of AuNPs.

To accurately measure the number of functionalized chemotherapeutic drug, PTX, connected on the surface of AuNPs (ca. 2 nm), a flexible hexaethylene glycol linker was coupled with phenol-terminated gold nanocrystals firstly, and then anchored at the C-7 position of paclitaxel at the other terminal [48] (Fig. 20.4). The thermogravimetric analysis (TGA) of the hybrid nanoparticles reveals the content of the covalently attached organic shell as nearly 67% by weight, which corresponds to ~70 molecules of paclitaxel per 1 nanoparticle. To carry cisplatin molecules for tumor treatment, a dendron having one thiol and three carboxyl group has been

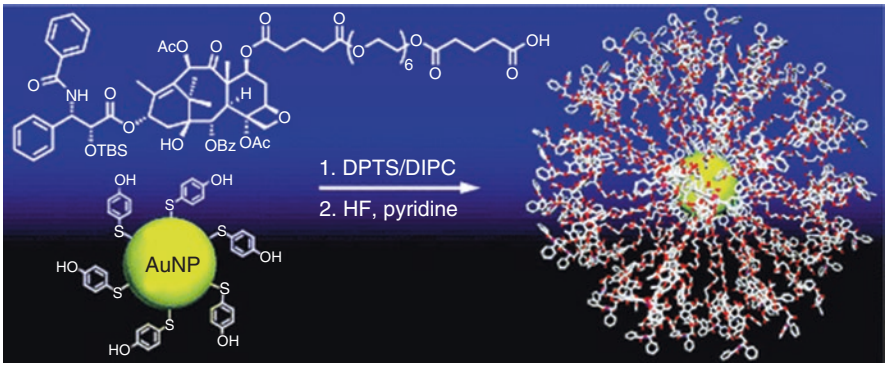


Fig. 20.4 Schematic illustration of covalent coupling of paclitaxel to 4-mercaptophenol-modified 2 nm gold nanoparticles. Reproduced from Gibson et al. [48] with permission from ACS Publications

attached on AuNPs via a simple S-Au bond, where Pt(II) is connected on terminated dendron ligand via the carboxyl-amino interaction [49]. PEGylation influences its drug loading, colloidal stability, and drug release, especially improves the stability of Pt(II)-complexed AuNP. It is noting that, the Pt(II) release over 10 days (examined at 0.5 Pt(II)/nm^2 drug loading) remained constant for non-PEGylated, 1 K-PEGylated, and 5 K-PEGylated conjugates.

Integrating multiple properties in an all-in-one system holds great promise for drug delivery, but the absence of technology to assemble highly functionalized devices has hindered this progress in nanomedicine. To overcome this challenge, poly(ethylene oxide)-β-poly(ε-caprolactone) (PEO-b-PCL) block polymers modified at the apolar PCL terminus with thioctic acid and at the polar PEO terminus with an acylhydrazide, amine, or azide moiety have been synthesized and coated on the surface of AuNPs [18]. Three different types of surface reactive groups, acylhydrazide, amine, or azide moieties, could be reacted with high efficiencies with modules having a ketone, isocyanate, or active ester and alkyne function, respectively, for surface functionalization. An oligo-arginine peptide to facilitate cellular uptake, a histidine-rich peptide to escape from lysosomes, and an Alexa Fluor 488 tag for imaging purposes were successfully conjugated on gold surface. This modular synthetic methodology provides facile and optimal configurations of nanocarriers for disease-specific drug delivery [11, 50].

3.3 Surface Modification by Click Chemistry

Click chemistry is an excellent, simple, and fast strategic method for surface modification because it does not affect the structure of NPs, this method based on the carbon-heteroatom bond [51]. In chemical synthesis, “click” chemistry is a class of

biocompatible small molecule reactions commonly used in bioconjugation, allowing the joining of substrates of choice with specific biomolecules. Recently, especially the development of copper-free click chemistry has attracted more attention because it increases the biosafety of click reaction in vivo.

For the conjugation of a cytotoxic drug, a cancer cell targeting ligand, and an imaging moiety, a multifunctional AuNP was prepared. Among all surface modifications, the glycan-based ligand for the cell surface receptor CD22 of B-cells used strain promoted azide–alkyne cycloaddition [52]. The surface attachment of CD22 endowed the rapid entry of the nanoparticle by receptor-mediated endocytosis, compared to nontargeted nanoparticles. To coat AuNPs with a functional copolymer, polydimethylacrylamide (DMA) modified with an alkyne monomer that allows the binding of azido-modified molecules by Cu(I)-catalyzed azide/alkyne 1,3-dipolar cycloaddition (CuAAC) was synthesized [53]. The polymeric backbone made the azido-modified protein possible. Anti-mouse IgG antibody was then attached on the particle surface and would realize gold labels in biosensing techniques (Fig. 20.5).

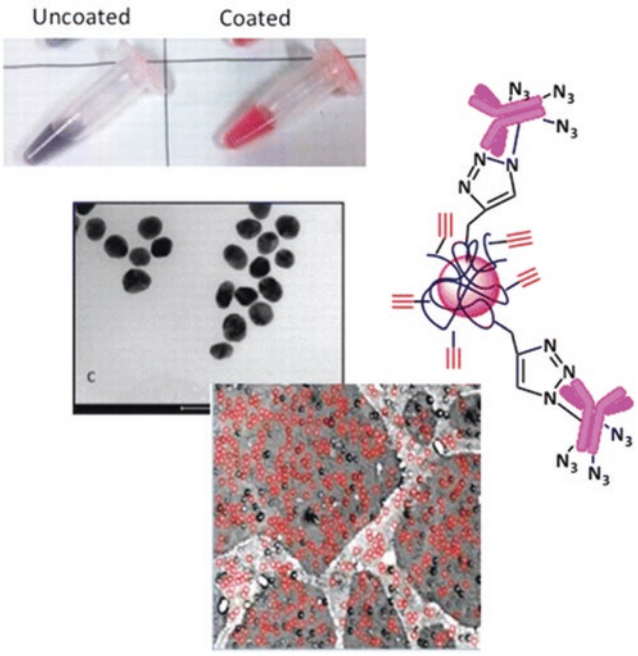


Fig. 20.5 AuNPs modified with a functional polymer and consequent derivatization of the polymer with an azido-modified antibody, as well as their photograph, TEM image, and interferometric reflectance imaging system (SP-IRIS) images of surfaces functionalized with different antibodies. Reproduced from [53] with permission from ACS Publications

4 Discussion and Outlook

In this chapter, we summarize the main points of noncovalent and covalent methods for surface modification of AuNPs for targeted drug delivery. The electrostatic interaction, complementation of base pairs, ligand exchange, and chemical reactions are efficient methods to adjust and improve the physicochemical properties and treatment performance of AuNP-based drug delivery systems. They each have advantages and disadvantages for practical use. A rational use of existing surface modification methods and development of new techniques are significantly important for further regulation of the treatment efficacy of nanoparticle-based therapeutic systems.

References

1. Yeh, Y.-C., Creran, B., & Rotello, V. M. (2012). Gold nanoparticles: Preparation, properties, and applications in bionanotechnology. *Nanoscale, 4*, 1871–1880.
2. Biju, V. P. (2014). Chemical modifications and bioconjugate reactions of nanomaterials for sensing, imaging, drug delivery and therapy. *Chemical Society Reviews, 45*, 744–764.
3. Giljohann, D. A., Seferos, D. S., Daniel, W. L., Massich, M. D., Patel, P. C., & Mirkin, C. A. (2010). Gold nanoparticles for biology and mdicine. *Angewandte Chemie, International Edition, 49*, 3280–3294.
4. Saha, K., Agasti, S. S., Kim, C., Li, X., & Rotello, V. M. (2012). Gold nanoparticles in chemical and biological sensing. *Chemical Reviews, 112*, 2739–2779.
5. Li, N., Zhao, P., & Astruc, D. (2014). Anisotropic gold nanoparticles: Synthesis, properties, applications, and toxicity. *Angewandte Chemie, International Edition, 53*, 1756–1789.
6. Perrault, S. D., & Chan, W. C. W. (2009). Synthesis and surface modification of highly monodispersed, spherical gold nanoparticles of 50-200 nm. *Journal of the American Chemical Society, 131*, 17042–17043.
7. Chen, T., Xu, S., Zhao, T., Zhu, L., Wei, D., Li, Y., Zhang, H., & Zhao, C. (2012). Gold nanocluster-conjugated amphiphilic block copolymer for tumor-targeted drug delivery. *ACS Applied Materials and Interfaces, 4*, 5766–5774.
8. He, L., Chen, T., You, Y., Hu, H., Zheng, W., Kwong, W.-L., Zou, T., & Che, C.-M. (2014). A cancer-targeted nano system for delivery of gold (III) complexes: Enhanced selectivity and apoptosis-inducing efficacy of a gold (III) porphyrin complex. *Angewandte Chemie, International Edition, 53*, 12532–12536.
9. Jang, H., Ryoo, S.-R., Kostarelos, K., Hanb, S. W., & Mina, D.-H. (2013). The effective nuclear delivery of doxorubicin from dextran-coated gold nanoparticles larger than nuclear pores. *Biomaterials, 34*, 3503–3510.
10. Verma, H. N., Singh, P., & Chavan, R. M. (2014). Gold nanoparticle: Synthesis and characterization. *Veterinary World, 7*, 72–77.
11. Brown, S. D., Nativo, P., Smith, J.-A., Stirling, D., Edwards, P. R., Venugopal, B., Flint, D. J., Plumb, J. A., Graham, D., & Wheate, N. J. (2010). Gold nanoparticles for the improved anticancer drug delivery of the active component of oxaliplatin. *Journal of the American Chemical Society, 132*, 4678–4684.
12. Yeh, Y.-C., Creran, B., & Rotello, V. M. (2012). Gold nanoparticles: Preparation, properties, and applications in bio nanotechnology. *Nanoscale, 4*, 1871–1880.
13. Park, J., Brust, T. F., Lee, H. J., Lee, S. C., Watts, V. J., & Yeo, Y. (2014). Polydopamine-based simple and versatile surface modification of polymeric nano drug carriers. *ACS Nano, 8*, 3347–3356.

14. Adams, S. A., Hauser, J. L., Allen, A. C., Lindquist, K. P., Ramirez, A. P., Oliver, S., & Zhang, J. Z. (2018). Fe3O4@SiO2 nanoparticles functionalized with gold and poly(vinylpyrrolidone) for bio-separation and sensing applications. *ACS Applied Nano Materials, 1*, 1406–1412.
15. Hu, J., Wu, T., Zhang, G., & Liu, S. (2012). Efficient synthesis of single gold nanoparticle hybrid amphiphilic triblock copolymers and their controlled self-assembly. *Journal of the American Chemical Society, 134*, 7624–7627.
16. Ghiassian, S., Gobbo, P., & Workentin, M. S. (2015). Water-soluble maleimide-modified gold nanoparticles (AuNPs) as a platform for cycloaddition reactions. *European Journal of Organic Chemistry*, (24), 5438–5447.
17. Kalies, S., Gentemann, L., Schomaker, M., Heinemann, D., Ripken, T., & Meyer, H. (2014). Surface modification of silica particles with gold nanoparticles as an augmentation of gold nanoparticle mediated laser perforation. *Biomedical Optics Express, 5*, 2686–2696.
18. Li, X., Guo, J., Asong, J., Wolfert, M. A., & Boons, G.-J. (2011). Multifunctional surface modification of gold-stabilized nanoparticles by bioorthogonal reactions. *Journal of the American Chemical Society, 133*, 11147–11153.
19. Lipka, J., Behnke, M. S., Sperling, R. A., Wenk, A., Takenaka, S., Schleh, C., Kissel, T., Parak, W. J., & Kreyling, W. G. (2010). Biodistribution of PEG-modified gold nanoparticles following intratracheal instillation and intravenous injection. *Biomaterials, 31*, 6574–6581.
20. Kodiyan, A., Silva, E. A., Kim, J., Aizenberg, M., & Mooney, D. J. (2012). Surface modification with alginate-derived polymers for stable, protein-repellent, long-circulating gold nanoparticles. *ACS Nano, 6*, 4796–4805.
21. Doane, T. L., & Burda, C. (2012). The unique role of nanoparticles in nanomedicine: Imaging, drug delivery and therapy. *Chemical Society Reviews, 41*, 2885–2911.
22. Caragheorgheopol, A., & Chechik, V. (2008). Mechanistic aspects of ligand exchange in au nanoparticles. *Physical Chemistry Chemical Physics, 10*, 5029–5041.
23. Veiseh, O., Kievit, F. M., Gunn, J. W., Ratner, B. D., & Zhanga, M. (2009). A ligand-mediated nanovector for targeted gene delivery and transfection in cancer cells. *Biomaterials, 30*, 649–657.
24. Chen, Y., Xianyu, Y., & Jiang, X. (2017). Surface modification of gold nanoparticles with small molecules for biochemical analysis. *Accounts of Chemical Research, 50*, 310–319.
25. Paciotti, G. F., Kingston, F. G. I., & Tamarkin, L. (2006). Colloidal gold nanoparticles: A novel nanoparticle platform for developing multifunctional tumor-targeted drug delivery vectors. *Drug Development Research, 67*, 47–54.
26. Ding, Y., Jiang, Z., Saha, K., Kim, C. S., Kim, S. T., Landis, R. F., & Rotello, V. M. (2014). Gold nanoparticles for nucleic acid delivery. *Molecular Therapy, 22*, 1075–1083.
27. Liang, J.-J., Zhou, Y.-Y., Wu, J., & Ding, Y. (2014). Gold nanoparticle-based drug delivery platform for antineoplastic chemotherapy. *Current Drug Metabolism, 15*, 620–631.
28. Bera, K., Maiti, S., Maity, M., Mandal, C., & Maiti, N. C. (2018). Porphyrin−gold nanomaterial for efficient drug delivery to cancerous cells. *ACS Omega, 3*, 4602–4619.
29. Khandelia, R., Jaiswal, A., Ghosh, S. S., & Chattopadhyay, A. (2013). Gold nanoparticle–protein agglomerates as versatile nanocarriers for drug delivery. *Small, 9*, 3494–3505.
30. Guo, S., Huang, Y., Jiang, Q., Sun, Y., Deng, L., Liang, Z., Du, Q., Xing, J., Zhao, Y., Wang, P. C., Dong, A., & Liang, X.-J. (2010). Enhanced gene delivery and siRNA silencing by gold nanoparticles coated with charge-reversal polyelectrolyte. *ACS Nano, 4*, 5505–5511.
31. Fytianos, K., Rodriguez-Lorenzo, L., Clift, M. J., Blank, F., Vanhecke, D., von Garnier, C., Petri-Fink, A., & Rothen-Rutishauser, B. (2015). Uptake efficiency of surface modified gold nanoparticles does not correlate with functional changes and cytokine secretion in human dendritic cells in vitro. *Nanomedicine, 11*, 633–644.
32. Schäffler, M., Sousa, F., Wenk, A., Sitia, L., Hirn, S., Schleh, C., Haberl, N., Violatto, M., Canovi, M., Andreozzi, P., Salmona, M., Bigini, P., Kreyling, W. G., & Krol, S. (2014). Blood protein coating of gold nanoparticles as potential tool for organ targeting. *Biomaterials, 35*, 3455–3466.
33. Alexander, C. M., Hamner, K. L., Maye, M. M., & Dabrowiak, J. C. (2014). Multifunctional DNA-gold nanoparticles for targeted doxorubicin delivery. *Bioconjugate Chemistry, 25*, 1261–1271.

34. Heuer-Jungemann, A., Kirkwood, R., El-Sagheer, A. H., Brown, T., & Kanaras, A. G. (2013). Copper-free click chemistry as an emerging tool for the programmed ligation of DNA-functionalised gold nanoparticles. *Nanoscale, 5*, 7209–7212.
35. Kyriazi, M.-E., Giust, D., El-Sagheer, A. H., Lackie, P. M., Muskens, O. L., Brown, T., & Kanaras, A. G. (2018). Multiplexed mRNA sensing and combinatorial-targeted drug delivery using DNA-gold nanoparticle dimers. *ACS Nano, 12*, 3333–3340.
36. Roca, M., & Haes, A. J. (2008). Probing cells with noble metal nanoparticle aggregates. *Nanomedicine UK, 3*, 555–565.
37. Chen, W., He, S., Pan, W. Y., Jin, Y., Zhang, W., & Jiang, X. Y. (2010). Strategy for the modification of electrospun fibers that allows diverse functional groups for biomolecular entrapment. *Chemistry of Materials, 22*, 6212–6214.
38. Liu, D. B., Qu, W. S., Chen, W. W., Zhang, W., Wang, Z., & Jiang, X. Y. (2010). Highly sensitive, colorimetric detection of mercury (II) in aqueous media by quaternary ammonium group-capped gold nanoparticles at room temperature. *Analytical Chemistry, 82*, 9606–9610.
39. Prisner, L., Bohn, N., Hahn, U., & Mews, A. (2017). Size dependent targeted delivery of gold nanoparticles modified with the IL-6R-specific aptamer AIR-3A to IL-6R-carrying cells. *Nanoscale, 9*, 14486–14498.
40. Dekiwadia, C. D., Lawrie, A. C., & Fecondo, J. V. (2012). Peptide-mediated cell penetration and targeted delivery of gold nanoparticles into lysosomes. *Journal of Peptide Science, 18*, 527–534.
41. Frigell, J., García, I., Gómez-Vallejo, V., Llop, J., & Penadés, S. (2014). 68Ga-labeled gold glyconanoparticles for exploring blood-brain barrier permeability: Preparation, biodistribution studies, and improved brain uptake via neuropeptide conjugation. *Journal of the American Chemical Society, 136*, 449–457.
42. Bao, Q.-Y., Geng, D.-D., Xue, J.-W., Zhou, G., Gu, S.-Y., Ding, Y., & Zhang, C. (2013). Glutathione-mediated drug release from tiopronin-conjugated gold nanoparticles for acute liver injury therapy. *International Journal of Pharmaceutics, 446*, 112–118.
43. Ding, Y., Zhou, Y.-Y., Chen, H., Geng, D.-D., Wu, D.-Y., Hong, J., Shen, W.-B., Hang, T.-J., & Zhang, C. (2013). The performance of thiol-terminated PEG-paclitaxel-conjugated gold nanoparticles. *Biomaterials, 34*, 10217–10227.
44. Gao, Y.-Y., Chen, H., Zhou, Y.-Y., Wang, L.-T., Hou, Y., Xia, X.-H., & Ding, Y. (2017). Intraorgan targeting of gold conjugates for precise liver cancer treatment. *ACS Applied Materials and Interfaces, 9*, 31458–31468.
45. Wu, D.-Y., Wang, H.-S., Hou, X.-S., Chen, H., Ma, Y., Hou, Y., Hong, J., & Ding, Y. (2018). Effects of gold core size on regulating the performance of doxorubicin-conjugated gold nanoparticles. *Nano Research, 11*, 3396–3410.
46. Cui, T., Liang, J.-J., Chen, H., Geng, D.-D., Jiao, L., Yang, J.-Y., Qian, H., Zhang, C., & Ding, Y. (2017). The Performance of doxorubicin-conjugated gold nanoparticles: Regulation of drug location. *ACS Applied Materials and Interfaces, 9*, 8569–8580.
47. Ruan, S., Yuan, M., Zhang, L., Hu, G., Chen, J., Cun, X., Zhang, Q., Yang, Y., He, Q., & Gao, H. (2015). Tumor microenvironment sensitive doxorubicin delivery and release to glioma using angiopep-2 decorated gold nanoparticles. *Biomaterials, 37*, 425–435.
48. Gibson, J. D., Khanal, B. P., & Zubarev, E. R. (2007). Paclitaxel-functionalized gold nanoparticles. *Journal of the American Chemical Society, 129*, 11653–11661.
49. Tan, J., Cho, T. J., Tsai, D.-H., Liu, J., Pettibone, J. M., You, R., Hackley, V. A., & Zachariah, M. R. (2018). Surface modification of cisplatin-complexed gold nanoparticles and its influence on colloidal stability, drug loading, and drug release. *Langmuir, 34*, 154–163.
50. Jang, H., Ryoo, S.-R., Kostarelos, K., Han, S. W., & Min, D.-H. (2013). The effective nuclear delivery of doxorubicin from dextran-coated gold nanoparticles larger than nuclear pores. *Biomaterials, 34*, 3503–3510.
51. Hua, C., Zhang, W. H., De Almeida, S. R., Ciampi, S., Gloria, D., Liu, G., Harper, J. B., & Gooding, J. J. (2012). A novel route to copper (II) detection using 'click' chemistry-induced aggregation of gold nanoparticles. *Analyst, 137*, 82–86.

52. Hudlikar, M. S., Li, X., Gagarinov, I. A., Kolishetti, N., Wolfert, M. A., & Boons, G.-J. (2016). Controlled multi-functionalization facilitates targeted delivery of nanoparticles to cancer cells. *Chemistry, 22*, 1415–1423.
53. Finetti, C., Sola, L., Pezzullo, M., Prosperi, D., Colombo, M., Riva, B., Avvakumova, S., Morasso, C., Picciolini, S., & Chiari, M. (2016). Click chemistry immobilization of antibodies on polymer coated gold nanoparticles. *Langmuir, 32*, 7435–7441.

Chapter 21
Surface Modification and Bioconjugation of Nanoparticles for MRI Technology

M. Azam Ali and Mohammad Tajul Islam

Abstract Nanomaterials (NPs) with precise biological functions have considerable potential for use in biomedical applications. Surface modification is one of the effective routes to impart such desired and precise biological functions to NPs. Introduction of various reactive functional groups on the surface of NPs are required to conjugate a spectrum of contrast agents (CAs), for the targeted imaging such as magnetic resonance imaging (MRI). Current state in surface modification of NPs for preparing CAs of MRI is summarized in this chapter. Chemistries involved in the bioconjugation and surface modification are discussed. Chemical and bioconjugate reactions to transform the surface of NPs such as silica NPs, gold NPs, and gadolinium NPs are highlighted. Coating is another important approach to enhance the functionalities of CAs for MRI application, therefore, light is thrown on the coating mechanism of organic polymers including dextran, chitosan, and copolymers.

Keywords Surface modification · Bioconjugation · Nanoparticles · Contrast agent · Magnetic resonance image

1 Introduction

Imaging techniques, which detect lesion information (e.g., type, location, and stage) offer tremendous opportunities both for clinical diagnostics and as a research tool [1]. Among all existing imaging techniques, magnetic resonance imaging (MRI) has emerged as one of the most powerful diagnostic tools in biomedicine primarily due to its exquisite soft tissue contrast, high spatial resolution, lack of ionizing radiation, unlimited signal penetration depth, and wide clinical applicability [2].

M. A. Ali (✉)
Centre for Bioengineering and Nanomedicine, Department of Food Science, Division of Sciences, University of Otago, Dunedin, New Zealand
e-mail: azam.ali@otago.ac.nz

M. T. Islam
Centre for Materials Science and Technology, University of Otago, Dunedin, New Zealand

Y. V Pathak (ed.), *Surface Modification of Nanoparticles for Targeted Drug Delivery*, https://doi.org/10.1007/978-3-030-06115-9_21

Another advantage of MRI is that it can obtain real-time images of the internal anatomy and physiology of living organisms in a noninvasive manner. Furthermore, MRI provides information not possible to access with other imaging modalities. Recently, three-dimension (3D) MRI has been shown to be much useful in the evaluation and diagnosis of brain and neural tissue anatomy using a single acquisition technique. Therefore, it has been extensively used for imaging brain and central nervous systems, for assessing cardiac function, and for detecting abnormal tissues such as tumors [2].

An MRI is the collection of pixels or voxels representing the nuclear magnetic resonance (NMR) signal intensity of the hydrogen atoms in water and fat of the body area of living organisms being imaged. MRI scanner applies a strong magnetic field around the area of the body to be imaged. The hydrogen nuclear spins are aligned in the direction of the external magnetic field (Fig. 21.1a). Figure 21.1b shows that when a resonant radiofrequency wave (5–100 MHz) is applied, some protons with low energy absorb the electromagnetic energy and flip their spin. After removal of the radio frequency, the protons gradually return to their normal spin. Protons simultaneously release the energy in the form of radio waves during resuming their original state, which are measured by receivers and made into the MR images. Returning process of the protons to their original state is known as relaxation. Relaxation is measured in two directions, longitudinal and transverse, and characterized by the time constants: spin–lattice relaxation time T1 or spin–spin relaxation time T2, respectively [3, 4].

However, MRI's inherent low sensitivity, little difference between normal and abnormal soft tissues in relaxation time, and resulting contrast usually provides poor anatomic descriptions, which hampers the visualization of subtle changes in tissues. Therefore, additional supplements have been used for further improvement of the contrast of imaging, and more accurate detection and diagnosis. The most

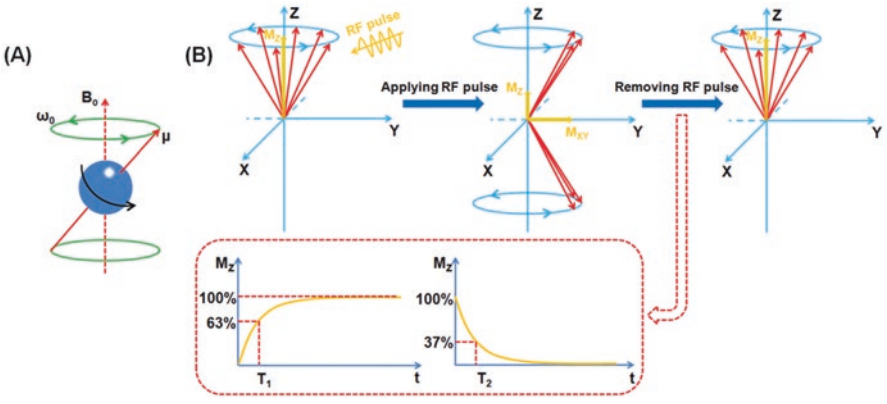


Fig. 21.1 Schematic illustration of the mechanisms of MRI. (**a**) Protons precessing under an external magnetic field B_0. (**b**) After the introduction of the RF pulse, protons are excited, with relaxation occurring following removal of the RF pulse. And the graphical representation of T1 relaxation and T2 relaxation. Reproduced from [3] with permission of Royal Society of Chemistry

effective supplement is a chemical compound known as a probe or a CA. CA is introduced to a living body prior to MRI scanning to enhance the signal difference between the region of interest (disease) and the background (normal tissues). In general, contrast agents can make the signal difference by modifying the water proton relaxation rates when present in micromolar concentrations, although recent advances can produce nanomolar detection levels [5]. Moreover, the interrelation between the contrast agent and the biological system often reveals biological and functional information [2].

Merbach et al. [6] reported in their book in 2013 that approximately 35% of clinical MRI scans took the assistance of CAs, although this is dependent on the type of CA, which is expected to increase signal intensity and/or imaged performances further with the emergence of novel and more effective CAs compared to commercially available CAs at present. CAs can be categorized into four class based on the MR mechanism to generate signal: T1, T2, PARACEST (paramagnetic chemical exchange saturation transfer), and hyperpolarization [1]. T1 and T2 are more commonly used CAs amongst these four. T1 contrast agents are known as positive contrast agents since they increases the signal intensity in T1-weighted images by shortening T1. On the other hand, T2 contrast agents reduces the signal intensity in T2-weighted images and hence called negative contrast agent [4]. One of the most common T1 CAs employed in clinical imaging is paramagnetic gadolinium ion complex while superparamagnetic iron oxide nanoparticle (SPION) is the typical example of T2 MRI contrast agent [2]. Unfortunately, CAs are not free from limitations. Being small molecules CAs shows limited efficacy. As a result, to obtain desired contrast relatively high dose of CAs is required [7]. To overcome this limitation, CAs were incorporated into nanoparticles via bioconjugation (Fig. 21.2). Nanoparticle acts as a carrier for the CAs and improve the relaxation time thereby efficiently amplify the contrast signal at locations of interest.

The synthesis of NPs requires an organic solvent or a complex aqueous mixture, which is unfortunately not compatible with direct biomedical use. Therefore, surface modification is essential to get colloidal stable NPs at physiological pH as well

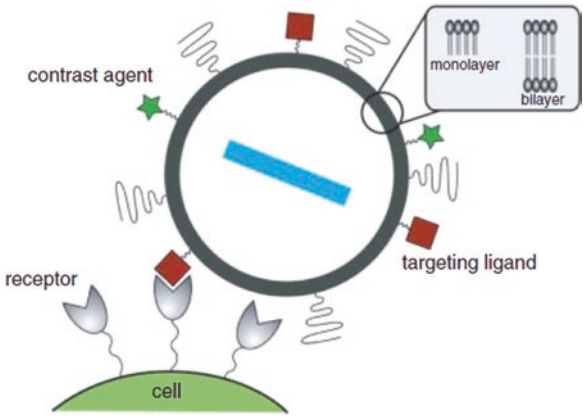


Fig. 21.2 Schematic representation of a lipid-based nanoparticle, which binds to a cell-surface receptor. The nanoparticle carries contrast agents (green), a payload of drug (blue), and is equipped with targeting ligands (red) for specific receptor recognition. Reproduced from Nicollay et al. (2013) with permission of Wiley

as targeting abilities and additional functionalities that demonstrate biocompatibility to the body tissues. The initial surface functionality can be introduced during synthesis of NPs. Otherwise the surface layer can be modified after synthesis either by chemical modification of the initial ligand or by complete ligand exchange. Hundreds of thousands of CAs such as gadolinium ions or iron oxides can be loaded per nanoscale CA structure. Furthermore, surface modification of nanoscale CAs can introduce various functionalities, such as targeting ligands for tumor targeting, dyes for multimodal imaging and drugs for constructing therapeutic nanoplatforms [3]. In this chapter, we introduce and report the recent developments of surface modification of various CAs including bioconjugation of nanoparticles for MRI technology.

2 Surface Modification Chemistry

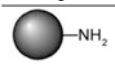
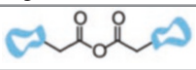
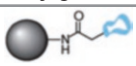
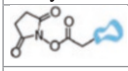
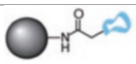
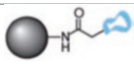
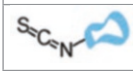
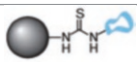
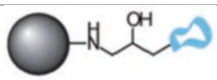
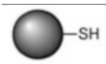
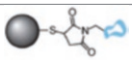
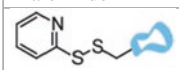
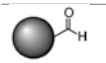
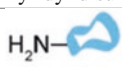
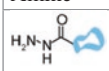
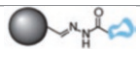
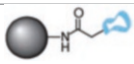
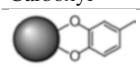
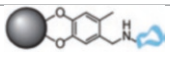
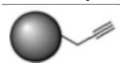
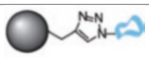
2.1 Covalent Linkages

Covalent linkage is one of the strong and stable bonds formed between functional groups found on the NP surface and conjugated ligands. Usually, these functional groups such as amino, carboxylic acid, and thiol groups are added to the NP surface via its polymer coating. Both the type and number of functional groups on each NP can be controlled during the coating of polymers. Functional groups are present either on the body of the natural or synthetic polymers chain (such as chitosan, dextran, PEI) or at their terminal ends (such as PEG). At the same time, total number of reactive groups can also be customized by adding specific number of binding sites per polymer chain. For example, superparamagnetic iron oxide nanoparticles (SPIONs), 38 nm, in particular have been extensively investigated as novel magnetic resonance imaging (MRI) CAs which coated with dextran have been reported with 62 reactive amino groups per NP [8]. While a larger SPION (64 nm) coated with PEG was reported to have 26 reactive amino groups per NP [9]. These same chemical groups are also found on the targeting, optical, or therapeutic agent to be covalently attached. To link the functional groups a host of chemistries are available, which are subdivided into direct reaction (Table 21.1), click chemistry (Table 21.1), and linker strategies (Table 21.2).

2.1.1 Direct Nanoparticle Conjugation

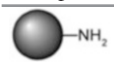
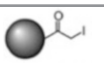
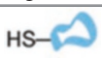
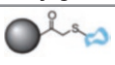
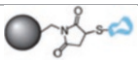
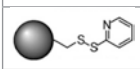
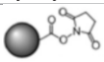
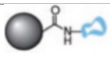
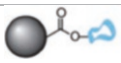
Direct reaction strategies are particularly suitable for small molecule conjugation. As listed in Table 21.1, functional groups such as amine, sulfhydryl, aldehyde, carboxyl, and active hydrogen functional groups present at the NP surfaces can be directly bonded or linked to reactive ligands by a number of reactions schemes, for example Mannich reaction, Michael addition, Schiff-base condensation and epoxide opening. A linkage reaction between the functional groups and ligand can also be possible, which is facilitated with the help of catalysts. In one notable study [8], 146 different small molecules were conjugated to 10-kDa dextran-coated

Table 21.1 Direct nanoparticle conjugation

Nanoparticle	Ligand	Conjugate	Reaction
 Amine	 Anhydride		Amide bond formation
	 Succinimidyl ester		
	 Carboxilic acid ester		
	 Isothiocyanate		Addition of amine to cyanates
	 Epoxide		Epoxide opening
 Sulfhydryl	 Maleimide		Michael addition
	 Pyridyl disulfide		Substitution
 Aldehyde	 Amine		Schiff-base condensation
	 Hydrazide		Imine formation
 Carboxyl	 Amine		Amide bond formation
 Active hydrogen	 Amine		Mannich reaction
 Alkyne	 Azide		Click chemistry

monocrystalline magnetic NP in array format to impart water solubility, conjugatability, biocompatibility, and chemical diversity. On an average, 60 small molecules (MW < 500 Da) with the chemical functional groups of primary amines, alcohols, carboxylic acids, sulfhydryls, and anhydrides were attached per 38-nm nanoparticle. Fourteen compounds showed significant uptake into cancer cells (up to 160×10^6 nanoparticles per cell for the most efficient compounds) for early detection of

Table 21.2 Linker chemistry conjugation

Nanoparticle	Linker chemistry	Ligand	Conjugate
 Amine	 Iodoacetyl		
	 Maleimide		
	 Pyridyl disulfide		
 Carboxyl	 EDC		
	 Thionyl chloride		

pancreatic cancer. The authors concluded that the efficacy of prepared materials might be attributed to the multivalent nature of the surface molecules (60 ligands per nanoparticle). However, the efficiency of these chemistries varies with the functional groups. Functionalized NPs obtained from direct conjugation methods are prone to intercalating or cross-linking, with the exception of amine functional groups. Specifically, disulfide linkage formation between NPs may be the reason for the cross-linking. Another reason might be binding amongst multiple NPs and a single ligand containing multiple amino functional groups. Moreover, there is a need for initial modification prior to conjugation since biomolecules are not natively reactive with NPs. Modification of biomolecules involves the risk of loss of biocompatibility and bioactivity. However, Schellenberger et al. [10] reported that only precise, limited modifications can be possible without losing the bioactivity. Therefore, care should be taken during chemical modification to limit the loss of biofunctionality and bioactivity. Unfortunately, glutaraldehyde, a common direct conjugation agent, has limited applicability for biomolecule-NP attachment as it denatures proteins and peptides. Tetraethylene glycol (TEG)-based phosphonate ligands were introduced in stable superparamagnetic iron oxide nanoparticles via both direct conjugation and ligand exchange process. The direct conjugation has found less toxic to cell whereas ligand exchange process lead to NP with greater magnetic properties [11] that illustrate effective performance for MRI imaged.

2.1.2 Click Chemistry

Sharpless et al. in the year 2005 developed "click" chemistry to generate substances by joining small units together with heteroatom links (C–X–C) [12]. Table 21.1 shows Cu-catalyzed azide–alkyne chemistries. The intention of the development of

click chemistry was to give an easier route for conjugations between bioactive surfaces and less harsh environment to biomolecule ligands [13]. Click reactions have several advantages such as they are fast, efficient, take place in aqueous environment at relatively neutral pH (mild reaction conditions). Bonds formed by click chemistry are water-soluble and obtained biocompatible linkages are electronically similar to amide bonds [14]. This method of attachment offers several unique features over other direct conjugation strategies. Specific conjugation at the desired location(s) on the reactive moiety is obtained as azide and alkyne reactive groups are highly specific to each other, and do not react with most functional groups. In addition, bonds formed by click chemistry are highly stable whereas amide bonds and disulfide linkages formed by other direct conjugation techniques are prone to cleavage by hydrolysis and reduction, respectively. Moreover, there is a very little risk of cross interaction among moieties at the NP surface. This is because of the rigidity of formed linkages, which helps to maintain conformation of reacted moieties in place. Thanks to those features for which production of highly oriented linkages, capable of optimal reaction activity and efficiency, is possible. Therefore, click chemistry is considered as a desired technique where orientation and stability of linkages are particularly important [15]. Click chemistry was implemented with SPIONs under mild reaction conditions with a reaction time of 5–8 h. Orthogonal to thiol- and amine-containing targeting motifs were effectively bound with NP at an efficiency of >90%, and the resultant linkages were stable in the complex in-vivo environments of the blood and tumor milieu [16]. However, there are some drawbacks of click chemistry in its implementation. Cu catalyst is required to advance the reaction, which may disturb the biocompatible nature of the formed linkage. A number of disorders such hepatitis, neurological disorders, kidney diseases, and Alzheimer's disease were found to be linked with the excessive Cu consumption [14]. To minimize this proper purifications are required to remove all of the catalyst from magnetic nanoparticle (MNP) solutions before use. Another issue is biodegradation. The highly stable linkages formed may render nonbiodegradability to MNP.

2.1.3 Linker Chemistry

Covalent linkage by linker chemistry can control the molecular orientation of bound ligands, which is very important to protect the functionality of targeting ligand. Cleaving of linkers selectively is possible for some applications such as ligand quantification, controlled release of drugs. Amine or carboxylic acid functionalized SPION surfaces have been modified with heterobifunctional molecules such as iodoacetyl, maleimide, pyridyl disulfide, and thionyl chloride followed by the reaction with reactive ligands as shown in Table 21.1. In this approach, usually a linker molecule is used to link or bind the functional group of a SPION surface with the sulfhydryl (SH) of a biomolecule. Cystine amino acid residues are suitable target for reaction in case of biomolecules containing proteins and peptides. If reactive cystine amino acids are not present, terminal primary amine groups can be thiolated with heterofunctional linker such as *N*-succinimidyl-*S*-acetylthioacetate [17]. Alternatively, a free sulfhydryl group can be grafted to chlorotoxin through

modification with Traut's reagent (2-mercaptoethylamine-HCl reagents) [18]. However, in the latter case to avoid any risk of undesired cross-linking prior to reaction with NPs, the introduced sulfhydryl group initially needs to be protected.

Oligonucleotide based molecules such as siRNA biomolecules containing sulfhydryls was synthesized and conjugated to SPIONs via linker chemistry. In brief, Cy5.5 succinimide ester was conjugated with SPION followed by another conjugation with a heterobifunctional cross-linker, *N*-succinimidyl 3-(2-pyridyldithio) propionate [19].

Complex biological molecules or biomacromolecules with multiple reactive sites can be suitably conjugated using linker chemistry method. To improve the MRI probe for efficient detection of gene expression, SPIONs were linked with targeting ligands using both linker chemistry and direct conjugation by Högemann et al. [20]. In linker chemistry method, SPIONs with a cross-linked dextran coat were conjugated to transferrin (Tf) through the linker molecule *N*-succinimidyl 3-(2-pyridyldithio)propionate. Direct conjugation was carried out by oxidative activation of the dextran coat with subsequent reduction of Schiff's base. The comparison study revealed that conjugation using linker chemistry provided approximately four-times Tf molecules attached per SPION. The resulted SPION showed 10 times more enhancement of binding and uptake by cells, and 16 times better for imaging gene expression. Higher number of active Tf proteins at the NPs surface obtained by linker chemistry provided better control over the binding sites used in ligand conjugations. Another advantage of linker chemistry is it minimizes the risk of adverse effect on bioactivity of the protein as relatively a milder reactive condition of this chemistry limits the oxidative conditions.

Disulfide linkages and reaction byproduct produced by Pyridyl disulfide (PD) heterobifunctional linkers are cleavable and quantifiable, respectively. Quantification enables the possibility of evaluation of reaction efficiency. Schellenberger et al. [21] demonstrated this utility in the preparation of SPIONs conjugated with annexin V by PD linker molecule. If the application medium is reducing environment then other stable linkers such as iodoacetyls or maleimides should be used as the bonds formed via PD linker chemistry are sensitive to reducing environments.

Amide linkages are obtained through *N*-ethyl-*N*′-(3dimethylaminopropyl)-carbodiimide hydrochloride (EDAC)/*N*-hydroxysuccinimide (NHS) linkers where SPIONs decorated with carboxylic acid groups covalently bonded to biomolecules bearing primary amines. This approach has been used in the attachment t-Boc-protected folic acid [22] to SPIONs. Figure 21.3 shows another example of EDAC/NHS linker chemistry for the linking of aptamers with SPION [23]. EDAC/NHS can effectively attach molecules that have only one amino group. However, in case of multiple amines it is difficult to control the binding orientation of ligands, often resulting into inactivation of the ligands. Therefore, sulfhydryl-based linker chemistry is the preferred conjugation technique for the attachment of peptides, proteins, antibodies, and enzymes to amino-decorated SPIONs.

There are some drawbacks of linker chemistry such as complexation of covalent bond formation between NPs or ligands, which needs stepwise NP modification prior to ligand attachment. Some linker chemistries have low yield due to the long reaction times and purifications between each step.

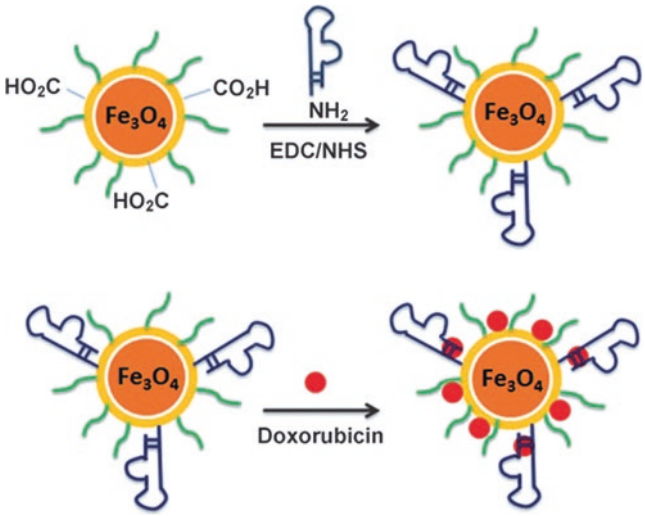
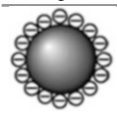
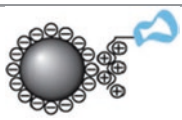
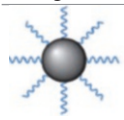
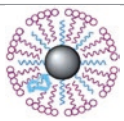
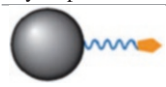
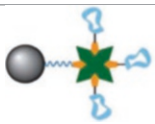


Fig. 21.3 Schematic illustration of the TCL-SPION–Apt bioconjugate system. Adapted from [23] with permission of Wiley

Table 21.3 Physical interaction attachment

Nanoparticle	Ligand	Functionalized nanoparticles	Reaction
 Charged surface			Electrostatic interaction
 Hydrophobic surface			Hydrophobic interaction
 Biotinylated			Biotin–avidin interaction

2.2 *Physical Interactions*

Physical interactions found in conjugation are mainly electrostatic, hydrophilic/hydrophobic, and affinity interactions that largely occurred onto the NPs surfaces (Table 21.3). These physical interactions offer several advantages. It allows for the design of very small targeted particles (<20 nm) on the basis of anionic nanoparticles. This surface modification and coupling techniques overcome the main disadvantages of SPIONs compared to gadolinium based probes. Other advantages of this coupling

technique include rapid binding, high efficiencies, and highly economic as no need for intermediate modification steps. Electrostatic interactions were found to be highly sufficient in complexing of plasmid DNA onto SPIONs. SPIONs were coated with cationic polymers of polyethylenimine (PEI) to be used as complexation agents for negatively charged plasmid DNA molecules [24]. The conjugate (SPION-PEI) is capable of assembling plasmid DNA into NPs with diameters ~100 nm and protecting the DNA from nuclease degradation. Highly efficient cells transfection with low toxicity was observed from the SPION-polyplexes. In addition, the T2 relaxation time of water was enhanced [25]. Electrostatic interactions were successfully applied to bind cationic proteins to an anionic SPION surface. The electrostatic attraction took place between the strongly positively charged peptide protamine and the anionic citrate shell of the electrostatically stabilize SPIONs [26].

Jain et al. [27] investigated the drug delivery and MRI properties of oleic acid-coated iron-oxide and pluronic-stabilized MNPs. Doxorubicin and paclitaxel drugs were loaded (alone or in combination) in MNPs with an efficiency of 74–95% and the drug release was sustained. Incorporated drugs showed marginal effects on physical (size and zeta potential), and magnetization properties of the MNPs. Hydrophobic interactions were the physical interaction force between the hydrophobic layers of drug and MNPs. However, hydrophobic interactions make NP sensitive to environmental conditions, decrease the T1 relaxation of MNPs slightly, and bring low control over molecular orientation of bound ligands. As a result, attachment of targeting ligands through these strategies is unattractive. On the other hand, affinity interactions, which is another form of physical interaction, can effectively bioconjugate targeting ligands to SPIONs [28]. Table 21.3 shows that SPION surfaces modified with streptavidin can be specifically bound to biotinylated molecules. The linkage formed is the strongest and highly stable of all noncovalent linkages chemistries. Unlike hydrophobic and electrostatic interactions, environmental conditions such as changes in pH, salinity, or hydrophilicity do not affect the affinity binding. Using this strategy Gunn et al. [29] produced high-affinity multivalent display of targeted SPIONs for immunotherapy applications.

3 Surface Chemistry Dependent on NP Material

The main chemistries developed for surface functionalization of silica NP, gold NP, quantum dots, and carbon nanotubes are summarized here. The most important reactions and recent highlights are mainly reported below, with a focus on the relationship between nanomaterial composition and functionalization method.

3.1 Silica NPs

One of the most widely used methods for the surface functionalization of NPs is silica coating. Silica NPs have gained interest for bioimaging application due to their straightforward and size-controlled synthesis, chemical and physical

stabilities, large surface area, hydrophilic surface, and well-defined surface chemistry. The same physicochemical properties also make them suitable as a protecting shell material for a wide number of nanomaterials. Such silica shells protect NPs against chemical and biochemical degradations, release of toxic ions, and activation of immune response along with hydrophilic, biocompatible and chemically active surfaces.

As-synthesized silica NPs possess highly hydrophilic surfaces, this is due to due to the presence of the silanol (Si-OH) groups on the surface of the particle, which make them one of the friendliest nanomaterials for biomedical applications including bioimaging. Furthermore, these silanol groups can chemically react with various reagents to render the silica hydrophobic. However, desired surface functional groups are required for the conjugation of contrast agents to the surface as well as inside the pores of silica NPs. The cocondensation process during the preparation of silica or post-synthesis surface modification is used to introduce reactive functional groups such as a primary or a secondary amino, carboxyl, hydroxyl, alkyl halogen, or azide group [30]. As-synthesized silica nanoparticles contain ample hydroxyl groups, which can be conjugated with isocyanates to form urethanes or carboxylic acids to form esters. Thiol-functionalized silica NPs can be obtained from the condensation of 3-mercaptopropyltriethoxy silane or its analogues with silanol groups on the surface of silica NPs. Thiol functionalized silica NPs can be further conjugated with gold nanoparticles, or biomolecules such as proteins, antibodies, peptides, polymers by reductive addition, disulfide coupling, or maleimide reaction. Thiol and maleimide functionalized molecules react with thiol groups on the surface of silica nanoparticles to form redox-active disulfide bonds, and thioether bonds, respectively [31]. Silica NPs can also be functionalized by introducing azide groups followed by click chemistry with alkyne substituted molecules.

Applications of silica NPs in bioimaging are vast due to their ability to accommodate MRI contrast agents and drug/DNA molecules to their adaptable surface and pores [32]. Chemical modifications of the surface of silica NPs can be conveniently chosen during the incorporation of various contrast agents. Various probes can be conjugated to silica NPs and efficiently delivered in different cell lines or injected in vivo [33]. Silica NPs can be coated with a dense layer of paramagnetic and PEGylated lipids in absence of coupling agent. The silica nanoparticles carrying a quantum dot in their center and were made target-specific by the conjugation of multiple αvβ3-integrin-specific RGD-peptides to enable their detection with both fluorescence techniques and MRI [34]. Figure 21.4 shows that higher signal intensity for the pellet of cells incubated with the RGD-conjugated Q-SiPaLCs (the bright white circle) was obtained as compared to the control cell pellets (gray circles). The differences in relaxation rate R1 (1/T1) clearly demonstrate the effective and specific targeting of this NPs agent to angiogenically activated endothelial cells (Fig. 21.4b). Core–shell-structured mesoporous silica nanoparticles was also synthesized to decrease T2 relaxation [35].

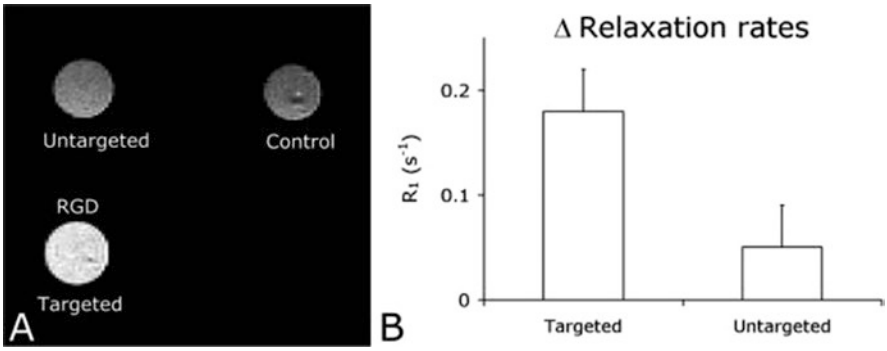


Fig. 21.4 (**a**) T1 weighted MRI of the different cell pellets revealed specific uptake of the targeted nanoparticles. (**b**) Difference in relaxation rates (R1) of the targeted and untargeted cell pellets compared to the relaxation rate of the control cell pellet. The differences in relaxation rate reflect the concentration of contrast agent in the untargeted and targeted cell pellets. Reprinted with permission from [34]. Copyright (2008) American Chemical Society

3.2 Gold NPs

Colloidal gold nanoparticles are highly suitable for bioimaging owing to their brilliant color and size-and-shape-dependent tunable surface plasmon. Other promising properties of gold NPs for bioimaging are the straightforward synthesis, well-defined surface chemistry, nontoxic nature, and the large one- and two-photon absorption cross sections. Optical and electronic properties of gold nanoparticles for bioimaging applications can be tuned by modifying their surface chemistry and aggregation state.

As-synthesized gold NPs do not have many types of surface capping ligands and functional groups. Therefore, gold NPs need to be decorated with desired functional groups via ligand exchange reactions and chemical modifications for enabling them for MRI applications. Usually the surface of as-synthesized gold NPs contains alkane thiols capping ligands. Giersig and Mulvaney [36] first reported that thiol plays an important role to facilitate the exchange reaction since it has high affinity for gold. The Au–S bond is relatively strong ($H = 253.6$ kJ/mol), though it is not as stable as the Si–O covalent bond ($H = 799.6$ kJ/mol) [37]. This makes the functionalization task easy by ligand exchange but colloidal stability overtime under highly saline conditions or in a biological environment is limited. Colloidal stability seems to be closely related to the ligand packing [38]. To overcome this limitation with multiple thiol groups have been synthesized on ligands to get more stable form, such as dihydrolipoic acid (DHLA) [39] or thioctic acid [40]. Multiple binding points provide increased stability allowing for more densely packed ligands where sulfur anchoring groups are structurally constrained [41]. Diazonium salts chemistry is another possible option, however, the drastic conditions of the in situ diazonium formation limits it potential application [42]. Gold can be coated via a reduction of gold precursors on SPIONs of selected sizes as seeds [43]. In addition to core–shell structures, gold coating on SPIONs with heterostructures are widely used in medical practice (Fig. 21.5) [44].

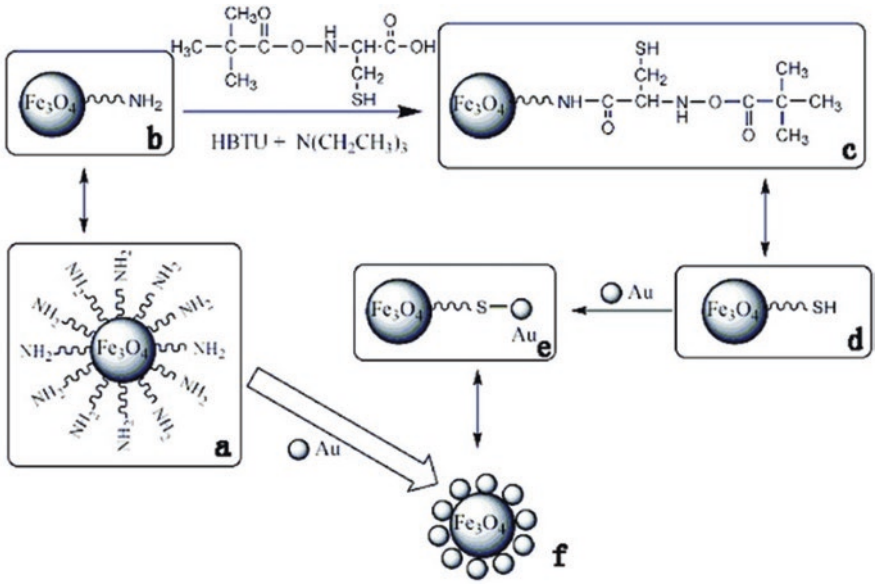


Fig. 21.5 Schematic illustration of bifunctional Au–Fe_3O_4 nanoparticle synthesis. Amino-functionalized Fe_3O_4 nanoparticles [(**a**), simplified as (**b**) for the convenience of illustration] were first modified by Boc-L-cysteine to have surface thiol groups [(**c**), simplified as (**d**)]. Gold nanoparticles were the conjugated onto the surface of Fe_3O_4 nanoparticles to form the expected Fe_3O_4–Au bifunctional nanoparticles [(**f**), simplified as (**e**)]. Reprinted with permission from [44]. Copyright (2007) American Chemical Society

3.3 Gadolinium NP

One promising new direction in the development of MRI contrast agents involves the labeling and/or loading of nanoparticles with gadolinium (Gd). Gadolinium ion (Gd^{3+}) and calcium ion (Ca^{2+}) have very similar ionic radius although former has higher positive charge. Ca^{2+}-requiring proteins such as calmodulin, calsequestrin, and calexitin cannot distinguish (Gd^{3+}) and (Ca^{2+}). Consequently, Gd^{3+} would quickly bind to Ca^{2+} channels [45]. Free or unchelated gadolinium ions have toxic effect on most biological systems and cannot be administered to a patient in their aqueous form. Potential toxicity of the gadolinium ions can be suppressed by binding them with a strongly coordinating ligand for clinical examinations. Currently approved gadolinium-based CAs for clinical MRI are given in Fig. 21.6. These existing forms of gadolinium ions can directly constitute NPs for MRI CAs. Gadolinium ion can also be used as a chelate incorporated into the nanocarriers.

There is a range of polymeric micelles designed to develop gadolinium-based nanoscale CAs for MRI. The most commonly polymeric micelles is developed by conjugating the gadolinium chelates to the hydrophilic layer, which modifies the relaxivity properties favorably. Nanoscale micelles based on poly(L-glutamic acid)-

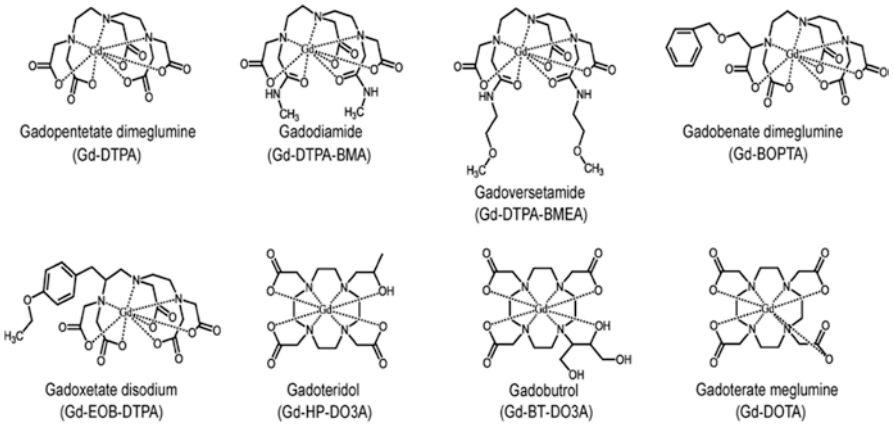


Fig. 21.6 Chemical structure of currently marketed gadolinium-based MRI CAs. Reproduced from [3] with permission of Royal Society of Chemistry

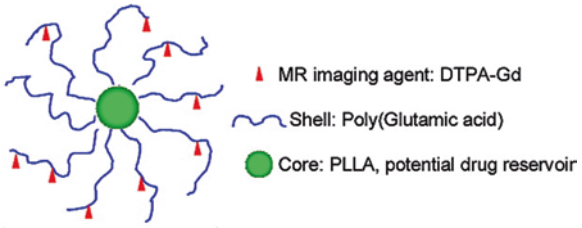


Fig. 21.7 Schematic model of the micellar structure with DTPA-Gd chelated to the shell layer. Adapted from with permission [46]. Copyright (2008) American Chemical Society

b-polylactide block copolymer was produced with paramagnetic Gd^{3+} ions chelated to their shell. The metal chelator *p*-aminobenzyldiethylenetriaminepenta(acetic acid) (DTPA) was used to readily conjugate to the side chain carboxylic acids of poly(L-glutamic acid). The resulting DTPA-Gd chelated spherical micelles (Fig. 21.7) exhibited significantly higher spin–lattice relaxivity than a small-molecular-weight MRI CA [46]. A suitable chelating agent such as 1,4,7,10-tetraazacyclododecane-1, 4,7,10-tetraacetic acid mono (*N*-hydroxysuccinimide ester) (DOTA-OSu) was used as an active chelating agent to conjugate gadolinium ions with a block copolymer, PEG-*b*-poly(L-lysine). After conjugation to all primary amine groups of the lysine residues through DOTA moieties, a polymeric micelle was obtained. The polymeric micelle-based MRI CA exhibited enhanced permeability and retention effect. A considerable amount of the polymeric micelle CA accumulated at solid tumors which doubled the MRI signal intensity [47].

Gadolinium can be prepared as hydrogel to be used as a CA in MRI. Gadolinium based nanohydrogel CA was prepared by incorporating Gd chelating cross-linkers into self-assembled pullulan nanogels to avoid repeated administration of CA and improve signal-to-noise ratios [48]. Effective and biosafe CA was prepared by attaching multiple Gd chelates to mesoporous silica nanoparticles (MSNs). The Gd^{3+} chelates were attached to the surface of dendrons via click chemistry. Resultant CA showed an approximately 11-fold increase in the relaxivity and enhancement of MR images [49].

3.4 Iron Oxide NPs

Typically, interaction between CAs and surrounding water protons typically originates and enhancement of MRI, which shorten the longitudinal (T1) or transverse (T2) relaxation time of nearby water molecules. Usually, T1 CAs are applied to increase signal intensity, resulting in a positive contrast enhancement (brighter image) in T1-weighted MRI, whereas T2 CAs can decrease signal intensity, providing a negative contrast enhancement (darker image) in T2-weighted MRI. Iron oxide NPs are generally considered safer than Gd-based CAs because iron is found in the human body, mostly stored as ferritin in the blood. Five unpaired electrons make Fe^{3+} a promising candidate for T1 CAs. Enhanced T1 contrast effects from small-sized iron oxide NP are obtained due to the presence of a large number of Fe^{3+} ions on the surface, which suppress T2 relaxation by their small magnetic moment [50].

The surface of iron oxide is rapidly oxidized by air so synthesized iron nanoparticles are not stable under aerobic atmosphere. Moreover, as synthesized, unmodified iron oxide NPs are not stable in vivo condition. Therefore, iron oxide NPs need to be coated. For instance, iron (Fe) core can be stabilized by controlling the surface oxidation using an oxygen transfer agent (i.e., trimethylamine *N*-oxide) [51]. Additionally, coating protects against iron oxide core agglomeration, provides chemical handles for the conjugation of targeting ligands, and avoids nonspecific cell interaction. A number of ways, including in situ coating, postsynthesis adsorption, and postsynthesis end grafting, can achieve coating [52]. Uniformly encapsulate coated cores are achieved in case of in situ and post synthesis modification with polysaccharides and copolymers whereas brush like extension anchored to the NP surface by the polymer end groups are obtained by grafting polymers (e.g., PEG). Alternatively, shell around the iron oxide core can be obtained using liposome and micelle-forming molecules for coating. Coating thickness and hydrophobicity are important as magnetic properties of the CAs are greatly affected by them [52, 53]. Thicker coatings can lower R2 relaxivities [53] and higher relaxivities can be obtained with hydrophilic coatings [54].

Iron oxide NPs can be made soluble in an aqueous solution using hydrophilic ligands with various anchoring groups, including carboxylic acids, catechol-based molecules (e.g., dopamine) [55], and bisphosphonates [56]. Silanization can be used for iron oxide materials. Iron oxide NPs was coated with a mixture of 3-aminopropylsilane (APS) and PEG-silane of different lengths by a ligand exchange reaction [57].

3.5 Quantum Dots

Quantum dots (QDs) is one of the most attractive nanomaterials in the biomedical fields due to their size-dependent tunable optical and electronic properties. Cell imaging is the main biological application of QDs. QDs can be categorized into two groups based on their cell biological applications such as nonspecific, and

cell-specific. Cell-specific and nonspecific mainly depend on the property of molecules recruited to the surface of QDs. Nonspecific extracellular and intracellular labeling can detect and image any cell type whereas biomarker specific targeted cell labeling is required for the detection and imaging of cancer cells and tumor milieu [58]. QDs are synthesized in the organic medium and are finished with highly hydrophobic aliphatic ligands such as alkyl phosphines, alkyl phosphine oxides, aliphatic amines, and aliphatic carboxylic acids. Therefore, ligand exchange reactions and surface modifications are necessary to make them suitable for biological applications.

Functionalizing semiconductor QD with biomolecules has some major challenges such as chemical instability in aqueous solutions, photo etching, toxicity due to the leaching out of cadmium ions, and unstable photoluminescence [59]. Surface coating with amphiphilic ligands or polymers can provide water soluble QDs. Ligand exchange, electrostatic adsorption, or covalent attachment are used for coupling small molecules or biomolecules to functional groups of polymer [60]. In another approach, Paquet et al. [61] produced biofunctionalized QDs by associating polyhistidine tags with Zn atoms present on the QD surface. Conjugation of growth hormones and antibodies with QDs is required for targeted labeling of cells. Low-cost alternatives such as hyaluronic acid and folic acid are also investigated [58].

3.6 Carbon Nanomaterials

Nowadays, carbon nanomaterials are emerging as an interesting class of nanostructures for biological imaging due to their favorable optoelectronic properties such as NIR fluorescence, photoluminescence and unique Raman signature [62]. Carbon nanotubes (CNT), graphene, carbon dots, and nanodiamonds are the main members of carbonaceous nanomaterials although some novel structures have been recently cited such as carbon nanohorns and nanoonions [63, 64]. However, as synthesized, these structures are hydrophobic and not biocompatible. Therefore, covalent or noncovalent conjugation of hydrophilic molecules to them is prerequisite to enable them for biological applications. In case of noncovalent conjugation which is mainly π–π stacking, and hydrophobic interactions, the optoelectronic properties of the functionalized carbon nanomaterials are not affected. Sonication with amphiphilic molecules or surfactants helps to disperse carbon nanomaterials. However, the resulting suspensions are toxic and thus generally not compatible with in vivo application. PEG-based surfactants are preferred for in vivo applications. In case of covalent conjugation of carbon nanomaterials, stable suspensions in water are possible to obtain. Cycloaddition and oxidation reactions have been tried to impart various functional groups to sp^2 carbon atoms. For example, oxidation by nitric acid generates carboxyl groups on the ends of CNTs, which can further react with a variety of functional groups. In addition, in situ reduction of aryl-diazonium salts or Diels–Alder cycloaddition, among other organic reactions, can be used to conjugate dyes, biomolecules, ligands, drugs, or other nanomaterials [65].

4 Organic Surface Coatings

4.1 Poly Ethylene Glycol (PEG)

Poly ethylene glycol (PEG) is a frequently used biocompatible linear synthetic polyether [66]. A low-molecular weight PEG is a clear, colorless, and viscous liquid. It is highly soluble with water and mostly soluble with alcohol and other organic solvents. Low-molecular weight PEG relatively low toxicity, which exhibited a good potential, and used in many cosmetic, nutraceutical, and pharmaceutical applications. Moreover, PEG can be prepared with a wide range of terminal functional groups as derivatives [67]. PEG along with its derivatives has been used clinically as excipients in FDA approved pharmaceutical formulations [68]. Being hydrophilic in biological fluids, PEG coating improve dispersity and blood circulation time of SPIONs [69]. PEG-coated (or PEGylated) SPIONs are not readily recognized by the reticuloendothelial system, hence, commonly regarded to as "stealth" nanoparticles [70]. This makes them suitable for target-specific cell labeling after modification with targeting ligands [71]. However, the same characteristic limits their use in imaging macrophages or other RES-related cells [72].

PEG polymers with molecular weight below 100,000 Da show amphiphilic characteristic and are soluble in water as well as in many organic solvents such as methylene chloride, ethanol, toluene, acetone, and chloroform. A variety of chemistries requiring the use of either aqueous or organic solvents are suitable for assembling at the SPION surface. For example, PEG can be coated onto SPIONs either by aqueous precipitation [73], or by grafting in the organic solvent (toluene) via a silane group [22]. Grafting in the organic solvent yielded a heterobifunctional PEG that have two ends. On end can be covalently attached to the SPION surface and the other end can be functionalized with targeting ligands, imaging reporter molecules, or therapeutic agents [18]. However, a PEG shell does not favor the uptake of SPIONs by most cells. Modification of the SPIONs with hyaluronic acid (HA) that act as a targeting moiety can solve this problem [74]. A recent study reported that different terminal groups of PEG could be used for fluorescence–MR dual-modality imaging guided cancer photothermal therapy [75].

4.2 Dextran

Dextran is a glycan composed of glucose subunits with a molecular weight ranging from 10 to 150 kDa. Its polar interactions (chelation and hydrogen bonding) provide a high affinity for iron oxide surfaces [76]. It is biocompatible as a result many of the clinically approved SPION preparations are dextran coated [77]. Molday and Mackenzie [78] first demonstrated the in situ coating preparation in 1982. Since then, different forms of dextran polymers such as carboxydextran and carboxymethyl dextran have been coated on SPIONs with varying hydrodynamic sizes [52].

Conventional dextran coatings are based on hydrogen bonding, which makes the polymer prone to detachment. However, cross-linked iron oxide (CLIO) can be a solution where coated polymers after SPION attachment are cross-linked using epichlorohydrin and ammonia [79]. Dextran coated CLIO nanoparticles were found to be a suitable platform for the synthesis of multifunctional imaging agents [80]. Although CLIOs improve circulation half-life in blood with no acute toxicity [81] due to the use of epichlorohydrin and their nonbiodegradability make them unsuitable for using in a clinical setting [82]. A multistep process using silane chemistry, which offer covalent bonding between dextran and SPION, can be an alternative of cross-linking [83].

4.3 Chitosan

Chitosan is a cationic, hydrophilic, and biodegradable natural polymer. It is derived by deacetylation of chitin obtained from the shells of crustaceans. Its large abundance in nature, ease of functionalization, biological activities, biocompatibility, high charge density, low toxicity toward mammalian cells, and ability to improve dissolution have made it a popular material for many biological applications [84]. Although chitosan and its derivatives have been used to develop polymeric nanoparticles through electrostatic complexation for decades [85], their use in magnetic nanoparticles is recent [86]. Direct and in situ coating of chitosan onto SPIONs is not easy as they are sparingly soluble at pH levels necessary to precipitate SPIONs [84]. However, chitosan-coated SPIONs was produced by adsorbing chitosan physically onto SPIONs coated with oleic acid which gave spherically shaped SPIONs with a diameter of 15 nm [87]. Chitosan-coated SPIONs can be used as gene delivery carrier in addition to CA due to the cationic nature of the chitosan that allows complexation with genetic material. For example, Bhattarai et al. [86] loaded anionic adenovirus vectors through electrostatic interactions on chitosan-coated SPIONs. These SPIONs showed enhanced gene transfection property. Moreover, chitosan possesses both amino and hydroxyl functional groups, which can be used for SPION functionalization with targeting, imaging, and therapeutic agents.

4.4 Liposomes and Micelles

Liposomes and micelles, spherical aggregates of amphiphilic molecules, are usually biocompatible as they are lipid bilayer of lamellar phase. Being bilayer structure liposomes can encapsulate SPIONs with resultant diameters ranging from 100 nm to 5 μm. Thus, liposome encapsulation can gather a certain number of MNPs for collective delivery to the target. For these reasons, liposome complexes become an ideal platform for delivery of contrast agents in MRI [88]. Liposomes and micelles coating can be done in two ways: postsynthesis incorporation or by in situ synthesizing. In the

first approach, water-soluble SPIONs can be attached to the aqueous center of the liposome [89], or alternatively, micelles can be coated around the structure to get hydrophobic SPIONs [90]. Second approach provides uniform NPs with a diameter of 15 nm where SPIONs can be precipitated in the liposomal core [91].

SPION coating with either liposomal or micellar structures has advantages over direct synthesis such as simple and easy surface modification, convenient encapsulation of pharmaceuticals inside the amphiphilic substructures, and chelation and protection of pharmaceuticals from the body until degraded in target cells [92]. However, there is a risk of coating agglomerates rather than discrete SPION cores in micellar or phospholipid structures, leading to poor physicochemical and magnetic properties [93].

4.5 Copolymers

Copolymers allow taking advantage of the distinct functionalities obtained from its constituents. Copolymer obtained by joining PEI and PEG polymers, can both form complex DNA to facilitate cell transfection (PEI functionality), and enable molecular targeting of cancer cells (PEG functionality) [94]. The advantages these copolymers provide can be applied to SPION coatings. For instance, a DNA delivering nanovector was developed, recently, by coating SPION with a copolymer of PEG-g-chitosan-g-PEI [95]. This study demonstrated that PEG, chitosan, and PEI polymers grafted together enabled the NP for DNA complexation, stabilization for in vivo use, and gene transfection. A unique pH-sensitive coating with a hydrophobic center layer was prepared using triblock PEG-poly(methacrylic acid)-poly(glycerol monomethacrylate) copolymer on SPIONs in situ. Coated NP was capable of encapsulating drug molecules and preferentially releasing the therapeutics in the acidic environment of the cellular endosome [96]. Similar copolymers were attach to the SPION surface by layer-by-layer deposition directed by matching of electrostatic interactions [97], hydrophilic/hydrophobic interactions [98], and covalently grafting polymer layers to base coatings [99].

5 Conclusion

Surface-functionalized NPs are extensively studied as CAs for MRI. This chapter mainly focuses on the surface modification of NPs such as silica NPs, gold NPs and quantum dots, carbon nanomaterials, and organic polymer coating including dextran, chitosan, PEG, copolymers through various bioconjugation reactions. Many biological applications of nanomaterials including imaging CAs, drug and gene delivery systems, biosensors, and nanomedicine share common functional groups, which are typically attached onto the surface of nanomaterials via suitable chemical or bioconjugation reaction to create nanofunctional nanodevices. However, that

there are some challenges and limitations including toxicity and biocompatibility is still a major issue to create nanomaterials using CAs for MRI application. Hence, multimodal and multifunctional NPs fabricated by the conjugation of various targeting molecules and CAs are extensively investigated for MRI on the way to treatment of cancer cells and tumors. Yet, controversial reports about toxicity and pharmacokinetics do not permit the clinical applications. Therefore, arguments of functionalized CAs that need to be addressed are biocompatibility, toxicity, in vivo and in vitro targeting efficiency, bioavailability, and renal and hepatobiliary clearance. The current scenario of research in the formulation and testing of CAs indicate that we may not to wait long for the complete transformation of conventional medicine into nanomedicine.

References

1. Smith, B. R., & Gambhir, S. S. (2017). Nanomaterials for in vivo imaging. *Chemical Reviews, 117*(3), 901–986. https://doi.org/10.1021/acs.chemrev.6b00073.
2. Na, H. B., & Hyeon, T. (2009). Nanostructured T1 MRI contrast agents. *Journal of Materials Chemistry, 19*(35), 6267–6273. https://doi.org/10.1039/B902685A.
3. Cao, Y., Xu, L., Kuang, Y., Xiong, D., & Pei, R. (2017). Gadolinium-based nanoscale MRI contrast agents for tumor imaging. *Journal of Materials Chemistry B, 5*(19), 3431–3461. https://doi.org/10.1039/C7TB00382J.
4. Lee, N., & Hyeon, T. (2012). Designed synthesis of uniformly sized iron oxide nanoparticles for efficient magnetic resonance imaging contrast agents. *Chemical Society Reviews, 41*(7), 2575–2589. https://doi.org/10.1039/C1CS15248C.
5. Hahn, M. A., Singh, A. K., Sharma, P., Brown, S. C., & Moudgil, B. M. (2011). Nanoparticles as contrast agents for in-vivo bioimaging: Current status and future perspectives. *Analytical and Bioanalytical Chemistry, 399*(1), 3–27. https://doi.org/10.1007/s00216-010-4207-5.
6. Merbach, A. S., Helm, L., & Toth, E. (2013). *The chemistry of contrast agents in medical magnetic resonance imaging*. Hoboken, NJ: Wiley.
7. Chan, K. W.-Y., & Wong, W.-T. (2007). Small molecular gadolinium(III) complexes as MRI contrast agents for diagnostic imaging. *Coordination Chemistry Reviews, 251*(17), 2428–2451. https://doi.org/10.1016/j.ccr.2007.04.018.
8. Weissleder, R., Kelly, K., Sun, E. Y., Shtatland, T., & Josephson, L. (2005). Cell-specific targeting of nanoparticles by multivalent attachment of small molecules. *Nature Biotechnology, 23*, 1418. https://doi.org/10.1038/nbt1159.
9. Veiseh, O., Kievit, F., Ellenbogen, R. G., & Zhang, M. (2011). Cancer cell invasion: Treatment and monitoring opportunities in nanomedicine. *Advanced Drug Delivery Reviews, 63*(8), 582–596. https://doi.org/10.1016/j.addr.2011.01.010.
10. Schellenberger, E. A., Weissleder, R., & Josephson, L. (2004b). Optimal modification of annexin V with fluorescent dyes. *Chembiochem: A European Journal of Chemical Biology, 5*(3), 271–274. https://doi.org/10.1002/cbic.200300741.
11. Lam, T., Avti, P. K., Pouliot, P., Maafi, F., Tardif, J. C., Rheaume, E., Lesage, F., & Kakkar, A. (2016). Fabricating water dispersible superparamagnetic iron oxide nanoparticles for biomedical applications through ligand exchange and direct conjugation. *Nanomaterials, 6*(6), E100. https://doi.org/10.3390/nano6060100.
12. Sharpless, K. B., Finn, M. G., & Kolb, H. C. (2001). Click chemistry: Diverse chemical function from a few good reactions. *Angewandte Chemie (International ed in English), 40*(11), 2004–2021. https://doi.org/10.1002/1521-3773(20010601)40:11%3C2004::AID-ANIE2004%3E3.0.CO;2-5.

13. Lutz, J. F., & Zarafshani, Z. (2008). Efficient construction of therapeutics, bioconjugates, biomaterials and bioactive surfaces using azide-alkyne "click" chemistry. *Advanced Drug Delivery Reviews, 60*(9), 958–970. https://doi.org/10.1016/j.addr.2008.02.004.
14. Hein, C. D., Liu, X. M., & Wang, D. (2008). Click chemistry, a powerful tool for pharmaceutical sciences. *Pharmaceutical Research, 25*(10), 2216–2230. https://doi.org/10.1007/s11095-008-9616-1.
15. Sun, E. Y., Josephson, L., & Weissleder, R. (2006). "Clickable" nanoparticles for targeted imaging. *Molecular Imaging, 5*(2), 122–128.
16. Maltzahn, v G., Ren, Y., Park, J.-H., Min, D.-H., Kotamraju, V. R., Jayakumar, J., Fogal, V., Sailor, M. J., Ruoslahti, E., & Bhatia, S. N. (2008). In vivo tumor cell targeting with "Click" nanoparticles. *Bioconjugate Chemistry, 19*(8), 1570–1578. https://doi.org/10.1021/bc800077y.
17. Veiseh, O., Sun, C., Gunn, J., Kohler, N., Gabikian, P., Lee, D., Bhattarai, N., Ellenbogen, R., Sze, R., Hallahan, A., Olson, J., & Zhang, M. (2005). Optical and MRI multifunctional nanoprobe for targeting gliomas. *Nano Letters, 5*(6), 1003–1008. https://doi.org/10.1021/nl0502569.
18. Conroy, S., Omid, V., Jonathan, G., Chen, F., Stacey, H., Donghoon, L., Raymond, S., Richard, G. E., Jim, O., & Miqin, Z. (2008). In vivo MRI detection of gliomas by chlorotoxin-conjugated superparamagnetic nanoprobes. *Small, 4*(3), 372–379. https://doi.org/10.1002/smll.200700784.
19. Medarova, Z., Pham, W., Farrar, C., Petkova, V., & Moore, A. (2007). In vivo imaging of siRNA delivery and silencing in tumors. *Nature Medicine, 13*, 372. https://doi.org/10.1038/nm1486.
20. Högemann, D., Josephson, L., Weissleder, R., & Basilion, J. P. (2000). Improvement of MRI probes to allow efficient detection of gene expression. *Bioconjugate Chemistry, 11*(6), 941–946. https://doi.org/10.1021/bc000079x.
21. Schellenberger, E. A., Sosnovik, D., Weissleder, R., & Josephson, L. (2004a). Magneto/optical annexin V, a multimodal protein. *Bioconjugate Chemistry, 15*(5), 1062–1067. https://doi.org/10.1021/bc049905i.
22. Kohler, N., Fryxell, G. E., & Zhang, M. (2004). A bifunctional poly(ethylene glycol) silane immobilized on metallic oxide-based nanoparticles for conjugation with cell targeting agents. *Journal of the American Chemical Society, 126*(23), 7206–7211. https://doi.org/10.1021/ja049195r.
23. Wang, A. Z., Vaishali, B., Christophoros, V. C., Frank, G., Frank, A., Liangfang, Z., Mariam, S., Kai, Y., Michael, J. C., Robert, L., Philip, W. K., Neil, H. B., Sangyong, J., & Omid, C. F. (2008). Superparamagnetic iron oxide nanoparticle-aptamer bioconjugates for combined prostate cancer imaging and therapy. *ChemMedChem, 3*(9), 1311–1315. https://doi.org/10.1002/cmdc.200800091.
24. Steitz, B., Hofmann, H., Kamau, S. W., Hassa, P. O., Hottiger, M. O., von Rechenberg, B., Hofmann-Amtenbrink, M., & Petri-Fink, A. (2007). Characterization of PEI-coated superparamagnetic iron oxide nanoparticles for transfection: Size distribution, colloidal properties and DNA interaction. *Journal of Magnetism and Magnetic Materials, 311*(1), 300–305. https://doi.org/10.1016/j.jmmm.2006.10.1194.
25. Park, I.-K., Ng, C.-P., Wang, J., Chu, B., Yuan, C., Zhang, S., & Pun, S. H. (2008). Determination of nanoparticle vehicle unpackaging by MR imaging of a T(2) magnetic relaxation switch. *Biomaterials, 29*(6), 724–732. https://doi.org/10.1016/j.biomaterials.2007.10.018.
26. Eyk, S., Jörg, S., Chris, R., Liset, U., Wolfdietrich, M., Matthias, T., & Bernd, H. (2008). Linking proteins with anionic nanoparticles via protamine: Ultrasmall protein-coupled probes for magnetic resonance imaging of apoptosis. *Small, 4*(2), 225–230. https://doi.org/10.1002/smll.200700847.
27. Jain, T. K., Richey, J., Strand, M., Leslie-Pelecky, D. L., Flask, C. A., & Labhasetwar, V. (2008). Magnetic nanoparticles with dual functional properties: Drug delivery and magnetic resonance imaging. *Biomaterials, 29*(29), 4012–4021. https://doi.org/10.1016/j.biomaterials.2008.07.004.

28. Pan, D., Caruthers, S. D., Hu, G., Senpan, A., Scott, M. J., Gaffney, P. J., Wickline, S. A., & Lanza, G. M. (2008). Ligand-directed nanobialys as theranostic agent for drug delivery and manganese-based magnetic resonance imaging of vascular targets. *Journal of the American Chemical Society, 130*(29), 9186–9187. https://doi.org/10.1021/ja801482d.
29. Gunn, J., Wallen, H., Veiseh, O., Sun, C., Fang, C., Cao, J., Yee, C., & Zhang, M. (2008). A multimodal targeting nanoparticle for selectively labeling T cells. *Small, 4*(6), 712–715. https://doi.org/10.1002/smll.200701103.
30. Wu, S.-H., Mou, C.-Y., & Lin, H.-P. (2013). Synthesis of mesoporous silica nanoparticles. *Chemical Society Reviews, 42*(9), 3862–3875. https://doi.org/10.1039/C3CS35405A.
31. Yang, P., Gai, S., & Lin, J. (2012). Functionalized mesoporous silica materials for controlled drug delivery. *Chemical Society Reviews, 41*(9), 3679–3698. https://doi.org/10.1039/C2CS15308D.
32. Tae-Jong, Y., Nam, Y. K., Eunha, K., Sung, K. J., Geol, K. B., Sang-Hyun, Y., Byeong-Hyeok, S., Myung-Haing, C., Jin-Kyu, L., & Bum, P. S. (2006). Specific targeting, cell sorting, and bioimaging with smart magnetic silica core–shell nanomaterials. *Small, 2*(2), 209–215. https://doi.org/10.1002/smll.200500360.
33. Tallury, P., Payton, K., & Santra, S. (2008). Silica-based multimodal/multifunctional nanoparticles for bioimaging and biosensing applications. *Nanomedicine, 3*(4), 579–592. https://doi.org/10.2217/17435889.3.4.579.
34. Koole, R., van Schooneveld, M. M., Hilhorst, J., Castermans, K., Cormode, D. P., Strijkers, G. J., de Mello Donegá, C., Vanmaekelbergh, D., Griffioen, A. W., Nicolay, K., Fayad, Z. A., Meijerink, A., & Mulder, W. J. M. (2008). Paramagnetic lipid-coated silica nanoparticles with a fluorescent quantum dot core: A new contrast agent platform for multimodality imaging. *Bioconjugate Chemistry, 19*(12), 2471–2479. https://doi.org/10.1021/bc800368x.
35. Wang, F., Chen, X., Zhao, Z., Tang, S., Huang, X., Lin, C., Cai, C., & Zheng, N. (2011). Synthesis of magnetic, fluorescent and mesoporous core-shell-structured nanoparticles for imaging, targeting and photodynamic therapy. *Journal of Materials Chemistry, 21*(30), 11244–11252. https://doi.org/10.1039/C1JM10329F.
36. Giersig, M., & Mulvaney, P. (1993). Preparation of ordered colloid monolayers by electrophoretic deposition. *Langmuir, 9*(12), 3408–3413. https://doi.org/10.1021/la00036a014.
37. Haynes, W. M. (2014). *CRC handbook of chemistry and physics*. Boca Raton, FL: CRC.
38. Hou, W., Dasog, M., & Scott, R. W. J. (2009). Probing the relative stability of thiolate- and dithiolate-protected au monolayer-protected clusters. *Langmuir, 25*(22), 12954–12961. https://doi.org/10.1021/la9018053.
39. Roux, S., Garcia, B., Bridot, J.-L., Salomé, M., Marquette, C., Lemelle, L., Gillet, P., Blum, L., Perriat, P., & Tillement, O. (2005). Synthesis, characterization of dihydrolipoic acid capped gold nanoparticles, and functionalization by the electroluminescent luminol. *Langmuir, 21*(6), 2526–2536. https://doi.org/10.1021/la048082i.
40. Pérez-Rentero, S., Grijalvo, S., Peñuelas, G., Fàbrega, C., & Eritja, R. (2014). Thioctic acid derivatives as building blocks to incorporate DNA oligonucleotides onto gold nanoparticles. *Molecules, 19*(7), 10495. https://doi.org/10.3390/molecules190710495.
41. Oh, E., Susumu, K., Mäkinen, A. J., Deschamps, J. R., Huston, A. L., & Medintz, I. L. (2013). Colloidal stability of gold nanoparticles coated with multithiol-poly(ethylene glycol) ligands: Importance of structural constraints of the sulfur anchoring groups. *The Journal of Physical Chemistry C, 117*(37), 18947–18956. https://doi.org/10.1021/jp405265u.
42. Gehan, H., Fillaud, L., Felidj, N., Aubard, J., Lang, P., Chehimi, M. M., & Mangeney, C. (2010). A general approach combining diazonium salts and click chemistries for gold surface functionalization by nanoparticle assemblies. *Langmuir, 26*(6), 3975–3980. https://doi.org/10.1021/la9033436.
43. WangWang, L. J., Fan, Q., Suzuki, M., Suzuki, I. S., Engelhard, M. H., Lin, Y., Kim, N., Wang, J. Q., & Zhong, C.-J. (2005). Monodispersed core–shell Fe3O4@Au nanoparticles. *The Journal of Physical Chemistry B, 109*(46), 21593–21601. https://doi.org/10.1021/jp0543429.

44. Bao, J., Chen, W., Liu, T., Zhu, Y., Jin, P., Wang, L., Liu, J., Wei, Y., & Li, Y. (2007). Bifunctional Au-Fe3O4 nanoparticles for protein separation. *ACS Nano, 1*(4), 293–298. https://doi.org/10.1021/nn700189h.
45. Fraum, T. J., Ludwig, D. R., Bashir, M. R., & Fowler, K. J. (2017). Gadolinium-based contrast agents: A comprehensive risk assessment. *Journal of Magnetic Resonance Imaging, 46*(2), 338–353. https://doi.org/10.1002/jmri.25625.
46. Zhang, G., Zhang, R., Wen, X., Li, L., & Li, C. (2008). Micelles based on biodegradable poly(l-glutamic acid)-b-polylactide with paramagnetic Gd ions chelated to the shell layer as a potential nanoscale MRI-visible delivery system. *Biomacromolecules, 9*(1), 36–42. https://doi.org/10.1021/bm700713p.
47. Shiraishi, K., Kawano, K., Minowa, T., Maitani, Y., & Yokoyama, M. (2009). Preparation and in vivo imaging of PEG-poly(L-lysine)-based polymeric micelle MRI contrast agents. *Journal of Controlled Release, 136*(1), 14–20. https://doi.org/10.1016/j.jconrel.2009.01.010.
48. Chan, M., Lux, J., Nishimura, T., Akiyoshi, K., & Almutairi, A. (2015). Long-lasting and efficient tumor imaging using a high relaxivity polysaccharide nanogel magnetic resonance imaging contrast agent. *Biomacromolecules, 16*(9), 2964–2971. https://doi.org/10.1021/acs.biomac.5b00867.
49. Guo, C., Hu, J., Bains, A., Pan, D., Luo, K., Li, N., & Gu, Z. (2016). The potential of peptide dendron functionalized and gadolinium loaded mesoporous silica nanoparticles as magnetic resonance imaging contrast agents. *Journal of Materials Chemistry B, 4*(13), 2322–2331. https://doi.org/10.1039/C5TB02709H.
50. Zhang, H., Li, L., Liu, X. L., Jiao, J., Ng, C.-T., Yi, J. B., Luo, Y. E., Bay, B.-H., Zhao, L. Y., Peng, M. L., Gu, N., & Fan, H. M. (2017). Ultrasmall ferrite nanoparticles synthesized via dynamic simultaneous thermal decomposition for high-performance and multifunctional T1 magnetic resonance imaging contrast agent. *ACS Nano, 11*(4), 3614–3631. https://doi.org/10.1021/acsnano.6b07684.
51. Peng, S., Wang, C., Xie, J., & Sun, S. (2006). Synthesis and stabilization of monodisperse Fe nanoparticles. *Journal of the American Chemical Society, 128*(33), 10676–10677. https://doi.org/10.1021/ja063969h.
52. Laurent, S., Forge, D., Port, M., Roch, A., Robic, C., Vander Elst, L., & Muller, R. N. (2008). Magnetic iron oxide nanoparticles: Synthesis, stabilization, vectorization, physicochemical characterizations, and biological applications. *Chemical Reviews, 108*(6), 2064–2110. https://doi.org/10.1021/cr068445e.
53. LaConte, L. E. W., Nitin, N., Zurkiya, O., Caruntu, D., O'Connor, C. J., Hu, X., & Bao, G. (2007). Coating thickness of magnetic iron oxide nanoparticles affects R2 relaxivity. *Journal of Magnetic Resonance Imaging, 26*(6), 1634–1641. https://doi.org/10.1002/jmri.21194.
54. Duan, H., Kuang, M., Wang, X., Wang, Y. A., Mao, H., & Nie, S. (2008). Reexamining the effects of particle size and surface chemistry on the magnetic properties of iron oxide nanocrystals: New insights into spin disorder and proton relaxivity. *The Journal of Physical Chemistry C, 112*(22), 8127–8131. https://doi.org/10.1021/jp8029083.
55. Gillich, T., Acikgöz, C., Isa, L., Schlüter, A. D., Spencer, N. D., & Textor, M. (2013). PEG-stabilized core–shell nanoparticles: Impact of linear versus dendritic polymer shell architecture on colloidal properties and the reversibility of temperature-induced aggregation. *ACS Nano, 7*(1), 316–329. https://doi.org/10.1021/nn304045q.
56. Lalatonne, Y., Paris, C., Serfaty, J. M., Weinmann, P., Lecouvey, M., & Motte, L. (2008). Bisphosphonates-ultra small superparamagnetic iron oxide nanoparticles: A platform towards diagnosis and therapy. *Chemical Communications*, (22), 2553–2555. https://doi.org/10.1039/B801911H.
57. Barrera, C., Herrera, A. P., Bezares, N., Fachini, E., Olayo-Valles, R., Hinestroza, J. P., & Rinaldi, C. (2012). Effect of poly(ethylene oxide)-silane graft molecular weight on the colloidal properties of iron oxide nanoparticles for biomedical applications. *Journal of Colloid and Interface Science, 377*(1), 40–50. https://doi.org/10.1016/j.jcis.2012.03.050.

58. Biju, V., Itoh, T., & Ishikawa, M. (2010). Delivering quantum dots to cells: Bioconjugated quantum dots for targeted and nonspecific extracellular and intracellular imaging. *Chemical Society Reviews, 39*(8), 3031–3056. https://doi.org/10.1039/B926512K.
59. Bilan, R., Fleury, F., Nabiev, I., & Sukhanova, A. (2015). Quantum dot surface chemistry and functionalization for cell targeting and imaging. *Bioconjugate Chemistry, 26*(4), 609–624. https://doi.org/10.1021/acs.bioconjchem.5b00069.
60. Banerjee, A., Grazon, C., Nadal, B., Pons, T., Krishnan, Y., & Dubertret, B. (2015). Fast, efficient, and stable conjugation of multiple DNA strands on colloidal quantum dots. *Bioconjugate Chemistry, 26*(8), 1582–1589. https://doi.org/10.1021/acs.bioconjchem.5b00221.
61. Paquet, C., Ryan, S., Zou, S., Kell, A., Tanha, J., Hulse, J., Tay, L.-L., & Simard, B. (2012). Multifunctional nanoprobes for pathogen-selective capture and detection. *Chemical Communications, 48*(4), 561–563. https://doi.org/10.1039/C1CC16245D.
62. Hong, G., Diao, S., Antaris, A. L., & Dai, H. (2015). Carbon nanomaterials for biological imaging and nanomedicinal therapy. *Chemical Reviews, 115*(19), 10816–10906. https://doi.org/10.1021/acs.chemrev.5b00008.
63. Karousis, N., Suarez-Martinez, I., Ewels, C. P., & Tagmatarchis, N. (2016). Structure, properties, functionalization, and applications of carbon nanohorns. *Chemical Reviews, 116*(8), 4850–4883. https://doi.org/10.1021/acs.chemrev.5b00611.
64. Marco, F., Roberto, M., Lyn, M., Kevin, F., Valentina, S., Giacomo, C., Luis, E., Eoin, M. S., & Silvia, G. (2015). Multi-functionalized carbon nano-onions as imaging probes for cancer cells. *Chemistry – A European Journal, 21*(52), 19071–19080. https://doi.org/10.1002/chem.201503166.
65. Biju, V. (2014). Chemical modifications and bioconjugate reactions of nanomaterials for sensing, imaging, drug delivery and therapy. *Chemical Society Reviews, 43*(3), 744–764. https://doi.org/10.1039/C3CS60273G.
66. Amstad, E., Zurcher, S., Mashaghi, A., Wong, J. Y., Textor, M., & Reimhult, E. (2009). Surface functionalization of single superparamagnetic iron oxide nanoparticles for targeted magnetic resonance imaging. *Small, 5*(11), 1334–1342. https://doi.org/10.1002/smll.200801328.
67. Mahato, R. I. (2004). *Biomaterials for delivery and targeting of proteins and nucleic acids*. Boca Raton, FL: CRC.
68. Fuertges, F., & Abuchowski, A. (1990). The clinical efficacy of poly(ethylene glycol)-modified proteins. *Journal of Controlled Release, 11*(1), 139–148. https://doi.org/10.1016/0168-3659(90)90127-F.
69. Xie, J., Xu, C., Kohler, N., Hou, Y., & Sun, S. (2007). Controlled PEGylation of monodisperse Fe3O4 nanoparticles for reduced non-specific uptake by macrophage cells. *Advanced Materials, 19*(20), 3163–3166. https://doi.org/10.1002/adma.200701975.
70. Harris, J. M., & Chess, R. B. (2003). Effect of pegylation on pharmaceuticals. *Nature Reviews Drug Discovery, 2*, 214. https://doi.org/10.1038/nrd1033.
71. Chen, X., Zhang, W., Laird, J., Hazen, S. L., & Salomon, R. G. (2008). Polyunsaturated phospholipids promote the oxidation and fragmentation of γ-hydroxyalkenals: Formation and reactions of oxidatively truncated ether phospholipids. *Journal of Lipid Research, 49*(4), 832–846. https://doi.org/10.1194/jlr.M700598-JLR200.
72. Papisov, M. I., Bogdanov, A., Schaffer, B., Nossiff, N., Shen, T., Weissleder, R., & Brady, T. J. (1993). Colloidal magnetic resonance contrast agents: Effect of particle surface on biodistribution. *Journal of Magnetism and Magnetic Materials, 122*(1), 383–386. https://doi.org/10.1016/0304-8853(93)91115-N.
73. Lutz, J.-F., Stiller, S., Hoth, A., Kaufner, L., Pison, U., & Cartier, R. (2006). One-pot synthesis of PEGylated ultrasmall iron-oxide nanoparticles and their in vivo evaluation as magnetic resonance imaging contrast agents. *Biomacromolecules, 7*(11), 3132–3138. https://doi.org/10.1021/bm0607527.
74. Li, L., Jiang, W., Luo, K., Song, H., Lan, F., Wu, Y., & Gu, Z. (2013). Superparamagnetic iron oxide nanoparticles as MRI contrast agents for non-invasive stem cell labeling and tracking. *Theranostics, 3*(8), 595–615. https://doi.org/10.7150/thno.5366.

75. Ma, Y., Tong, S., Bao, G., Gao, C., & Dai, Z. (2013). Indocyanine green loaded SPIO nanoparticles with phospholipid-PEG coating for dual-modal imaging and photothermal therapy. *Biomaterials, 34*(31), 7706–7714. https://doi.org/10.1016/j.biomaterials.2013.07.007.
76. Tartaj, P., Morales, M. P., Veintemillas-Verdaguer, S., Gonzalez-Carreño, T., & Serna, C. J. (2006). Synthesis, properties and biomedical applications of magnetic nanoparticles. *Handbook of Magnetic Materials, 16*(5), 403–482.
77. Weissleder, R., Elizondo, G., Wittenberg, J., Lee, A. S., Josephson, L., & Brady, T. J. (1990). Ultrasmall superparamagnetic iron oxide: An intravenous contrast agent for assessing lymph nodes with MR imaging. *Radiology, 175*(2), 494–498. https://doi.org/10.1148/radiology.175.2.2326475.
78. Molday, R. S., & Mackenzie, D. (1982). Immunospecific ferromagnetic iron-dextran reagents for the labeling and magnetic separation of cells. *Journal of Immunological Methods, 52*(3), 353–367. https://doi.org/10.1016/0022-1759(82)90007-2.
79. Josephson, L., Tung, C.-H., Moore, A., & Weissleder, R. (1999). High-efficiency intracellular magnetic labeling with novel superparamagnetic-Tat peptide conjugates. *Bioconjugate Chemistry, 10*(2), 186–191. https://doi.org/10.1021/bc980125h.
80. Tassa, C., Shaw, S. Y., & Weissleder, R. (2011). Dextran-coated iron oxide nanoparticles: A versatile platform for targeted molecular imaging, molecular diagnostics, and therapy. *Accounts of Chemical Research, 44*(10), 842–852. https://doi.org/10.1021/ar200084x.
81. Wunderbaldinger, P., Josephson, L., & Weissleder, R. (2002). Crosslinked iron oxides (CLIO): A new platform for the development of targeted MR contrast agents. *Academic Radiology, 9*(Suppl 2), S304–S306.
82. McCarthy, J. R., & Weissleder, R. (2008). Multifunctional magnetic nanoparticles for targeted imaging and therapy. *Advanced Drug Delivery Reviews, 60*(11), 1241–1251. https://doi.org/10.1016/j.addr.2008.03.014.
83. Mornet, S., Portier, J., & Duguet, E. (2005). A method for synthesis and functionalization of ultrasmall superparamagnetic covalent carriers based on maghemite and dextran. *Journal of Magnetism and Magnetic Materials, 293*(1), 127–134. https://doi.org/10.1016/j.jmmm.2005.01.053.
84. Kumar, M. N. V. R., Muzzarelli, R. A. A., Muzzarelli, C., Sashiwa, H., & Domb, A. J. (2004). Chitosan chemistry and pharmaceutical perspectives. *Chemical Reviews, 104*(12), 6017–6084. https://doi.org/10.1021/cr030441b.
85. Janes, K. A., Calvo, P., & Alonso, M. J. (2001). Polysaccharide colloidal particles as delivery systems for macromolecules. *Advanced Drug Delivery Reviews, 47*(1), 83–97. https://doi.org/10.1016/S0169-409X(00)00123-X.
86. Bhattarai, S. R., Kim, S. Y., Jang, K. Y., Lee, K. C., Yi, H. K., Lee, D. Y., Kim, H. Y., & Hwang, P. H. (2008). Laboratory formulated magnetic nanoparticles for enhancement of viral gene expression in suspension cell line. *Journal of Virological Methods, 147*(2), 213–218. https://doi.org/10.1016/j.jviromet.2007.08.028.
87. Hee Kim, E., Sook Lee, H., Kook Kwak, B., & Kim, B.-K. (2005). Synthesis of ferrofluid with magnetic nanoparticles by sonochemical method for MRI contrast agent. *Journal of Magnetism and Magnetic Materials, 289*, 328–330. https://doi.org/10.1016/j.jmmm.2004.11.093.
88. Kim, M.-J., Jang, D.-H., Lee, Y.-I., Jung, H. S., Lee, H.-J., & Choa, Y.-H. (2011). Preparation, characterization, cytotoxicity and drug release behavior of liposome-enveloped paclitaxel/Fe3O4 nanoparticles. *Journal of Nanoscience and Nanotechnology, 11*(1), 889–893. https://doi.org/10.1166/jnn.2011.3267.
89. Martina, M.-S., Fortin, J.-P., Ménager, C., Clément, O., Barratt, G., Grabielle-Madelmont, C., Gazeau, F., Cabuil, V., & Lesieur, S. (2005). Generation of superparamagnetic liposomes revealed as highly efficient MRI contrast agents for in vivo imaging. *Journal of the American Chemical Society, 127*(30), 10676–10685. https://doi.org/10.1021/ja0516460.

90. Yang, J., Lee, T.-I., Lee, J., Lim, E.-K., Hyung, W., Lee, C.-H., Song, Y. J., Suh, J.-S., Yoon, H.-G., Huh, Y.-M., & Haam, S. (2007). Synthesis of ultrasensitive magnetic resonance contrast agents for cancer imaging using PEG-fatty acid. *Chemistry of Materials, 19*(16), 3870–3876. https://doi.org/10.1021/cm070495s.
91. De Cuyper, M., & Joniau, M. (1988). Magnetoliposomes. *European Biophysics Journal, 15*(5), 311–319. https://doi.org/10.1007/bf00256482.
92. Mulder, W. J. M., Strijkers, G. J., van Tilborg, G. A. F., Griffioen, A. W., & Nicolay, K. (2006). Lipid-based nanoparticles for contrast-enhanced MRI and molecular imaging. *NMR in Biomedicine, 19*(1), 142–164. https://doi.org/10.1002/nbm.1011.
93. Dagata, J. A., Farkas, N., Dennis, C. L., Shull, R. D., Hackley, V. A., Yang, C., Pirollo, K. F., & Chang, E. H. (2008). Physical characterization methods for iron oxide contrast agents encapsulated within a targeted liposome-based delivery system. *Nanotechnology, 19*(30), 305101.
94. Veiseh, O., Kievit, F. M., Gunn, J. W., Ratner, B. D., & Zhang, M. (2009). A ligand-mediated nanovector for targeted gene delivery and transfection in cancer cells. *Biomaterials, 30*(4), 649–657. https://doi.org/10.1016/j.biomaterials.2008.10.003.
95. Kievit, F. M., Veiseh, O., Bhattarai, N., Fang, C., Gunn, J. W., Lee, D., Ellenbogen, R. G., Olson, J. M., & Zhang, M. (2009). PEI-PEG-chitosan copolymer coated iron oxide nanoparticles for safe gene delivery: Synthesis, complexation, and transfection. *Advanced Functional Materials, 19*(14), 2244–2251. https://doi.org/10.1002/adfm.200801844.
96. Guo, M., Yan, Y., Zhang, H., Yan, H., Cao, Y., Liu, K., Wan, S., Huang, J., & Yue, W. (2008). Magnetic and pH-responsive nanocarriers with multilayer core-shell architecture for anticancer drug delivery. *Journal of Materials Chemistry, 18*(42), 5104–5112. https://doi.org/10.1039/B810061F.
97. Thünemann, A. F., Schütt, D., Kaufner, L., Pison, U., & Möhwald, H. (2006). Maghemite nanoparticles protectively coated with poly(ethylene imine) and poly(ethylene oxide)-block-poly(glutamic acid). *Langmuir, 22*(5), 2351–2357. https://doi.org/10.1021/la052990d.
98. Bulte, J. W. M., de Cuyper, M., Despres, D., & Frank, J. A. (1999). Short- vs. long-circulating magnetoliposomes as bone marrow-seeking MR contrast agents. *Journal of Magnetic Resonance Imaging, 9*(2), 329–335. https://doi.org/10.1002/(SICI)1522-2586(199902)9:2<329::AID-JMRI27>3.0.CO;2-Z.
99. Xiang, J. J., Tang, J. Q., Zhu, S. G., Nie, X. M., Lu, H. B., Shen, S. R., Li, X. L., Tang, K., Zhou, M., & Li, G. Y. (2003). IONP-PLL: A novel non-viral vector for efficient gene delivery. *The Journal of Gene Medicine, 5*(9), 803–817. https://doi.org/10.1002/jgm.419.
100. Nicollay, K., Strijkers, G. and Grull, H. (2013). Gd-Containing Nanoparticles as MRI Contrast Agents. In The Chemistryof Contrast Agents in Medical Magnetic Resonance Imaging (eds A. Merbach, L. Helm and E. Toth). https://doi.org/10.1002/9781118503652.ch11.

Chapter 22
Surface-Modified Lanthanide Nanomaterials for Drug Delivery

Nitya R. Chawda, S. K. Mahapatra, and I. Banerjee

Abstract The chapter highlights the importance and possibility of detrimental effects of lanthanide-based nanomaterials, surface capping, and toxicity. Lanthanide-based nanomaterials serve multimodality approach such as diagnosis and therapy. This speciality makes them superior over their other counterparts like transition metals and organic-based materials. The loose ions leached from the contrast agent used for magnetic resonance imaging (MRI) causes possibility of nephrotoxicity leading to nephrogenic systemic fibrosis (NSF). It also indulges the readers to understand synthesis mechanism for elongated mixed-phase rare-earth oxides and hydroxide nanostructures. The basic principle of transition and crystallization temperatures required for rare-earth-based oxide formation is specially highlighted and explained in detail using X-ray diffractometer (XRD), thermogravimetric analyzer (TGA), differential scanning calorimetry (DSC), and Fourier-transform infrared spectroscopy (FTIR) data. Furthermore, the lanthanide nanorods have been employed as contrast agent in MRI and drug delivery studies. The promising results like longitudinal proton relaxivity (r_1) value of 13.3 mM^{-1} s^{-1} and drug-loading capacity of 5-flurouracil are ~28% for FA-capped Gd_2O_3 nanorods in comparison to bare sample (~9%) that is best endowed to its unique structure and skillful capping. These advantages of well-capped FA-Gd_2O_3 nanorods are promising candidates for simultaneous bioimaging and drug delivery system due to the presence of FA over its surface. It will server further as potentially equipped candidate for clinical applications for targeted diagnosis and therapy which in combination is to be known as theranostics.

Keywords Drug delivery · Paramagnetic · Contrast agent · Nanomaterials Magnetic resonance imaging (MRI)

N. R. Chawda · I. Banerjee (✉)
School of Nano Sciences, Central University of Gujarat, Gandhi Nagar, Gujarat, India
e-mail: indrani.banerjee@cug.ac.in

S. K. Mahapatra
Department of Physical Sciences, Central University of Punjab, Bhatinda, Punjab, India

Y. V Pathak (ed.), *Surface Modification of Nanoparticles for Targeted Drug Delivery*, https://doi.org/10.1007/978-3-030-06115-9_22

1 Introduction

Nanotechnology has found enormous importance in biomedical sciences toward effective diagnosis, therapy, and elimination of diseases. The advancement of science and technology has developed the need for multitasking and multifunctional systems which can replace older, costlier, and time- and resource-consuming conventional systems and methods [1]. As per the American Cancer Society's estimation of 2017, around 1650 people die every day in the worlds most advanced country of United States due to cancer [2]. Owing to such a fatal threat, continuous as well as considerable efforts are made for expanding nanomaterials as next-generation promise for biomedical medicines and drug delivery systems. Nanomaterials possessing multimodality such as diagnosis and therapy, like imaging and targeted drug delivery, could certainly fulfil a promise and would reduce other side effects [3]. It is also essential to design such systems in order to enhance targeting strategies and improve biodistribution, enhanced blood circulation, and penetration depth of existing body barriers, and to reduce required volume needed for distribution [4]. Therefore, nanomaterials (NMs) which could response to light and magnet can contribute largely to diagnostics and image-guided drug delivery. However, most of the NMs designed for drug delivery lack optical properties (e.g., fluorescence and photothermal response) and magnetic properties of gadolinium-based lanthanides (paramagnetic property) as contrast agents used for magnetic resonance imaging (MRI). In this context, luminescence and magnetic properties of lanthanide materials are superior and useful over traditional quantum dots, green fluorescent proteins, and organic dyes [5].

Lanthanides also referred as "rare-earth" elements ranging from lanthanum (57) to lutetium (71) are widely utilized in cancer diagnosis (imaging) and drug delivery (therapy) [6–8]. The Ln^{3+} ions possess redox stability which makes them suitable for cellular applications with the additional benefit from their luminescent properties ascribed to 4f–5d charge transfer and f–f transitions [9]. Lanthanide-based nanomaterials (Ln-NMs) have one of the most outstanding properties of upconversion [6, 10]. The process is related to the nonlinear anti-stocks process, by which it can effectively convert either two or more low-energy continuous near-infrared (NIR) photons into high-energy photons, when embedded in suitable inorganic host matrix [11]. The main reason for rapid demand of upconversion nanoparticles (UCNPs) for biomedical purposes is its excitation wavelength (e.g., 980 nm) which falls under "optical transparency window" (700–1100 nm) [6]. It also supports for various advantages such as no photodamage to living organisms, low autofluorescence background, high signal-to-noise ratio, detection sensitivity, and high penetration depth in animal tissues [12, 13]. The UCNPs are superior to other NMs due to their chemical stability, photostability which is also free of on-off blinking, measurable single-particle excitation [14], multi-color emission (i.e., red, green, blue), multimodal imaging (i.e., positron emission tomography (PET), magnetic resonance imaging (MRI) [15], and upconversion luminescence (UCL)) [16] and would

also help for photo-triggered drug delivery [17, 18]. Surface-modified lanthanide-based nanomaterials used for drug delivery can act as excellent imaging probe to locate cancer site as well as act as efficient carrier for targeting site with therapeutic agents [19, 20]. Such a multimodal Ln-NMs are ideal to clarify pharmacokinetics and pharmacodynamics by additionally providing rational support for decisions of individual patients [21].

The model drug used in the present study is 5-fluorouracil (5-FU). It is an acidic water soluble drug along with antineoplastic agent. It is one of the most commonly used chemotherapeutic compounds for various tumors such as breast, colorectal, gastric, pancreatic, ovarian, head, and neck cancer [22, 23]. Apart from its various applications, it is an antimetabolite of pyrimidine analogue and is used to inhibit synthesis of DNA and RNA during the S-phase of the cell cycle. Some of the limitations of 5-FU are short biological half-life due to rapid metabolism and nonselective action against healthy cells [24]. Therefore, it is significant to design target-specific nanocarrier to the cancerous cells with sustained release so as to minimize side effects to nearby healthy cells [25].

The present chapter explains the formation mechanism of FA-capped Gd_2O_3 nanorods for its bimodal application. The FA-capped Gd_2O_3 nanorods are used for bimodal application as contrast agent for MRI bioimaging and FA-capped Gd_2O_3 nanorods-5-FU system for drug delivery. The FA-Gd_2O_3 was synthesized by co-precipitation method using FA as capping and stabilizing agent. The FA was chosen due to its biocompatibility, site specificity, fluorescence property, antitumor effect, and G-quartet-forming property [26]. It binds with folate receptors, which are widely found in most cancer cells [27], and it is interesting to note that the folate receptor density increases at the advanced stage of cancer [28]. The selected model drug 5-FU is very less explored with gadolinium oxide nanocarriers; one of the report by Zhang et al. showed results of 5-FU loaded with nanosheets of Gd_2O_3 and Sm_2O_3 for drug delivery applications. The nanosheets being bare nanocarriers could not have provided them with efficient loading, and the pH-triggered release showed maximum release within 24 h [29]. In the present study, the capping agent itself acts as growth modulator for anisotropic growth of nanoparticle, stabilizing them for longer time and improving the dispersity in aqueous solvent with respect to time. It enhances biocompatibility and acts as fluorescent probe for tracking and diagnosis. The restricted core particle being paramagnetic in nature is used for MRI studies and reduces toxicity inhibiting release of Gd^{3+} ions by formation of stable capping over the surface of oxide nanorods. The oxides with low solubility product having higher stability that chelated complexes, hence compared to commercially available Gd^{3+}-chelated compounds like Magnevist [30–36] oxides of gadolinium helps in reducing toxicity due to leaching of free Gd^{3+} ions. The compatibility and morphology add to the multifunctional activity of the present system. The FA-capped Gd_2O_3 nanorods were employed for bioimaging as contrast agent for in vitro MRI studies and were also effectively used for loading and release of 5-FU in simulated physiological medium of PBS having pH 7.4 at 37 °C.

2 Surface Functionalization of Nanomaterials

Surface tailoring of nanomaterials is a challenging and most significant task for desired applications of synthesized nanomaterials. The clinical applicability of NMs depends on suitable surface functionalization or modification, while the change in different properties and their extent of effects also depends on choice of ligands or molecules. Surface coatings of NMs are attained with two different modes either in situ (along with nucleation, while formation of NMs) or ex situ (post-synthesis, after NMs are obtained). The procedure of linking surface molecules is carried out either through end-grafting (anchored through one end of the single molecule, e.g., PEG and PLGA-PEO) or surface encapsulation (through multiple connections carrying multiple active groups, e.g., silica) [37]. The properties like stability, biocompatibility (toxicity), magnetization, relaxivity, optical emission, and contrast ability are purely dependent on efficiency and mode of capping materials with the suitable selection of ligands. For example, Cho Rong Kim and co-workers investigate longitudinal (r_1) and transverse (r_2) water–proton relaxivities of ultrasmall gadolinium oxide (Gd_2O_3) nanoparticles by varying surface-coating ligand size and found that both r_1 and r_2 values decreased with increasing ligand size [38]. Tamilmani Vairapperumal et al. reported catechin-capped gadolinium-doped $LaVO_4$ nanoparticles which showed increase of saturation magnetization from paramagnetic to superparamagnetic due to capping [39]. Tirusew Tegafaw et al. have investigated ligand-chain effects of hydrophilicity on the relaxometric properties of ultrasmall Gd_2O_3 nanoparticles [40]. These are various factors of the surface modification that play crucial role in its functionality like colloidal stability, biocompatibility, and role of functionalized moieties or ligands for fulfilling selective, targeting, and specific interactions for enhancing applicability of NMs for multimodality. Therefore synthesis of monodispersed, biocompatible surface-functionalized nanoparticles is of utmost importance for biomedical applications [34, 41].

The surface functionalization induces imaging, detection, therapeutic, and targeted capabilities to NMs. The multi-functionalities of nanomedicine lie in simultaneous detection, diagnosis, and treatment along with deep tissue penetration, enhanced contrast, specific targeting, and externally triggered drug delivery [42]. These applications are only possible if surfaces are tailored with specific surface molecules.

3 Materials and Methods

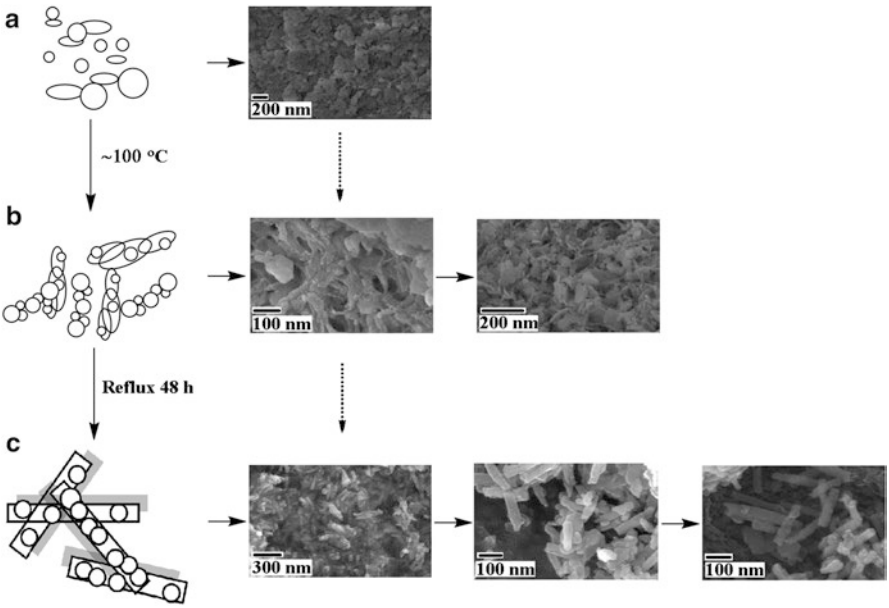
3.1 Materials

Gadolinium nitrate hexahydrate ($Gd(NO_3)_3{\cdot}6H_2O$) and 5-flurouracil (5-FU) were purchased from Sigma-Aldrich of purity 99.9% and ≥99%, respectively; sodium hydroxide (NaOH) and hydrogen peroxide (30% W/V) were procured from

S.D. Fine Chem. Ltd., Mumbai, India. Nitric acid was purchased from Molychem (India) Ltd., folic acid was purchased from HiMedia chemicals India, and urea was purchased from Rankem (Delhi India). All the chemicals were used without further purification.

3.2 Synthesis of Folic Acid (FA)-Capped Gadolinium Oxide Nanorods (Gd_2O_3·NRs)

The folic acid (FA)-capped gadolinium oxide (Gd_2O_3) nanorods were synthesized using co-precipitation method at ~100 °C. At room temperature, aqueous solutions of NaOH was added to aqueous solution of $Gd(NO_3)_3$ dropwise at the rate of 60 μL min^{-1} with stirring speed of 900 rpm. The resultant white powder of $Gd(OH)_3$ was obtained via first step of synthesis. In the second step, urea and H_2O_2 were mixed to form an adduct solution which controls the release of oxygen from H_2O_2 as required for oxidation of $Gd(OH)_3$ to Gd_2O_3 where FA was used as capping agent. In first step, 25 mL of 175 mM aqueous NaOH was added dropwise to 25 mL of 58 mM aqueous $Gd(NO_3)_3$ at the rate of 60 μL min^{-1} with stirring speed of 500 rpm. The resulting solution turned to turbid milky white suspension. After the completion of reaction, the mixture was centrifuged at 6000 rpm with repeated wash using chilled water to neutralize the pH and then dried to get $Gd(OH)_3$. $Gd(OH)_3$ was weighed according to stoichiometry and transferred to a 250 mL round-bottom flask followed by addition of 20 mL of water with stirring speed of 750 rpm until dispersed. Another solution containing 100 mg FA, 100 mg urea, and 30 mL of 30% H_2O_2 was added to the previous solution at the rate of 700 μL min^{-1} with 750 rpm stirring speed. The complete mixture of dispersed solution was then refluxed at 100 °C for 48 h at 2000 rpm on adding 10 mL 30% H_2O_2 after every 12 h of interval to get FA-Gd_2O_3. The pH was measured after adding each solution to the reaction mixture which was alkaline (~pH 9–10) which is preferred for elongated structures as reported in literatures [43, 44]. The FA controlled the growth uniformity at a preferred direction. The carboxyl functionality of FA structure interacts with the charge density of available surface which the gadolinium hydroxide gets converted to a gadolinium oxide. The resulting FA-Gd_2O_3 was centrifuged at 8000 rpm to remove the unreacted urea and FA with repeated washing using water and ethanol. The bulky structure of FA does not allow other ions involved in nucleation and ripening to come in vicinity once FA is attached over the surface. This growth formulation helps in proceeding along a single-oriented direction (Scheme 22.1). Other conditions of temperature, pH, and concentration of Gd^{3+} ions also contribute to it. The obtained sample was dried in vacuum oven at 50 °C for 12 h and stored for further characterizations. The Milli-Q water (Millipore SAS 67/20 Mosheim) of 10^{-7} S cm^{-1} was used throughout.



Scheme 22.1 (**a**) Random flakes of Gd_2O_3, (**b**) Flakes of Gd_2O_3 attached to form nanorods and (**c**) Complete growth of Gd_2O_3 nanorods

3.3 Characterization of Folic Acid (FA)-Capped Gadolinium Oxide Nanorods (Gd_2O_3·NRs)

To study the morphology of FA-Gd_2O_3, field-emission scanning electron microscopy (FESEM) QUANTA 200 FEG from FEI Netherlands was used. The X-ray diffractometer (XRD) pattern of FA-GdNRs was performed on PANalytical *X'Pert Pro* instrument of Cu-Kα wavelength (λ = 1.54 Å). The samples were scanned over a 2θ range of 10–80° with a step size of 0.013°. UV-Visible spectrophotometer (UV-1800 Shimadzu) was used for primary capping analysis. The FTIR spectra were recorded with PerkinElmer Spectrum 65 series of FTIR spectrophotometer. For FTIR, 1.5–2.5 mg of the sample was mixed with 185 mg KBr (AR, Sigma USA) for making pellets in press machine (model Mp-15) at 3–6 kg/cm^2 for 2–5 min. After a background scan with KBr pallet, the samples were analyzed at 4000–400 cm^{-1}. The TG-DSC analysis for all the samples was recorded using TG-DSC-Mettler-Toledo Star-3 system in a platinum crucible under inert N_2 atmosphere at 10 K min^{-1} and temperature range from room temperature to 1000 °C.

Surface capping with folic acid was investigated by FTIR spectra using KBr pallet at 4000–400 cm^{-1} to find the peak shifting by comparative study of pure capping agent, bare sample without FA and FA-Gd_2O_3. The thermogravimetric analyzer TGA curve recorded from 45 °C to 1000 °C in inert condition of nitrogen with air-flow over powder samples was used to estimate the amount of capping by mass loss.

3.4 Determination of Free Gd^{3+} Ions by Xylenol Orange and UV-Visible Spectrophotometry

Two millilitre of 0.1 mM of xylenol orange solution in acetate buffer (pH 5.88) was added to 50 μL of supernatant of bare-Gd_2O_3 and FA-Gd_2O_3 dispersant (1 mg mL^{-1}). The mixture was placed for UV-visible (350–650 nm). The amount of ions was calculated from fitted equation of absorbance ratio (λ_{max} = 578 and 437.5 nm) using calibration curve. The UV-1800 Shimadzu spectrophotometer was used for entire study at 298.15 K with an average of three scans.

3.5 The Gd^{3+} Ions Leaching Studies from Nanorods by Inductive Coupled Plasma-Optical Emission Spectroscopy (ICP-OES)

The sample preparation of FA-Gd_2O_3 was carried out by acid digestion method using nitric acid (i.e., HNO_3 70%) with heating over open flame till the clear and transparent solution was obtained. All the samples were later dissolved in ~2.0% HNO_3 for further detection using a Perkin Elmer ICP-OES.

4 Results and Discussion

4.1 Powdered X-Ray Diffractometer Analysis

Figure 22.1 illustrates powdered X-ray diffraction spectra of the samples. The XRD spectra showed mixture of amorphous and crystalline phase as no clear or perfect sharp peak was found. The diffraction spectra of as-prepared product could not be fitted to simple gadolinium hydroxide or oxide in ICDD or JCPDS database. The crystalline temperature for Gd_2O_3 was determined to be above 560 °C from TGA analysis presented in the following section. However, the as-synthesized sample was calcinated at 400 °C, so complete transition from hexagonal $Gd(OH)_3$ to cubic Gd_2O_3 was not achieved, and the sample showed mixed phases of hydroxides and oxides of gadolinium. The characteristic peaks corresponding to the mixed phases of hydroxide and oxide have been represented by asterisk (*) and octothorp (#), respectively, in the XRD spectra. The planes corresponding to (100), (110), (101), (200), (111), (201), (210), (300), (211), (102), (112), (310), and (131) represent the hexagonal phase of $Gd(OH)_3$ according to JCPDS card No. 83-2037. The planes corresponding to (211), (222), (400), (440), and (622) indicates formation of cubic Gd_2O_3 according to JCPDS card No. 43-1014. The XRD pattern of as-prepared FA-Gd_2O_3 presents three distinctive peaks at 28.1° corresponding to (110) and at

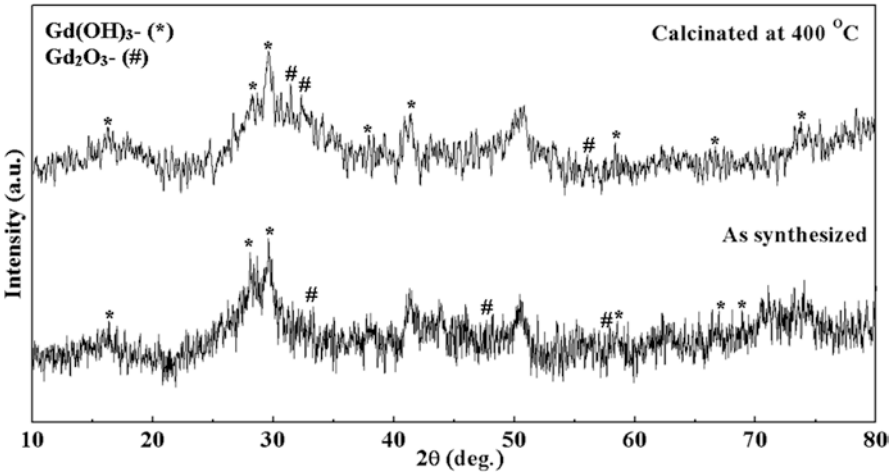


Fig. 22.1 XRD patterns of as-synthesized folic acid-capped-gadolinium oxide (FA-Gd_2O_3) and calcinated at 400 °C folic acid-capped-gadolinium oxide (FA-Gd_2O_3)

29.5° corresponding to (101) of $Gd(OH)_3$, respectively, and at 28.7° corresponding to (222) of Gd_2O_3 which are also indexed in XRD pattern of annealed samples at 400 °C. The intermediate phase of GdOOH planes indexed to (112) and (310) are also obtained supporting the phase transition observed in TGA analysis. The broad peaks are indicative of small crystallite size [45, 46]. The microstrain (ε) and average crystallite size (d) were calculated from Williamson-Hall (W-H) expression also known as Uniform Deformation Model (UDM):

$$\beta \cos\theta = \frac{0.9\lambda}{d} + 4\varepsilon \sin\theta \tag{22.1}$$

where "β" is full width at half maximum abbreviated as (FWHM) of the diffraction peaks in radian, "θ" is the Bragg diffraction angle in radian, "λ" is the wavelength of Cu-Kα X-rays used having value of 0.154 nm, "d" is the crystallite size along the specified direction, and "ε" is microstrain associated with nanorods. Equation 22.1 gives straight line equation when plotted for $\beta \cos\theta$ versus $\sin\theta$. The slope gives the microstrain (ε), and its intercept ($0.9\lambda/d$) gives the crystallite size "d." The obtained microstrain for as-prepared sample is ~17.4×10^{-3}, and annealed sample at 400 °C possessed negative microstrain of ~7.8×10^{-3} which are in agreement with the earlier reports [47]. The negative microstrain after annealing is due to the relaxed state of nanocrystallites present in the nanorods. The decrease in microstrain on heat treatment is attributed to the closing of the pores and reduction of defects. The average crystallite sizes (d) estimated for the as-prepared and annealed samples were 33 nm and 3.4 nm, respectively. The decrease in the crystallite size on annealing is due to the coalescence or aggregation of the nanocrystallites by higher-thermal energy reducing the surface-free energy and providing driving forces for growth of

the crystals. The microstrain and crystallite size determination derived from the Uniform Deformation Model (UDM) showed that the microstrain is inversely proportional to the crystallite size on application of heat treatment which is in agreement with the reported data [47].

4.2 *Field-Emission Scanning Electron Microscopy (FESEM) Imaging*

Figure 22.2a shows FESEM image of synthesized samples of FA-Gd system. The Schematic 22.1 gives the insight of the growth mechanism and stepwise evolution of the nanorods via oriented grain attachment mechanism from the nanoparticle seeds followed by the formation of nanoflakes. The particle seeds and flakes start nucleating and growing along a preferred direction which is governed by the capping agents along with the pH and time of the reaction mixture. The nanorods generated at the final stage of synthesis and calcined were ~200 nm in length and ~20 nm in diameter. It could be speculated that the particles get attached via oriented attachment mechanism [48]. The micrograph clearly illustrates formation of particles at initial stage followed by coalescence and growth along a preferred direction through surface effects resulting in elongated shapes once common boundaries

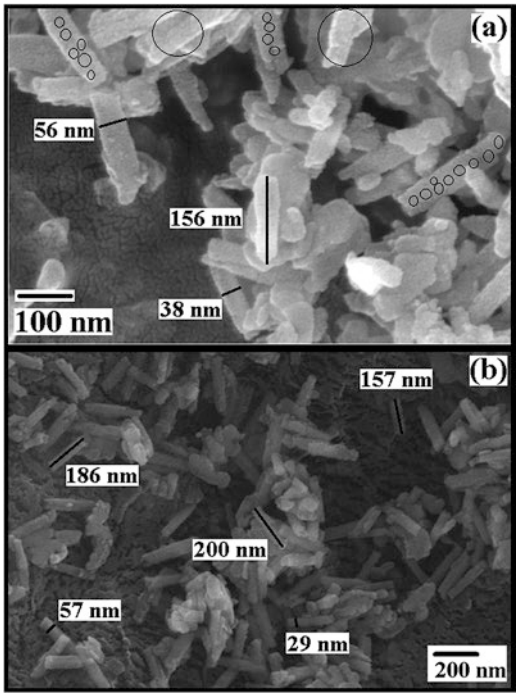


Fig. 22.2 FESEM image of folic acid-capped-gadolinium oxide ($FA\text{-}Gd_2O_3$) nanorods. (**a**) Image showed particle attachment for elongated structure. (**b**) The formation and well-defined size of nanorods at low magnification

get eliminated. The factors like dipole–dipole interaction, perfect lattice match, and vanishing of adjoining interface between the particles act as the driving force for such unidirectional surface growth [48]. The capping agent present in reaction mixture also assists dipole–dipole interaction. The functional part of folic acid known as glutamate is attached to the Gd_2O_3 surface via carboxylate ($–COO^–$) functionality. The synthesis procedure involves reaction mixture to be continuously stirred which makes ions involving in Brownian motion which tend the particles to colloid and assist for their coalescence and growth in elongated direction through common crystallographic orientation. It is anticipated that reaction time and choice of suitable capping agent could assist in growth of nanoparticles in such organized manner.

4.3 Thermogravimetric Analysis

The significance of surface tailoring has already been discussed, and, therefore, surface capping was estimated by simultaneous TGA-DSC analysis of the as-synthesized sample. The average weight loss is predicted from the TGA curves given in Fig. 22.3. The plot explains four distinct weight loss phenomena depicting the transformation of gadolinium hydroxide to cubic gadolinium oxide crystallizing above 560 °C. The initial mass loss of 1.1 mg till 100 °C is attributed to the surface dehydration of the FA-capped sample. A loss of 1.82 mg till 260 °C is accounted for the melting of surface capping of folic acid whose melting point is 250 °C. Therefore, the estimated capping amount was 1.82 mg which is 0.05 mg mg^{-1} of FA-Gd_2O_3. This weight loss is followed by a loss of 2.84 mg till 400 °C attributed to the phase transition of $Gd(OH)_3$ to intermediate phase of GdOOH in the form of $Gd(OH)_3 \rightarrow GdOOH + H_2O$ for which corresponding endothermic peak in DSC plot is at 337 °C (Fig. 22.4). The final weight loss of 3.97 mg is observed for the second step of dehydration in the form of $2GdOOH \rightarrow Gd_2O_3 + H_2O$ for which

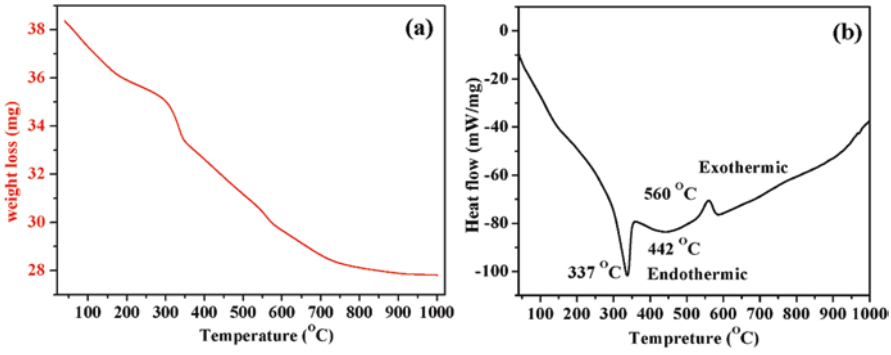


Fig. 22.3 Plots of TGA and DSC curves of FA-Gd_2O_3. (**a**) TGA of folic acid-capped-gadolinium oxide (FA-Gd_2O_3) and (**b**) DSC of folic acid-capped-gadolinium oxide (FA-Gd_2O_3)

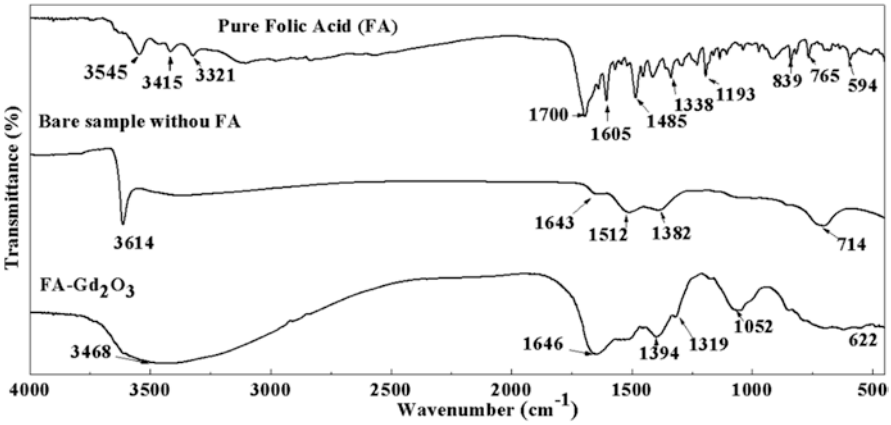


Fig. 22.4 Comparative FTIR spectra of pure folic acid (FA), bare-gadolinium oxide (Gd2O3), and folic acid-capped-gadolinium oxide (FA-Gd_2O_3)

corresponding exothermic peak in DSC plot is at 560 °C [49]. These weight losses are indicative of phase transition and crystallization temperatures of the gadolinium-based samples [50]. The crystallization temperature is supportive to understand the incomplete crystallization as evidenced in XRD analysis where due to lack of crystallization no significant peak intensity is obtained for sample calcinated at 400 °C. The apparent peak at 560 °C associated with named transformation and therefore calcination of gadolinium-based oxide should be carried out at ~600–700 °C or above for perfectly crystalline samples. The surface capping estimated could help in assuming chemical stability and in turn biocompatibility of FA-Gd_2O_3.

4.4 Fourier-Transform Infrared Spectroscopy (FTIR) Analysis

The FTIR (Fig. 22.4) plays a significant role in validating capping analysis showed by UV-vis spectra (Fig. 22.5). Stretching frequencies at ~3545, 3614, and 3468 cm^{-1} are attributed to –O–H stretching vibration of pure FA, bare-Gd_2O_3, and FA-Gd_2O_3, respectively. The peak at ~3415, 3321, and 1605 cm^{-1} corresponds to N–H stretching and vibration, ~1700 cm^{-1} for C=O stretching, ~1485 cm^{-1} for absorption band of phynyl ring, ~1338 cm^{-1} for amide-III of FA, ~1193 cm^{-1} for C–O stretching vibration, and ~800–600 cm^{-1} for C–C stretching for pure FA. The pure FA shows amide I and III vibrational peaks at 1605 and 1338 cm^{-1}, respectively. The peak shifting in this domain can be regarded as conclusive for successful capping of FA over Gd_2O_3 surface. The peak at ~1646 and 1394 cm^{-1} shows shift of ~41 and 56 cm^{-1} inferring successful capping of Gd_2O_3 [26].

4.5 *UV-Visible Spectrophotometer and Toxicity Studies through Leaching of Gd^{3+} Ions*

The UV-vis spectra of the aqueous dispersion of pure FA and FA-Gd_2O_3 have been compared and presented in Fig. 22.5. The pure FA shows two UV-Vis absorbance at 282 nm and 365 nm for the n → π* transitions of C=O bond while π → π* transition of aromatic C–C ring. The shift in the absorbance spectra of FA-Gd_2O_3 to 276 nm and 362 nm assures conjugation of FA over Gd_2O_3.

The leaching of Gd^{3+} ions was estimated using xylenol orange dye as indicator and measured by UV-visible spectroscopy. The coordination chemistry of free Ln^{3+} ions with disodium salt of xylenol orange is reported [51]. The Ln^{3+} ions in its free state coordinate with the xylenol orange and deprotonate the complex to change the color of solution from reddish orange to violet and as a result of which the absorption are shifted to higher wavelength [52]. Figure 22.5c shows the spectra of xylenol orange solution in pH 5.8. The varying (increase) concentration of Gd^{3+} ions cause the absorption peak at ca.437.5 nm to decrease its band intensity which is indicated

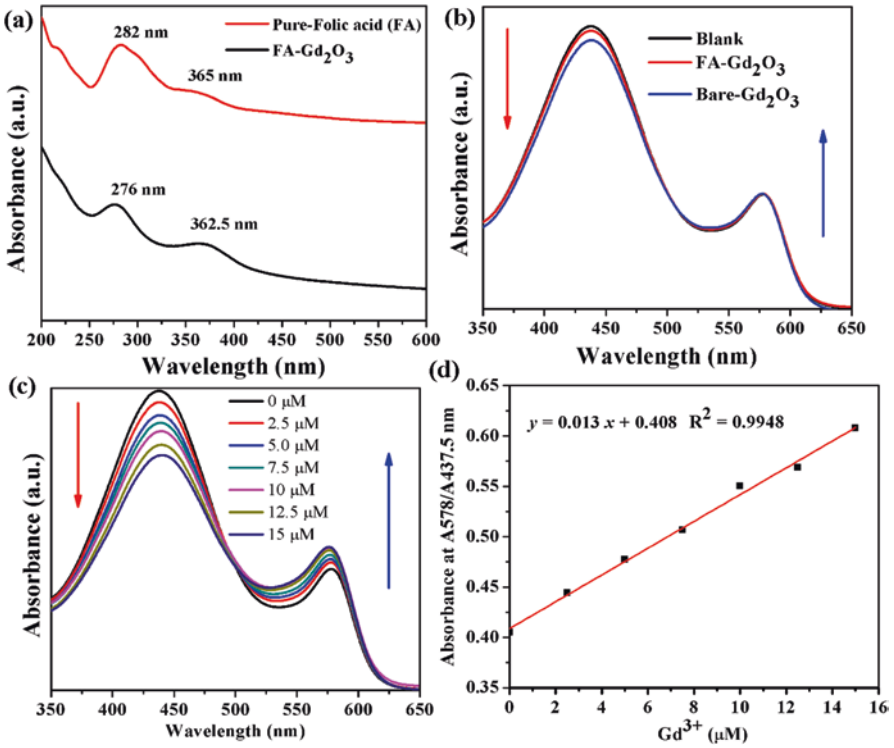


Fig. 22.5 (**a**) Comparative UV-vis spectra of pure folic acid (FA) and folic acid-capped-gadolinium oxide nanorods (FA-Gd_2O_3). (**b**) Spectrophotometric detection of leached Gd^{3+} ions by UV-visible spectroscopy using xylenol orange from bare Gd_2O_3 and FA-Gd_2O_3 and compared with blank. (**c**) Calibration curve obtained by increase in Gd^{3+} ions concentration from 0 to 15 μM (**d**) calibration plot of absorbance (A578/A437.5 nm) versus concentration of Gd^{3+} ions

in graph by red arrow heading downward. Simultaneously the absorption peak at ca.578 nm starts to increase its band intensity demonstrated by blue arrow heading upward. The shift in the observed absorption peaks help in plotting the calibration plot to calculate the unknown concentration in the solution of xylenol orange at pH 5.8. Figure 22.5d shows calibration plot, which is plotted by ratio of absorbance at A578/A437.5 nm versus varying concentration of Gd^{3+} ions in micromolar (μM). As per the fitted equation of calibration plot (Fig. 22.5d), concentration was found to be 1.58 μM and 0.49 μM for bare-Gd_2O_3 and FA-Gd_2O_3, respectively. The calculation using the mentioned concentration demonstrated 0.019% and 0.006% of leached Gd^{3+} ions from bare-Gd_2O_3 and FA-Gd_2O_3 samples, respectively. To validate our data of the leached Gd^{3+} ions as detected using xylenol orange through UV-vis spectroscopy, the leaching study was also performed with ICP-OES. The results from ICP-OES showed no leaching of Gd^{3+} ions in pH 7.4 PBS medium.

4.6 Inductive Coupled Plasma-Optical Emission Spectroscopy Analysis

The inductive coupled plasma-optical emission spectroscopy (ICP-OES) was used for estimation of Gd^{3+} ions composition in bare-Gd_2O_3 (1.33 g L^{-1}) and FA-Gd_2O_3 (1.77 g L^{-1}), respectively. The results are in well agreement with the calculated synthesis precursor's ratios, and therefore synthesis could be tuned by varying precursor's molar ratio to achieve other desired products.

4.7 In Vitro Longitudinal Relaxivity Measurement

The Gd-based samples are MR sensitive, and therefore we tested our samples and compared between bare and FA-capped Gd_2O_3. Literature report suggests the role of confinement of the gadolinium oxide nanoparticles to boost the T_1 contrast ability [53]. It is interesting to note the role of bare and capped Gd_2O_3 used as contrast agent. Therefore, the ability of the bare Gd_2O_3 nanorods and FA-capped Gd_2O_3 nanorods were evaluated. To enhance T_1 contrast of the present samples 1.5 Tesla human scanner (Siemens Essenza 1.5 T) with Inverse Recovery (IR) sequence and setting the parameters TR = 2000 ms for acquiring 30 points with time interval from 500 to 5000 ms, repetition time = 3 were used. The r_1 values for bare Gd_2O_3 nanorods and FA-capped Gd_2O_3 nanorods were 11.57 mM^{-1} s^{-1} and 13.3 mM^{-1} s^{-1}, respectively. This increment in the r_1 value accounted for ~13% increase in r_1 values could be due to sufficient capping to confine the Gd^{3+} ions which may have been loosely bound over the surface of nanoparticles. It is also supported by the previous experiment of Gd^{3+} ions leached from bare nanorods in larger amount, ~3 times than FA-capped Gd_2O_3 nanorods. This leaching in bare samples could have reduced the MR intensity than FA-capped sample. Our results are best supported by the previous reports, where PVP-capped gadolinium oxide spherical particles showed r_1 value of ~10.28 mM^{-1} s^{-1} which could be due to the fact of ligand size of capping as

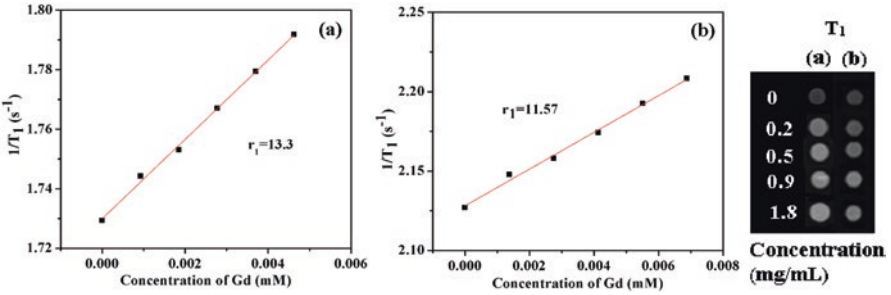


Fig. 22.6 Analysis of relaxation rate R_1 ($1/T_1$) against gadolinium ion concentration for (**a**) FA-GdNRs and (**b**) bare-GdNRs with T_1-weighted MR images

discussed earlier [54, 55]. The value is ~22.7% lower than the present work. Figure 22.6 shows the comparative T_1 images of the bare Gd_2O_3 nanorods, and FA-capped Gd_2O_3 nanorods revealed an enhancement of MR signals with increase in concentration. The T_1 images always starts from dark to gray with the increase in concentration of gadolinium compounds. It is clearly observed from the color of the T_1-weighted phantom images of bare Gd_2O_3 nanorods and FA-capped Gd_2O_3 nanorods, which increases with concentration of the samples.

4.8 Drug Loading and Release

The drug-loading and release profile was performed using 5-FU as model drug. 5-FU is used for colon cancer, cervical cancer, esophageal cancer, pancreatic cancer, stomach cancer, and breast cancer. The drug was dissolved in water at 2 mg/mL concentration (6.28 mmol) and added to ~2.5 mg bare-Gd_2O_3 and FA-Gd_2O_3, respectively. The loading was carried out by incubating the dispersant for 12 h in shaker-incubator at shaking speed of 200 shaking per minute for sufficient loading. The amount of loading was calculated by using supernatant of the centrifuged dispersant mixture of 5-Fu and bare/FA-Gd_2O_3. The obtained absorbance was fitted to its calibration plot equation and later to Eq. 22.2. For calibration curve, solutions of 5-FU in phosphate buffer (1 mM, pH 7.4) from 1 to 19 μg/mL was used which gave a good linear correlation ($R^2 = 0.999$). The supernatant gave the concentration of the remaining drug after loading was completed. The calibration curve and loading amount was estimated from its UV-vis absorbance at characteristic peak of 266 nm. The obtained values were further used for drug loading by the equation:

$$\%\text{Loading} = \frac{\text{Amount of drug in NPs}}{\text{Amount of NPs}} \times 100 \tag{22.2}$$

The calculated amount was ~9% and ~28% for bare Gd_2O_3 and FA-Gd_2O_3, respectively. The loading efficiency was calculated using theoretical loading from equation:

$$\text{Theoritical loading}(\%) = \frac{\text{Amount of drug added}}{\text{Amount of NPs}} \times 100 \tag{22.3}$$

The loading efficiency for bare Gd_2O_3 was ~4.5% and ~14% for FA-Gd_2O_3 using the equation as

$$\%\text{Loading efficiency} = \frac{\text{Actual loading}}{\text{Theoritical loading}} \times 100 \tag{22.4}$$

The drug-loading capacity was found to get enhanced with FA capping. This could be attributed to the increase in surface area and the surface functionalization sites associated with capping molecule. The 5-flurouracil (5-FU) with its simple structure is a low-molecular-weight drug (130.08 Dalton) having size of 3 × 6 Å which could fit well and easily get trapped by high-molecular-weight capping and targeting agent like FA (441.40 Dalton) [56, 57].

The drug release profile for 5-FU-loaded bare Gd_2O_3 and FA-Gd_2O_3 is shown in Fig. 22.7. The drug-loaded particles were re-dispersed in water to fill in dialysis bag

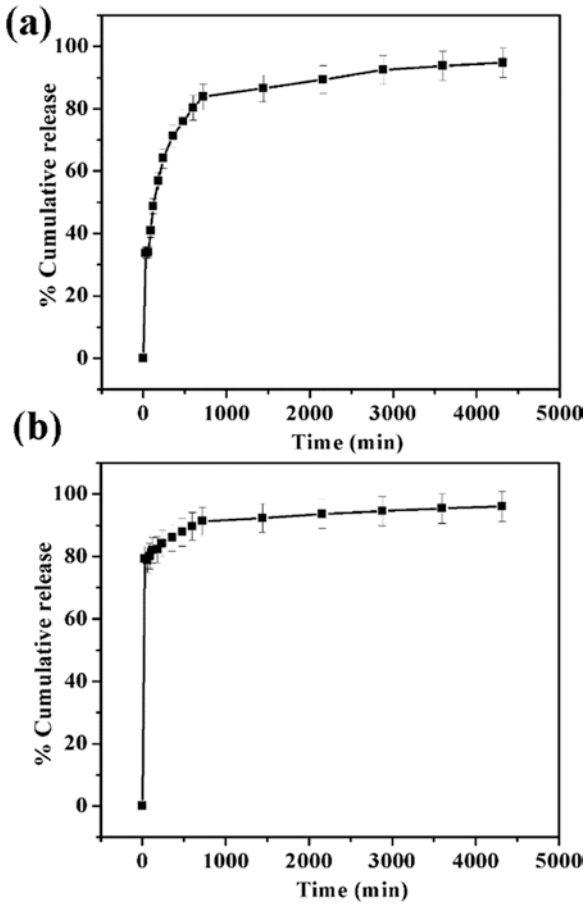


Fig. 22.7 Five-FU release profile of bare-gadolinium oxide (Gd_2O_3) and folic acid-capped-gadolinium oxide (FA-Gd_2O_3)

(MWCO 12,000 Daltons) against 30 mL of phosphate-buffered saline (PBS) buffer solution (pH 7.4) in 50 mL centrifuge tube and kept at 100 rpm and 37 °C. The drug release amount was measured by pipetting 2 mL of aqueous releasing buffer medium at mentioned time intervals and scanned by UV-vis spectroscopy. The initial burst release could be due to loosely bound drug molecules to the surface. The cumulative release percentage for bare Gd_2O_3 and FA-Gd_2O_3 was around 94 and 96% in 72 h, respectively. Although the release of drug was higher initially for FA-Gd_2O_3, later, the sustained release was observed. This release profile could be highly beneficial for long-term dose-dependent therapy.

5 Conclusion

The chapter has included the detailed explanation on diverse properties of lanthanide-based materials over their counterparts like transition metals and organic molecules like dyes. It has also highlighted the need of morphology and shape of nanomaterials for biomedical applications. The basic understanding of capping and surface tailoring has been discussed. The best possible mechanism for the elongated anisotropic shape of the lanthanide-based oxide has been discussed thoroughly. The special focus has been made in the transition and crystallizing temperature for the conversion from the hexagonal phase to cubic phase and explained with XRD, TGA, DSC, and FTIR. This detailed analysis has been used for understanding the use of the sample for their application as MRI contrast agent and drug delivery.

References

1. Mattoussi, H., & Rotello, V. M. (2013). Inorganic nanoparticles in drug delivery. *Advanced Drug Delivery Reviews, 65*(5), 605–606.
2. Siegel, R. L., Miller, K. D., & Jemal, A. (2017). Cancer statistics, 2017. *CA: A Cancer Journal for Clinicians, 67*(1), 7–30.
3. Shen, J., Zhao, L., & Han, G. (2013). Lanthanide-doped upconverting luminescent nanoparticle platforms for optical imaging-guided drug delivery and therapy. *Advanced Drug Delivery Reviews, 65*(5), 744–755.
4. Rizvi, S. A. A., & Saleh, A. M. (2018). Applications of nanoparticle systems in drug delivery technology. *Saudi Pharmaceutical Journal, 26*(1), 64–70.
5. Zhang, Y., Wei, W., Das, G. K., & Yang Tan, T. T. (2014). Engineering lanthanide-based materials for nanomedicine. *Journal of Photochemistry and Photobiology C: Photochemistry Reviews, 20*, 71–96.
6. Duan, C., Liang, L., Li, L., Zhang, R., & Xu, Z. P. (2018). Recent progress in upconversion luminescence nanomaterials for biomedical applications. *Journal of Materials Chemistry B, 6*(2), 192–209.
7. Opoku-Damoah, Y., Wang, R., Zhou, J., & Ding, Y. (2016). Versatile nanosystem-based cancer theranostics: Design inspiration and predetermined routing. *Theranostics, 6*(7), 986–1003.
8. Teo, R. D., Termini, J., & Gray, H. B. (2016). Lanthanides: Applications in cancer diagnosis and therapy. *Journal of Medicinal Chemistry, 59*(13), 6012–6024.

9. Ha-Thi, M.-H., Delaire, J. A., Michelet, V., & Leray, I. (2010). Sensitized emission of luminescent lanthanide complexes based on a phosphane oxide derivative. *The Journal of Physical Chemistry-A, 114*(9), 3264–3269.
10. Yang, D., Ma, P., Hou, Z., Cheng, Z., Li, C., & Lin, J. (2015). Current advances in lanthanide ion (Ln^{3+})-based upconversion nanomaterials for drug delivery. *Chemical Society Reviews, 44*(6), 1416–1448.
11. Kwon, O. S., Song, H. S., Conde, J., Kim, H. I., Artzi, N., & Kim, J. H. (2016). Dual-color emissive upconversion nanocapsules for differential cancer bioimaging in vivo. *ACS Nano, 10*(1), 1512–1521.
12. Shan, S.-N., Wang, X.-Y., & Jia, N.-Q. (2011). Synthesis of $NaYF_4$:Yb^{3+}, Er^{3+} upconversion nanoparticles in normal microemulsions. *Nanoscale Research Letters, 6*(1), 539.
13. Chen, G., Qiu, H., Prasad, P. N., & Chen, X. (2014). Upconversion nanoparticles: Design, nanochemistry, and applications in theranostics. *Chemical Reviews, 114*(10), 5161–5214.
14. Gao, W., Dong, J., Yan, X., Liu, L., Liu, J., & Zhang, W. (2017). An further enhancement in red upconversion emission in single $LiYbF_4$:Ho^{3+} microparticle through codoping Ce^{3+} ions. *Journal of Luminescence, 192*, 513–519.
15. Xue, Z., Yi, Z., Li, X., Li, Y., Jiang, M., Liu, H., et al. (2017). Upconversion optical/magnetic resonance imaging-guided small tumor detection and in vivo tri-modal bioimaging based on high-performance luminescent nanorods. *Biomaterials, 115*, 90–103.
16. Li, X., Yi, Z., Xue, Z., Zeng, S., & Liu, H. (2017). Multifunctional $BaYbF_5$:Gd/Er upconversion nanoparticles for in vivo tri-modal upconversion optical, X-ray computed tomography and magnetic resonance imaging. *Materials Science and Engineering-C, 75*, 510–516.
17. Dai, Y., Xiao, H., Liu, J., Yuan, Q., Ma, P., Yang, D., et al. (2013). In vivo multimodality imaging and cancer therapy by near-infrared light-triggered trans-platinum pro-drug-conjugated upconverison nanoparticles. *Journal of the American Chemical Society, 135*(50), 18920–18929.
18. Fedoryshin, L. L., Tavares, A. J., Petryayeva, E., Doughan, S., & Krull, U. J. (2014). Near-infrared-triggered anticancer drug release from upconverting nanoparticles. *ACS Applied Materials & Interfaces, 6*(16), 13600–13606.
19. Chowdhuri, A. R., Laha, D., Pal, S., Karmakar, P., & Sahu, S. K. (2016). One-pot synthesis of folic acid encapsulated upconversion nanoscale metal organic frameworks for targeting, imaging and pH responsive drug release. *Dalton Transactions, 45*(45), 18120–18132.
20. Ma, J., Huang, P., He, M., Pan, L., Zhou, Z., Feng, L., et al. (2012). Folic acid-conjugated LaF3:Yb,Tm@SiO2 nanoprobes for targeting dual-modality imaging of upconversion luminescence and X-ray computed tomography. *The Journal of Physical Chemistry-B, 116*(48), 14062–14070.
21. Walker, D. K. (2004). The use of pharmacokinetic and pharmacodynamic data in the assessment of drug safety in early drug development. *British Journal of Clinical Pharmacology, 58*(6), 601–608.
22. Bashir, S., Teo, Y. Y., Naeem, S., Ramesh, S., & Ramesh, K. (2017). Correction: pH responsive *N*-succinyl chitosan/poly (acrylamide-co-acrylic acid) hydrogels and in vitro release of 5-fluorouracil. *PLoS One, 12*(9), e0185505.
23. Toloudi, M., Apostolou, P., & Papasotiriou, I. (2015). Efficacy of 5-FU or oxaliplatin monotherapy over combination therapy in colorectal cancer. *Journal of Cancer Therapy, 6*, 345–355.
24. Rejinold, N. S., Muthunarayanan, M., Chennazhi, K. P., Nair, S. V., & Jayakumar, R. (2011). 5-Fluorouracil loaded fibrinogen nanoparticles for cancer drug delivery applications. *International Journal of Biological Macromolecules, 48*(1), 98–105.
25. Bagheri, A., Arandiyan, H., Boyer, C., & Lim, M. (2016). Lanthanide-doped upconversion nanoparticles: Emerging intelligent light-activated drug delivery systems. *Advancement of Science, 3*(7), 1500437.
26. Xing, P., Chu, X., Ma, M., Li, S., & Hao, A. (2014). Supramolecular gel from folic acid with multiple responsiveness, rapid self-recovery and orthogonal self-assemblies. *Physical Chemistry Chemical Physics, 16*(18), 8346–8359.

27. Sen, G. S., Kuzelka, J., Singh, P., Lewis, W. G., Manchester, M., & Finn, M. G. (2005). Accelerated bioorthogonal conjugation: A practical method for the ligation of diverse functional molecules to a polyvalent virus scaffold. *Bioconjugate Chemistry, 16*(6), 1572–1579.
28. Elnakat, H., & Ratnam, M. (2004). Distribution, functionality and gene regulation of folate receptor isoforms: Implications in targeted therapy. *Advanced Drug Delivery Reviews, 56*(8), 1067–1084.
29. Zhang, X., Ge, J., Xue, Y., Lei, B., Yan, D., Li, N., et al. (2015). Controlled synthesis of ultrathin lanthanide oxide nanosheets and their promising pH-controlled anticancer drug delivery. *Chemistry-A European Journal, 21*(34), 11954–11960.
30. Rogosnitzky, M., & Branch, S. (2016). Gadolinium-based contrast agent toxicity: A review of known and proposed mechanisms. *Biometals, 29*, 365–376.
31. FDA. (2015). *FDA evaluating the risk of brain deposits with repeated use of gadolinium-based contrast agents for magnetic resonance imaging (MRI)*, 7–10.
32. FDA Drug Safety. (2017, May 22). *FDA safety announcement-FDA identifies no harmful effects to date with brain retention of gadolinium-based contrast agents for MRIs: Review to continue*, 2–5.
33. FDA. (2017). *FDA warns that gadolinium-based contrast agents (GBCAs) are retained in the body; requires new class warnings*, 1–4.
34. Cho, M., Sethi, R., Ananta Narayanan, J. S., Lee, S. S., Benoit, D. N., Taheri, N., et al. (2014). Gadolinium oxide nanoplates with high longitudinal relaxivity for magnetic resonance imaging. *Nanoscale, 6*(22), 13637–13645.
35. Ranga, A., Agarwal, Y., & Garg, K. J. (2017). Gadolinium based contrast agents in current practice: Risks of accumulation and toxicity in patients with normal renal function. *Indian Journal of Radiology Imaging, 27*(2), 141–147.
36. Aime, S., & Caravan, P. (2009). Biodistribution of gadolinium-based contrast agents, including gadolinium deposition. *Journal of Magnetic Resonance Imaging, 30*(6), 1259–1267.
37. Wang, J., Li, W., & Zhu, J. (2014). Encapsulation of inorganic nanoparticles into block copolymer micellar aggregates: Strategies and precise localization of nanoparticles. *Polymer, 55*(5), 1079–1096.
38. Kim, C. R., Baeck, J. S., Chang, Y., Bae, J. E., Chae, K. S., & Lee, G. H. (2014). Ligand-size dependent water proton relaxivities in ultrasmall gadolinium oxide nanoparticles and in vivo T_1 MR images in a 1.5 T MR field. *Physical Chemistry Chemical Physics, 16*(37), 19866–19873.
39. Vairapperumal, T., Saraswathy, A., Ramapurath, J. S., Kalarical Janardhanan, S., & Balachandran Unni, N. (2016). Catechin tuned magnetism of Gd-doped orthovanadate through morphology as T_1-T_2 MRI contrast agents. *Scientific Reports, 6*(1), 34976.
40. Tegafaw, T., Xu, W., Lee, S. H., Chae, K. S., Cha, H., Chang, Y., et al. (2016). Ligand-size and ligand-chain hydrophilicity effects on the relaxometric properties of ultrasmall Gd_2O_3 nanoparticles. *AIP Advances, 6*(6), 065114.
41. Cao, Y., Xu, L., Kuang, Y., Xiong, D., & Pei, R. (2017). Gadolinium-based nanoscale MRI contrast agents for tumor imaging. *Journal of Materials Chemistry B, 5*(19), 3431–3461.
42. Ma, X., Zhao, Y., & Liang, X.-J. (2011). Theranostic nanoparticles engineered for clinic and pharmaceutics. *Accounts of Chemical Research, 44*(10), 1114–1122.
43. Hemmer, E., Venkatachalam, N., Hyodo, H., & Soga, K. (2012). The role of pH in PEG-b-PAAc modification of gadolinium oxide nanostructures for biomedical applications. *Advances in Materials Science and Engineering, 2012*, 1–15.
44. Lee,K., Bae, Y., & Byeon, S. (2010). pH Dependent Hydrothermal Synthesis and Photoluminescence of Gd_2O_3:Eu Nanostructures. *Nanowires Science and Technology* Edited by Nicoleta Lupu, https://doi.org/10.5772/39489.
45. Majeed, S., & Shivashankar, S. A. (2014). Rapid, microwave-assisted synthesis of Gd_2O_3 and Eu:Gd_2O_3 nanocrystals: Characterization, magnetic, optical and biological studies. *Journal of Materials Chemistry B, 2*(34), 5585.
46. Mekuria, S. L., Debele, T. A., & Tsai, H. (2017). Encapsulation of gadolinium oxide nanoparticle (Gd_2O_3) contrasting agents in PAMAM Dendrimer templates for enhanced magnetic resonance imaging in vivo. *ACS Applied Materials & Interfaces, 9*(8), 6782–6795.

47. Rocha, L. A., Schiavon, M. A., Ribeiro, S. J. L., Gonçalves, R. R., & Ferrari, J. L. (2015). Eu^{3+}-doped SiO_2–Gd_2O_3 prepared by the sol–gel process: Structural and optical properties. *Journal of Sol-Gel Science and Technology, 76*(2), 260–270.
48. Hazarika, S., & Mohanta, D. (2016). Oriented attachment (OA) mediated characteristic growth of Gd_2O_3 nanorods from nanoparticle seeds. *Journal of Rare Earths, 34*(2), 158–165.
49. Kang, J., Min, B., & Sohn, Y. (2015). Synthesis and characterization of $Gd(OH)_3$ and Gd_2O_3 nanorods. *Ceramics International, 41*(1), 1243–1248.
50. Chaudhary, S., Kumar, S., Umar, A., Singh, J., Rawat, M., & Mehta, S. K. (2017). Europium-doped gadolinium oxide nanoparticles: A potential photoluminescencent probe for highly selective and sensitive detection of Fe^{3+} and Cr^{3+} ions. *Sensors and Actuators B: Chemical, 243*, 579–588.
51. Barge, A., Cravotto, G., Gianolio, E., & Fedeli, F. (2006). How to determine free Gd and free ligand in solution of Gd chelates: A technical note. *Contrast Media & Molecular Imaging, 1*(5), 184–188.
52. Zhang, S., Jiang, Z., Liu, X., Zhou, L., & Peng, W. (2013). Possible gadolinium ions leaching and MR sensitivity over-estimation in mesoporous silica-coated upconversion nanocrystals. *Nanoscale, 5*(17), 8146.
53. Chaudhary, A., Gupta, A., & Nandi, C. K. (2015). Anisotropic gold nanoparticles for the highly sensitive colorimetric detection of glucose in human urine. *RSC Advances, 5*(51), 40849–40855.
54. Xu, W., Miao, X., Oh, I., Chae, K. S., Cha, H., Chang, Y., et al. (2016). Dextran-coated ultrasmall Gd $_2O_3$ nanoparticles as potential T_1 MRI contrast agent. *ChemistrySelect, 1*(19), 6086–6091.
55. Vahdatkhah, P., Madaah Hosseini, H. R., Khodaei, A., Montazerabadi, A. R., Irajirad, R., Oghabian, M. A., et al. (2015). Rapid microwave-assisted synthesis of PVP-coated ultrasmall gadolinium oxide nanoparticles for magnetic resonance imaging. *Chemical Physics, 453–454*, 35–41.
56. Du, P.-Y., Gu, W., & Liu, X. (2016). A three-dimensional Nd(III)-based metal–organic framework as a smart drug carrier. *New Journal of Chemistry, 40*(11), 9017–9020.
57. Leelakanok, N., Geary, S., & Salem, A. (2018). Fabrication and use of PLGA-based formulations designed for modified release of 5-fluorouracil. *Journal of Pharmaceutical Sciences, 107*(2), 513–528.

Chapter 23
Engineering of Targeted Nanoparticles by Using Self-Assembled Biointegrated Block Copolymers

Shoaib Iqbal, M. Naveed Yasin, and Heather Sheardown

Abstract Polymer-based nanoparticle delivery systems have attracted a lot of attention recently due to their chemical versatility offering precise engineering for targeted drug delivery. However, surface functionalization of these nanoparticles could lead to poor control over their properties. A strategy to overcome this shortcoming is synthesis of self-assembled biointegrated block copolymers which offer reproducible preparation, quantitative control over ligand density and easy production. These polymers are prepared by well-established polymerization and conjugation chemistries. The resultant nanoparticles are prepared by self-assembly of these polymers in a single step where fine tuning of these nanoparticles is accomplished by varying the composition of block polymers.

Keywords Self-assembled nanoparticles · Targeted drug delivery · Bio-inspired nanoparticles

1 Introduction

Various polymeric delivery systems are being employed for a number of therapeutic applications at the moment. The properties of these systems have been adjusted to improve their delivery to target site; for instance, surface PEGylation or hydrophilic coating of these systems provide stealth properties for longer blood circulation, while positive surface charge can improve internalization into cancer cells. Among various polymeric systems explored for therapeutic delivery applications, polymeric nanoparticles (NPs) and micelles (MCs) have recently attracted a lot of attention.

S. Iqbal
Department of Chemical and Biomolecular Engineering, Clemson University, Clemson, South Carolina, USA
e-mail: shoaibi@clemson.edu

M. Naveed Yasin (✉) · H. Sheardown
Department of Chemical Engineering, Faculty of Engineering, McMaster University, Hamilton, ON, Canada
e-mail: yasinn@mcmaster.ca; sheardow@mcmaster.ca

Y. V Pathak (ed.), *Surface Modification of Nanoparticles for Targeted Drug Delivery*, https://doi.org/10.1007/978-3-030-06115-9_23

This is due to broad scope for chemically modifying these polymeric systems for targeted therapeutic delivery.

The concept of drug targeting was introduced first by Paul Ehrlich in 1906, and tremendous progress has been made since then to develop NPs and MCs for this purpose. The development of these polymeric systems for targeted delivery helps to improve therapeutic index of drugs, limiting dose-related toxicities and suboptimal efficacy [1, 2]. Active targeting of these systems to specific tissues can be achieved by complexing them with targeting ligand (small molecule, peptide, aptamer, antibody/antibody fragment) that will recognize cell surface receptors in that tissue [3]. An added advantage of this receptor-specific interaction is increased uptake due to receptor mediated endocytosis (RME) as shown in Fig. 23.1. Designing NPs with targeting moieties on their surfaces helps in endosomal/lysosomal escape leading to the release of payloads into the cytosol and improvement of therapeutic efficacy.

The successful therapeutic application of polymeric NPs is determined by their well-defined biological behavior in vivo which is largely dependent on their properties such as size, surface modification, and shape [5, 6]. These properties along with mechanical strength and functionalization by targeting ligands have been widely accepted as important parameters in determining delivery efficacy [7, 8]. Engineering polymeric NPs is challenged by the influence of these properties which are interrelated and may impact other during the process of development. For targeted NPs, there is an additional consideration of attaching targeting ligand. The conventional approach of formulating targeted NPs involves formation of NPs with encapsulated drug first followed by surface functionalization to render the NPs stealth (e.g., PEGylation) and attachment of targeting ligands. This approach involves use of either functionalized biomaterials which simultaneously offer NPs

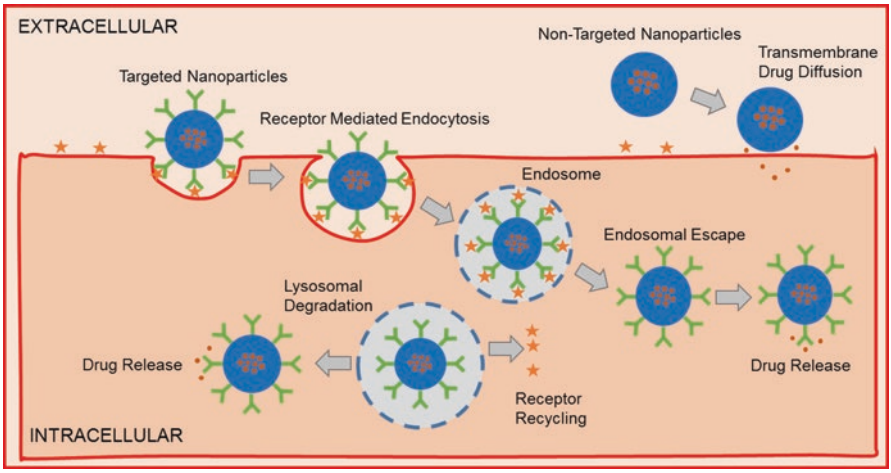


Fig. 23.1 Schematic illustration of differences in cellular uptake and payload release for targeted and nontargeted polymeric nanoparticles, reproduced with permission from [4] (Copyright Sci Forschen Inc. 2016)

with both stealth and targeted features [9–11], or assembling stealth NPs first followed by conjugation with a targeting ligands to achieve targeting ability [12, 13]. The associated disadvantages of the latter approach are the requirement for an excess amount of reactants, multistep manufacturing and purification procedures, and decreased control over the final NPs properties leading to batch-to-batch variations [2, 14, 15].

A simple yet rational approach for targeted NPs is to avoid post NP synthesis modification steps by developing prefunctionalized biomaterials having all of the desired components present and engineering them to self-assemble into targeted NPs. These prefunctionalized biomaterials which are constituted by self-assembled biointegrated block copolymers offer precise engineering of NPs without any requirement for post-particle modifications due to preattached ligands and their intrinsic ability to self-assemble into particles. Also, this approach offers advantages of well-controlled and -characterized polymer blocks, simpler conjugation of ligands, quantitative control over ligand density on the NP surface, assembly into different macromolecular architectures, and simple purification procedures which are amenable to scale-up production without batch-to-batch variation [16–18]. Such a strategy offers formulation of distinct NPs with minimal variability leading to possibility of optimizing biophysicochemical properties. An associated disadvantage of the preconjugation strategy of ligand conjugation with copolymer is the loss of ligand bioactivity during NP preparation leading to poor targeting performance. Table 23.1 shows some of the ligand-conjugated block copolymers for various therapeutic applications.

2 Synthesis of Biointegrated Block Copolymers

The synthesis of biointegrated block copolymers is essentially similar to the conventional polymerization methods used for preparing variety of block copolymers. Multiple types of synthetic approaches and conjugation chemistries have been developed to expand the breadth of synthetic approaches in developing biointegrated functional biomaterials. The diversity of available methods and conjugation with versatile targeting ligands ensures well-characterized and controlled polymer structures with adjustable properties for different therapeutic delivery applications. Repertoire of synthetic strategies and methodologies include both step and chain growth polymerization approaches. Using these methods a number of block polymers with different topologies and functional characteristics can be prepared [48].

The synthesis strategies mainly include (1) addition of monomers by controlled (living) polymerization and (2) using coupling reactions to exploit the chain ends of different polymer chain segments [49]. Controlled polymerization offers a facile approach for obtaining versatile block polymers owing to their compatibility with range of monomers, high functional group tolerance and easy experimental setup [50]. Some of the controlled polymerization techniques that has been described in literature include atom transfer racial polymerization (ATRP), reversible addition-

Table 23.1 Biointegrated block copolymers based nanoparticles and micelles to deliver drugs for various therapeutic applications

Ligand type	Polymer construction	Application	References
Protein	**EGF**-PEG-PCL micelles	Breast cancer	[19]
	RGD/Tf-HPAE-*co*-PLA/DPPE nanoparticles	Cervical cancer	[20]
Aptamer	**A10**-PEG-PLGA/PEG-PLGA micelles	Prostate cancer	[21]
	A10-PEG-PLGA-H40 micelles	Prostate cancer	[22]
Peptide	**cRGD**-PEG-PLA micelles	Brain glioma	[23]
	cRGDyk-PEG-PTMC micelles	Brain glioma	[24]
	cRGD-PEO-PCL micelles	Bladder cancer	[25]
	cRGD-PEG-b-PLL(2IT)	Cervical cancer	[26]
	Oct-PEG-PCL micelles	Breast cancer	[27]
	AP-PEG-PLA/mPEG-PAE micelles	Breast cancer	[28]
	cRGD-PLL-PLGA-mPEG nanoparticles	Breast cancer	[29]
	PEG-Dlink$_m$-**R9**-PCL	Lung cancer	[30]
Saccharide/ polysaccharide	**Gal**-PEG-PLA, **FITC**-PEG-PLA, mPEG-PLA, P(NIPAAm-*co*-MAAc)-*g*-PLA	Hepatoma	[31]
	Gal-PEG-PCL/PEG-SS-PCL micelles	Hepatoma	[32]
	Gal-PEG-PCL/PEG-PAC-PCL micelles	Hepatoma	[33]
	Poly(CL-*co*-OPEA-**gal**) nanoparticles	Hepatoma	[34]
	HA-PTX micelles	Colon and Breast cancer	[35]
	HA-PEG-PLGA nanoparticles	Ehrlich ascites cancer	[36]
	HA-SS-DOCA micelles	Breast cancer	[37]
Small drug molecules	**Folate**-PEG-PLGA/mPEG-PLGA-DOX micelles	Epithelial cancer	[38]
	Folate-PEG-PCL	Cancer	[39]
	Folate-PEG-PAsp(DIP)-CA micelles	Hepatoma	[40]
	Folate-PEG-b-PCL-hyd-DOX micelles	Epithelial cancer	[41]
	FA-PEEP-b-PBYP micelles	Carcinoma	[42]
	FA-PEG$_{2k}$-pD-pDPB/PEG$_{20k}$-peptide-pD-pDPB mixed micelles	Breast cancer	[43]
	Biotin-PEG-PLA/PLGA nanoparticles	Epithelial cancer	[44]
	Biotin-PEG-PLA/PLGA-PEI nanoparticles	Epithelial cancer	[45]
	ACUPA-PEG-PLA/PEG-PLGA micelles	Prostate cancer	[46]
	pLA-b-p(MAA-**PBA**)	Ocular mucosa	[47]

fragmentation chain transfer (RAFT) and many others. The biointegrated block polymers synthesis involves an additional functionalization step at their hydrophilic chain end(s) with ligands [51]. The approaches for functionalization of these block copolymer include (1) click chemistry mediated post-polymerization functionalization by modifying polymer pendant groups (post-polymerization

functionalization); (2) modification of chain end by employing functional initiating systems or functional terminating agents (end-chain functionalization). Using a single strategy or a combination of these strategies offers desired properties of resultant biointegrated block copolymers for biomaterials applications. However, each of these methods has its own pros and cons; for example, post-polymerization functionalization offers flexibility in modification by preventing the entire bulk of material from being affected. Directly polymerizing functional monomers is attractive but is hampered by polymerization conditions and parameters like in radical polymerization. Also, introduction of functional moieties in cyclic monomers is challenging due to the incompatibilities of the imparted functionalities [52]. The presence of functionalizable end groups attached to linear and terminal units of block copolymers allow for the convenience of end-capped with small organic molecules/targeting moieties to fabricate novel functional polymeric materials. The nature of the end group on the polymer backbone has an influence on the polymer degradability. Chain end groups have also been used as sites for functionalization of polymers to achieve targeted delivery of payloads [42]. To achieve therapeutic efficacy, precise structural tuning of polymers is important to prevent unpredictable properties and behavior. The overall advantage of synthesizing biointegrated block polymers is that they can be easily purified and characterized prior to their assembly into NPs. They offer better control of surface-density of exposed ligands due to adjustable ratios of functionalized to nonfunctionalized block copolymers [21]. They also offer decoration of NPs with multiple targeting ligands by assembling differently functionalized polymers into a single platform. A highly functionalized corona of NPs can be obtained by allowing self-assembly of a ligand-bearing copolymer. The only drawback of this approach is that the self-assembly behaviour might be affected by the end-functionalization of block copolymer which needs to be tested experimentally.

A biointegrated amphiphilic triblock copolymer (TCP) composed of PLGA-b-PEG-b-A10 aptamer was synthesized in two steps by conjugating carboxyl-capped PLGA (PLGA-acid) with amine terminals of heterobifunctional PEG (amine-PEG-acid) followed by conjugation of carboxyl ends of PLGA-b-PEG-acid with amine ends of A10 PSMA Apt as shown in Fig. 23.2 [21]. The formation of block copolymer and the ligand conjugation was straight forward using 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) and *N*-hydroxysulfosuccinimide (sulfo-NHS) coupling reaction.

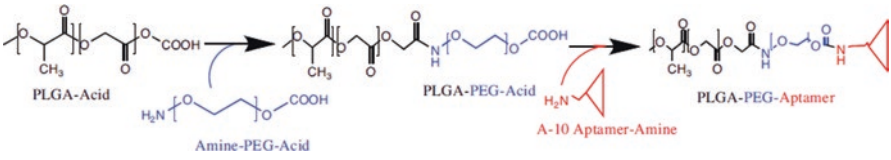


Fig. 23.2 Synthesis of PLGA-b-PEG-b-Apt TCP in a two-step conjugation reactions, reproduced with permission from [21] (Copyright The National Academy of Sciences of the USA 2008)

A ring-opening polymerization (ROP), photoinduced thiol-ene reaction and amidation reaction was used to synthesize Gal-conjugated biodegradable poly-(Ɛ-caprolactone-*co*-phosphoester) random copolymer [poly(CL-*co*-OPEA-$_{Gal}$)] [34] as shown in Fig. 23.3. Gal was covalently conjugated to pendant groups via combination of photoinduced thiol-ene reaction and amidation reaction.

RAFT polymerization has seen rapid growth due to its superior compatibility with a broader range of functionalities, one-pot synthesis and high tolerance of impurities. A reversible addition−fragmentation chain transfer (RAFT) polymerization reaction has been proposed for the synthesis of pLA-b-p(MAA-PBA) copolymers also termed LMP copolymer in a single step reaction [47] as shown in Fig. 23.4. The triblock copolymer synthesized by using Azobis(isobutyronitrile) (AIBN) as a RAFT agent showed excellent control over copolymer compositions and molecular weights by varying the molar feed rations of the monomers. The targeting moiety PBS polymerized at the end of the chain in higher density offering this polymer block as an excellent platform for mucoadhesive targeted delivery to eye.

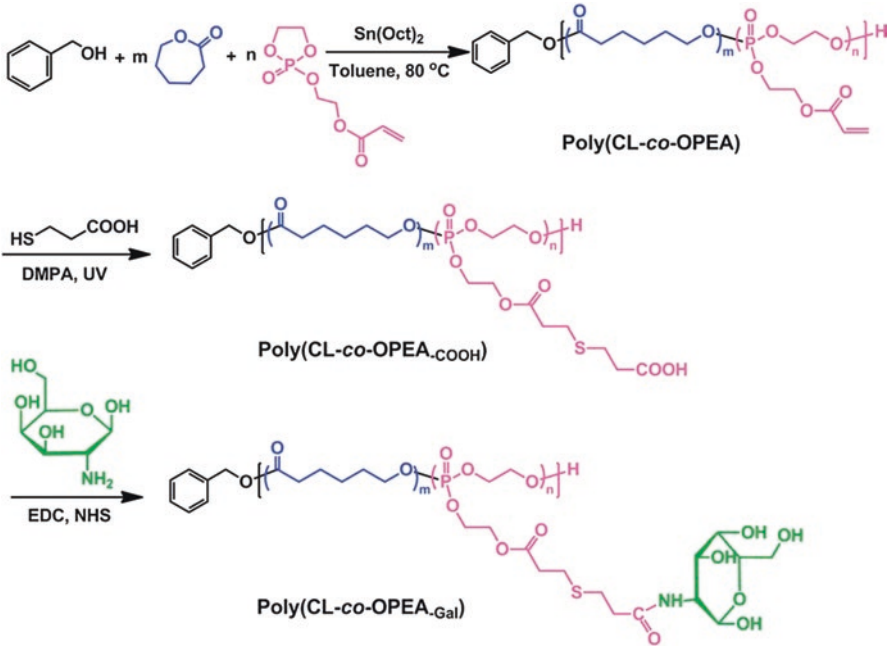


Fig. 23.3 Synthesis routes of galactosamine-conjugated random copolymer poly(CL-*co*-OPEA-$_{Gal}$), reproduced with permission from [34] (Copyright The Royal Society of Chemistry 2014)

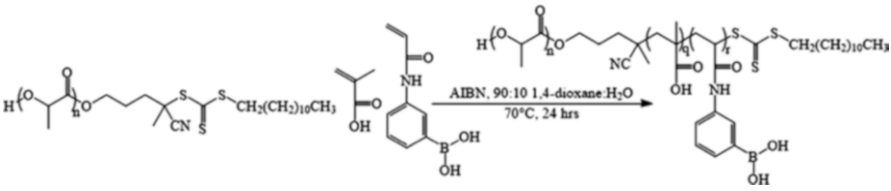


Fig. 23.4 Synthesis routes of LMP block copolymers by RAFT polymerization, reproduced with permission from (47) (Copyright The American Chemical Society 2016)

3 Preparation of Targeted Nanoparticles by Biointegrated Block Copolymers

The guiding principles to formulate targeted nanoparticles from biointegrated block copolymers are essentially similar to those used for nontargeted nanoparticles. Owing to the amphiphilic nature of these polymer blocks, they self-assemble themselves into nanoparticles architecture upon introduction into aqueous environment [53]. From delivery standpoint, the diameters of these nanoparticles should be less than 200 nm, systemically stable, allow immune evasion and nonspecific uptake and finally destabilize upon uptake by target cells. From commercial standpoint, the preparation should be cost effective and easy to scale-up production employing simple components with robust methods.

Targeted NPs from biointegrated block copolymers have been produced by numerous methods depending on the nature of ligand-conjugated polymer blocks and payload types. These methods include solvent evaporation/emulsion based preparation, nanoprecipitation, and dialysis method. Due to single step preparation using these block polymers, multiple processing steps are avoided, leading to NPs with well-controlled and reproducible characteristics. The simplicity of methodology offers convenient scale up of these nanoparticles. Post preparation treatment steps can be easily performed to increase or decrease the final concentration of NPs. A major advantage of using biointegrated block copolymers is well-controlled surface density of the targeting ligands by varying the ratio of ligand-conjugated and nonconjugated block polymers during self-assembly. Using distinct ratios of PLGA-b-PEG-b-Apt TCP (ligand-conjugated polymer block) and PLGA-b-PEG DCP (nonconjugated polymer block) during NP preparation by nanoprecipitation, the surface density of Apt was precisely and reproducibly changed [21] as shown in Fig. 23.5.

A multifunctional micelle composed of a graft copolymer (i.e., poly(*N*-isopropyl acrylamide-*co*-methacryl acid)-*g*-poly(D,L-lactide) (P(NIPAAm-*co*-MAAc)-*g*-PLA) (environmentally sensitive), a diblock copolymer [i.e., methoxy poly(ethylene glycol)-b-poly(D,L-lactide) (mPEG-PLA)], and two functionalized diblock copolymers [i.e., galactosamine-PEG-PLA (Gal-PEG-PLA) and fluorescein isothiocyanate-PEG-PLA (FITC-PEG-PLA)] was prepared by using a dialysis method in a single step formulation process as shown in Fig. 23.6. Due to single

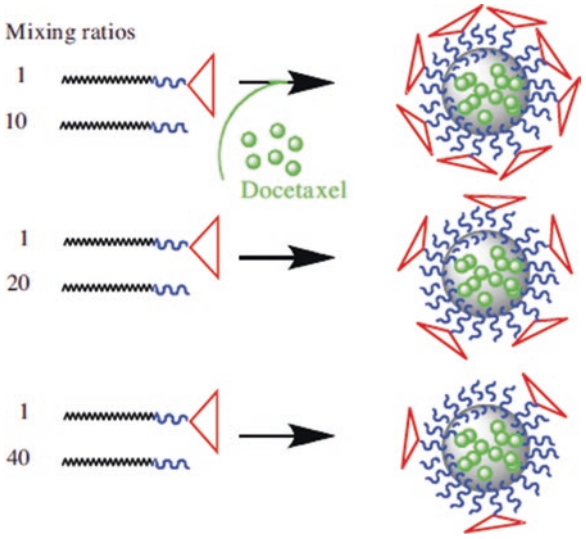


Fig. 23.5 Preparation of PLGA-b-PEG-b-Apt TCP-based polymeric NPs with well-controlled surface density, reproduced with permission from [21] (Copyright The National Academy of Sciences of the USA 2008)

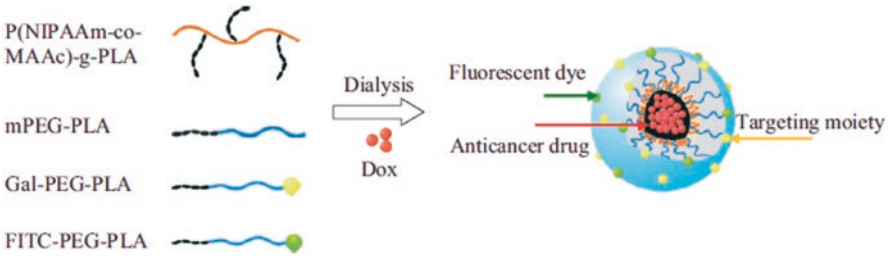


Fig. 23.6 Preparation of multifunctional micelle made of two functionalized diblock copolymers, a graft copolymer and a diblock copolymer, reproduced with permission from [31] (Copyright WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim 2007)

step formulation of NPs and MCs by these block polymers, the optimization of nanocarriers with desired features is easily achievable and these systems hold great potential for scale up production.

4 Functionalized Biointegrated Block Copolymers for Therapeutic Applications

Functionalized polymers have attracted considerable interest among biomaterials scientists due to the characteristic features of these materials in combining the properties of defined reactive functionalities present in their architecture. In such polymers, the reactive sites of the attached functional groups can be used as

attachment site for targeting ligands or biomolecules. Such functionalized materials have found numerous applications in targeted drug delivery, tissue engineering and gene delivery.

4.1 Targeted Drug Delivery in Cancer

Due to the functionalization advantages of polyphosphoester (PPE) based polymers for targeted delivery, a galactosamine (Gal)-functionalized novel drug delivery carrier (i.e., [poly(CL-*co*-OPEA-$_{Gal}$)]) was developed for improved hepatoma-targeting delivery of doxorubicin (DOX) [34]. Pendant end groups of the 2-(2-Oxo-1,3,2-dioxaphospholoyloxy) ethyl acrylate (OPEA) were functionalized with galactosamine using the terminal –COOH of (CL-*co*-OPEA-$_{COOH}$) to construct liver targeted delivery system as shown in Fig. 23.7. This galactosylated biointegrated block

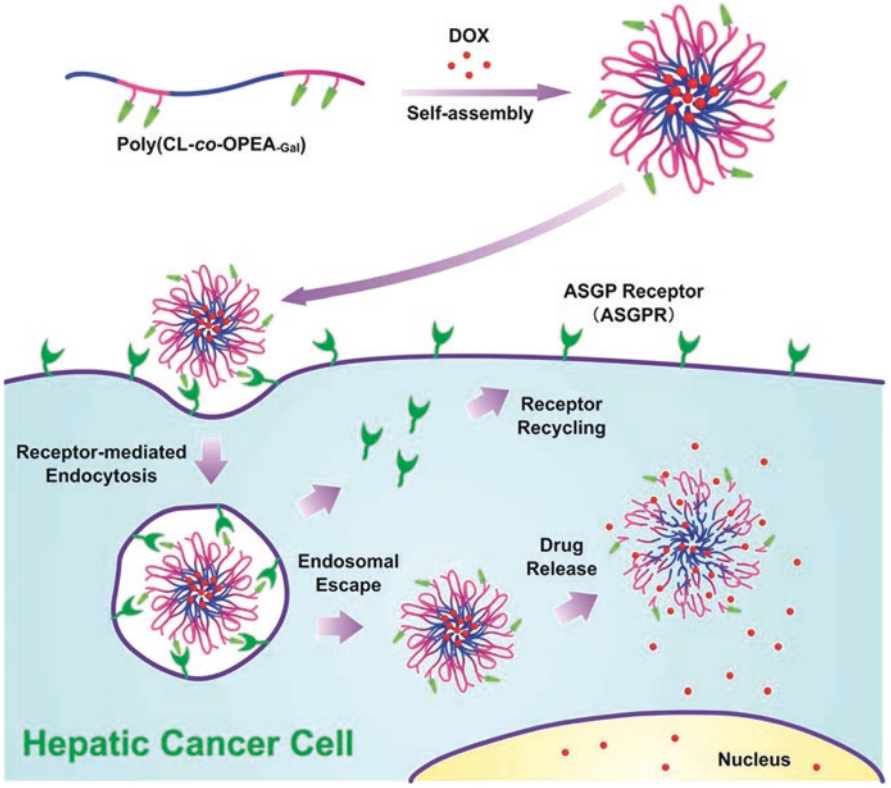


Fig. 23.7 Schematic illustration of the preparation of hepatoma targeting DOX-loaded poly(CL-co-OPEA-Gal) nanoparticles (designated as DOX/Gal-NPs) and the receptor-mediated intracellular drug release, reproduced with permission from [34] (Copyright The Royal Society of Chemistry 2014)

copolymer could self-assemble into micelles in an aqueous environment with the core constituted by the hydrophobic PC, corona by hydrophilic polyphosphoester and Gal moieties on the micellar surface. Using this micellar platform offered anti-tumor drug delivery for hepatoma-targeting with improved hepatic internalization, leading to improved therapeutic efficiency.

A novel water-soluble polymeric prodrug, paclitaxel–poly(ethyl ethylene phosphate) conjugated with folic acid molecules (PTX-PEEP-FA) was reported as an amphiphilic drug delivery platform [54]. The chain end hydroxyl groups were conjugated with folic acid (FA) via esterification. Apart from acting as a targeted prodrug, the system showed usefulness to load other hydrophobic drugs thereby acting as dual delivery platform with sustained release and targeting efficacy. To impart additional stability to micellar delivery platform, core cross-linked PPE micelles (i.e., PBYP-b-PEEP-FA with acid-cleavable acetal groups (ACCL-FA) loaded with DOX) were reported as shown in Fig. 23.8 [42]. Cross-linking of the core occurred via azide–alkyne cycloaddition (CuAAC) "Click" reaction between azides of acid-cleavable N_3-*a*-TEG-*a*-N_3 and the alkynyl groups on the PPE. Acetal linkage cleavage under acidic conditions, PPE degradability, and FA targetability offered benefits to this system.

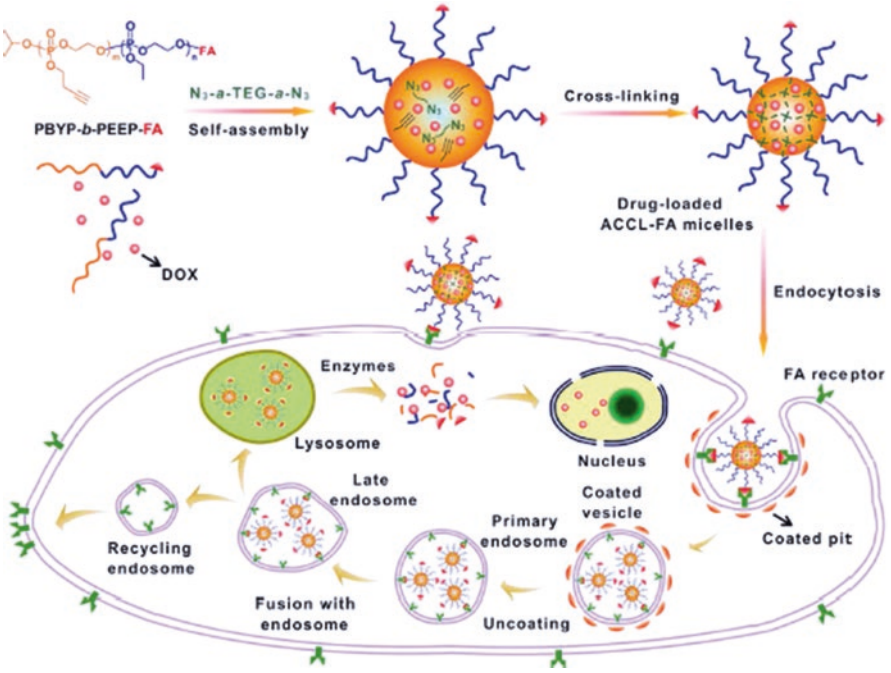


Fig. 23.8 Illustration of folate-conjugated and core cross-linked polyphosphoester micelles for efficient intracellular release of hydrophobic anticancer drugs triggered by enzymes and acidic microenvironment inside the tumor tissue, reproduced with permission from [42] (Copyright The Royal Society of Chemistry 2014)

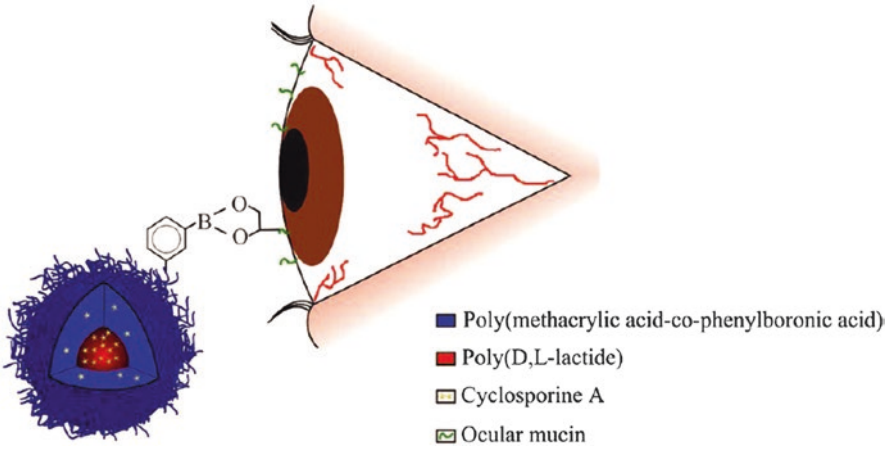


Fig. 23.9 Illustration of PBA-conjugated polymeric micelles for drug delivery to anterior segment of eye, reproduced with permission from [47] (Copyright The American Chemical Society 2015)

4.2 Targeted Drug Delivery to the Mucosa

In an effort to improve the corneal retention of NPs and thereby ocular bioavailability, targeted NPs with mucoadhesive ligands (i.e., phenylboronic acid (PBA)) which can form complexes with diols of sialic acid at physiological pH [55] have been reported [56]. A mucoadhesive PBA decorated polymeric micelle has been reported for ocular drug delivery to anterior segment of eye [47] as shown in Fig. 23.9. The mucoadhesive property of micelles was directly related to the PBA content in the micelles. These micelles showed promising potential to improve the bioavailability of topically applied ophthalmic drugs.

4.3 Targeted Delivery of Genes as Therapeutic Agents

Therapeutic delivery of siRNA holds great potential for the treatment of diseases via inhibition of protein expression. Several biointegrated block copolymers have been reported to offer delivery of siRNA payloads along with other functional demands. A peptide–block polymer conjugate [i.e., cRGD-PEG-b-PLL (2IT)] upon mixing with siRNA spontaneously formed multifunctional micellar structures [26]. The biointegrated polymer segment offered siRNA binding segment, a hydrophilic segment and a targeting ligand. This multifunctional micellar platform offered improved control of NP formation, increased stability and improved biological activity.

A dual targeted nanocarrier for siRNA was formulated from two different block polymers with similar core blocks but different corona functionalities [43]. These smart polymer nanoparticles were formed by combining FA-PEG-pDMAEMA-b-

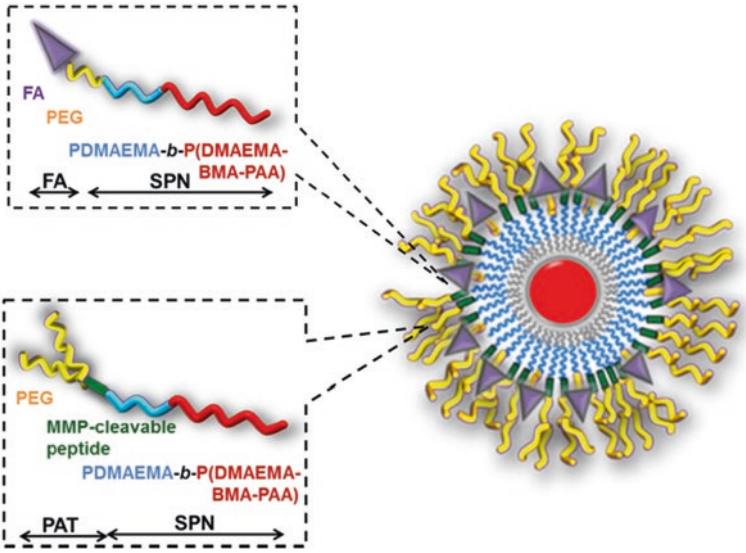


Fig. 23.10 Schematic of mixed micelle formed from combining FA-PEG_{2k}-pD-pDBP and PEG_{20k}-peptide-pD-pDBP mixed micelles, reproduced with permission from [43] (Copyright The American Chemical Society 2015)

p(DMAEMA-BMA-PAA) and PEG-MMP7-pDMAEMA-b-p(DMAEMA-BMA-PAA) polymer blocks which self-assembled into micelles (Fig. 23.10). The folic acid offered active targeting while MMP7 offered proximity-activated targeting (PAT), that is, shielding nonspecific interactions with cells/proteins by proteolytically removable PEG to these NPs.

A functional degradable bridged bond-based copolymer has been developed for the active targeted siRNA delivery [29]. The block copolymer (i.e., PEG-$Dlink_m$-R9-PCL) self-assembled into nanoparticles in an aqueous solution. The bridged bond ($Dlink_m$) was degraded in an extracellular pH (pH_e), leading to deshielding of PEG and exposure of nano-arginine (R9) for NPs uptake by the target tumor cells for improved siRNA delivery.

5 Concluding Remarks

Polymeric nanoparticles and micelles have shown promise as delivery vehicles for various therapeutic applications, yet they need to be properly designed in order to achieve maximum efficacy. The delivery efficiency of these nanocarriers can be increased by functionalizing their surfaces with targeting ligands with high binding affinity to the target cells. When decorating NPs with targeting ligands, the characteristic features and properties of the resultant nanocarriers are not well

controlled, leading to unpredictable behavior during application. This problem can be solved by the use of self-assembled biointegrated block polymers.

Prefunctionalized ligand-conjugated block copolymers offer precisely engineered nanocarriers for downstream therapeutic applications. Owing to single step NP preparation, the batch-to-batch variability is minimized thereby offering high reproducibility in these polymers. The ability to self-assemble provides straightforward preparation with the possibility to scale-up production. Quantitative control over ligand density can be achieved by simply varying the feed ratios of these polymers. Qualitatively, highly functionalized nanocarriers can be prepared by self-assembly of a ligand-conjugated copolymer only. Multifunctionality can be achieved by using two or more ligand-conjugated copolymers.

Developing advanced multifunctional biointegrated copolymers broadens the application potential of these materials. The synthesis approaches for these materials are well established, offering good control over molecular weights and polydispersity. These materials are characterized well before using them for self-assembly, offering better control of NP characteristics. Using blend of polymerization and conjugation chemistries results in polymers with desired functional features. Such an engineering approach allows for application of these nanocarriers for different types of payloads (e.g., gene delivery), besides its common application in drug delivery. Recently, the targeting ligands are engineered with stimuli-responsive linkers which allows deshielding of stealth PEG blocks after reaching a target and allowing the target ligand to interact with cell receptors for better uptake and internalization.

Despite these advantages, there are some associated problems when engineering these polymers which need to be addressed for their successful application. Due to end functionalization, the self-assembly of these block copolymers might be affected. This will lead to failure of materials for subsequent use and they need to be tested initially before moving further. Due to the biological nature of targeting ligand, it is likely to believe that the conjugated ligand might have reduced or lost bioactivity after conjugation. This problem should also be addressed before considering future prospects of these materials.

References

1. Langer, R. (1998). Drug delivery and targeting. *Nature-London*, 5–10.
2. Gu, F. X., Karnik, R., Wang, A. Z., Alexis, F., Levy-Nissenbaum, E., Hong, S., et al. (2007). Targeted nanoparticles for cancer therapy. *Nano Today, 2*(3), 14–21.
3. Alexis, F., Pridgen, E., Molnar, L. K., & Farokhzad, O. C. (2008). Factors affecting the clearance and biodistribution of polymeric nanoparticles. *Molecular Pharmaceutics, 5*(4), 505–515.
4. Gad, A., Kydd, J., Piel, B., & Rai, P. (2016). Targeting cancer using polymeric nanoparticle mediated combination chemotherapy. *International Journal of Nanomedicine and Nanosurgery, 2*(3).
5. Petros, R. A., & DeSimone, J. M. (2010). Strategies in the design of nanoparticles for therapeutic applications. *Nature Reviews Drug Discovery, 9*(8), 615.
6. Albanese, A., Tang, P. S., & Chan, W. C. (2012). The effect of nanoparticle size, shape, and surface chemistry on biological systems. *Annual Review of Biomedical Engineering, 14*, 1–16.

7. Yuan, H., & Zhang, S. (2010). Effects of particle size and ligand density on the kinetics of receptor-mediated endocytosis of nanoparticles. *Applied Physics Letters, 96*(3), 033704.
8. Verma, A., & Stellacci, F. (2010). Effect of surface properties on nanoparticle–cell interactions. *Small, 6*(1), 12–21.
9. Leamon, C. P., Cooper, S. R., & Hardee, G. E. (2003). Folate-liposome-mediated antisense oligodeoxynucleotide targeting to cancer cells: Evaluation in vitro and in vivo. *Bioconjugate Chemistry, 14*(4), 738–747.
10. Popielarski, S. R., Pun, S. H., & Davis, M. E. (2005). A nanoparticle-based model delivery system to guide the rational design of gene delivery to the liver. 1. Synthesis and characterization. *Bioconjugate Chemistry, 16*(5), 1063–1070.
11. Chiu, S.-J., Liu, S., Perrotti, D., Marcucci, G., & Lee, R. J. (2006). Efficient delivery of a Bcl-2-specific antisense oligodeoxyribonucleotide (G3139) via transferrin receptor-targeted liposomes. *Journal of Controlled Release, 112*(2), 199–207.
12. Farokhzad, O. C., Jon, S., Khademhosseini, A., Tran, T.-N. T., LaVan, D. A., & Langer, R. (2004). Nanoparticle-aptamer bioconjugates: A new approach for targeting prostate cancer cells. *Cancer Research, 64*(21), 7668–7672.
13. Sun, B., Ranganathan, B., & Feng, S.-S. (2008). Multifunctional poly (D, L-lactide-co-glycolide)/montmorillonite (PLGA/MMT) nanoparticles decorated by Trastuzumab for targeted chemotherapy of breast cancer. *Biomaterials, 29*(4), 475–486.
14. Farokhzad, O. C., Cheng, J., Teply, B. A., Sherifi, I., Jon, S., Kantoff, P. W., et al. (2006). Targeted nanoparticle-aptamer bioconjugates for cancer chemotherapy in vivo. *Proceedings of the National Academy of Sciences, 103*(16), 6315–6320.
15. Cheng, J., Teply, B. A., Sherifi, I., Sung, J., Luther, G., Gu, F. X., et al. (2007). Formulation of functionalized PLGA–PEG nanoparticles for in vivo targeted drug delivery. *Biomaterials, 28*(5), 869–876.
16. Kubowicz, S., Baussard, J. F., Lutz, J. F., Thünemann, A. F., von Berlepsch, H., & Laschewsky, A. (2005). Multicompartment micelles formed by self-assembly of linear ABC triblock copolymers in aqueous medium. *Angewandte Chemie International Edition, 44*(33), 5262–5265.
17. Li, G., Shi, L., Ma, R., An, Y., & Huang, N. (2006). Formation of complex micelles with double-responsive channels from self-assembly of two diblock copolymers. *Angewandte Chemie International Edition, 45*(30), 4959–4962.
18. Reynhout, I. C., Cornelissen, J. J., & Nolte, R. J. (2007). Self-assembled architectures from biohybrid triblock copolymers. *Journal of the American Chemical Society, 129*(8), 2327–2332.
19. Lee, H., Hu, M., Reilly, R. M., & Allen, C. (2007). Apoptotic epidermal growth factor (EGF)-conjugated block copolymer micelles as a nanotechnology platform for targeted combination therapy. *Molecular Pharmaceutics, 4*(5), 769–781.
20. Xu, Q., Liu, Y., Su, S., Li, W., Chen, C., & Wu, Y. (2012). Anti-tumor activity of paclitaxel through dual-targeting carrier of cyclic RGD and transferrin conjugated hyperbranched copolymer nanoparticles. *Biomaterials, 33*(5), 1627–1639.
21. Gu, F., Zhang, L., Teply, B. A., Mann, N., Wang, A., Radovic-Moreno, A. F., et al. (2008). Precise engineering of targeted nanoparticles by using self-assembled biointegrated block copolymers. *Proceedings of the National Academy of Sciences, 105*(7), 2586–2591.
22. Xu, W., Siddiqui, I. A., Nihal, M., Pilla, S., Rosenthal, K., Mukhtar, H., et al. (2013). Aptamer-conjugated and doxorubicin-loaded unimolecular micelles for targeted therapy of prostate cancer. *Biomaterials, 34*(21), 5244–5253.
23. Zhan, C., Gu, B., Xie, C., Li, J., Liu, Y., & Lu, W. (2010). Cyclic RGD conjugated poly (ethylene glycol)-co-poly (lactic acid) micelle enhances paclitaxel anti-glioblastoma effect. *Journal of Controlled Release, 143*(1), 136–142.
24. Jiang, X., Sha, X., Xin, H., Chen, L., Gao, X., Wang, X., et al. (2011). Self-aggregated pegylated poly (trimethylene carbonate) nanoparticles decorated with c(RGDyK) peptide for targeted paclitaxel delivery to integrin-rich tumors. *Biomaterials, 32*(35), 9457–9469.
25. Zhou, D., Zhang, G., & Gan, Z. (2013). C(RGDfK) decorated micellar drug delivery system for intravesical instilled chemotherapy of superficial bladder cancer. *Journal of Controlled Release, 169*(3), 204–210.

26. Christie, R. J., Matsumoto, Y., Miyata, K., Nomoto, T., Fukushima, S., Osada, K., et al. (2012). Targeted polymeric micelles for siRNA treatment of experimental cancer by intravenous injection. *ACS Nano, 6*(6), 5174–5189.
27. Zhang, Y., Zhang, H., Wang, X., Wang, J., Zhang, X., & Zhang, Q. (2012). The eradication of breast cancer and cancer stem cells using octreotide modified paclitaxel active targeting micelles and salinomycin passive targeting micelles. *Biomaterials, 33*(2), 679–691.
28. Wu, X. L., Kim, J. H., Koo, H., Bae, S. M., Shin, H., Kim, M. S., et al. (2010). Tumor-targeting peptide conjugated pH-responsive micelles as a potential drug carrier for Cancer therapy. *Bioconjugate Chemistry, 21*(2), 208–213.
29. Liu, P., Qin, L., Wang, Q., Sun, Y., Zhu, M., Shen, M., et al. (2012). cRGD-functionalized mPEG-PLGA-PLL nanoparticles for imaging and therapy of breast cancer. *Biomaterials, 33*(28), 6739–6747.
30. Sun, C.-Y., Shen, S., Xu, C.-F., Li, H.-J., Liu, Y., Cao, Z.-T., et al. (2015). Tumor acidity-sensitive polymeric vector for active targeted siRNA delivery. *Journal of the American Chemical Society, 137*(48), 15217–15224.
31. Huang, C. K., Lo, C. L., Chen, H. H., & Hsiue, G. H. (2007). Multifunctional micelles for cancer cell targeting, distribution imaging, and anticancer drug delivery. *Advanced Functional Materials, 17*(14), 2291–2297.
32. Zhong, Y., Yang, W., Sun, H., Cheng, R., Meng, F., Deng, C., et al. (2013). Ligand-directed reduction-sensitive shell-sheddable biodegradable micelles actively deliver doxorubicin into the nuclei of target cancer cells. *Biomacromolecules, 14*(10), 3723–3730.
33. Yang, R., Meng, F., Ma, S., Huang, F., Liu, H., & Zhong, Z. (2011). Galactose-decorated cross-linked biodegradable poly (ethylene glycol)-b-poly (ε-caprolactone) block copolymer micelles for enhanced hepatoma-targeting delivery of paclitaxel. *Biomacromolecules, 12*(8), 3047–3055.
34. Tao, Y., He, J., Zhang, M., Hao, Y., Liu, J., & Ni, P. (2014). Galactosylated biodegradable poly(ε-caprolactone-co-phosphoester) random copolymer nanoparticles for potent hepatoma-targeting delivery of doxorubicin. *Polymer Chemistry, 5*(10), 3443–3452.
35. Lee, H., Lee, K., & Park, T. G. (2008). Hyaluronic acid-paclitaxel conjugate micelles: Synthesis, characterization, and antitumor activity. *Bioconjugate Chemistry, 19*(6), 1319–1325.
36. Yadav, A. K., Mishra, P., Mishra, A. K., Mishra, P., Jain, S., & Agrawal, G. P. (2007). Development and characterization of hyaluronic acid–anchored PLGA nanoparticulate carriers of doxorubicin. *Nanomedicine: Nanotechnology, Biology and Medicine, 3*(4), 246–257.
37. Li, J., Huo, M., Wang, J., Zhou, J., Mohammad, J. M., Zhang, Y., et al. (2012). Redox-sensitive micelles self-assembled from amphiphilic hyaluronic acid-deoxycholic acid conjugates for targeted intracellular delivery of paclitaxel. *Biomaterials, 33*(7), 2310–2320.
38. Yoo, H. S., & Park, T. G. (2004). Folate receptor targeted biodegradable polymeric doxorubicin micelles. *Journal of Controlled Release, 96*(2), 273–283.
39. Yang, X., Chen, Y., Yuan, R., Chen, G., Blanco, E., Gao, J., et al. (2008). Folate-encoded and Fe3O4-loaded polymeric micelles for dual targeting of cancer cells. *Polymer, 49*(16), 3477–3485.
40. Wang, W., Cheng, D., Gong, F., Miao, X., & Shuai, X. (2012). Design of multifunctional micelle for tumor-targeted intracellular drug release and fluorescent imaging. *Advanced Materials, 24*(1), 115–120.
41. Guo, X., Shi, C., Wang, J., Di, S., & Zhou, S. (2013). pH-triggered intracellular release from actively targeting polymer micelles. *Biomaterials, 34*(18), 4544–4554.
42. Hu, J., He, J., Cao, D., Zhang, M., & Ni, P. (2015). Core cross-linked polyphosphoester micelles with folate-targeted and acid-cleavable features for pH-triggered drug delivery. *Polymer Chemistry, 6*(17), 3205–3216.
43. Li, H., Miteva, M., Kirkbride, K. C., Cheng, M. J., Nelson, C. E., Simpson, E. M., et al. (2015). Dual MMP7-proximity-activated and folate receptor-targeted nanoparticles for siRNA delivery. *Biomacromolecules, 16*(1), 192–201.
44. Patil, Y., Sadhukha, T., Ma, L., & Panyam, J. (2009). Nanoparticle-mediated simultaneous and targeted delivery of paclitaxel and tariquidar overcomes tumor drug resistance. *Journal of Controlled Release, 136*(1), 21–29.

45. Patil, Y. B., Swaminathan, S. K., Sadhukha, T., Ma, L., & Panyam, J. (2010). The use of nanoparticle-mediated targeted gene silencing and drug delivery to overcome tumor drug resistance. *Biomaterials, 31*(2), 358–365.
46. Hrkach, J., Von Hoff, D., Ali, M. M., Andrianova, E., Auer, J., Campbell, T., et al. (2012). Preclinical development and clinical translation of a PSMA-targeted docetaxel nanoparticle with a differentiated pharmacological profile. *Science Translational Medicine, 4*(128), 128ra39–128ra39.
47. Prosperi-Porta, G., Kedzior, S., Muirhead, B., & Sheardown, H. (2016). Phenylboronic-acid-based polymeric micelles for Mucoadhesive anterior segment ocular drug delivery. *Biomacromolecules, 17*(4), 1449–1457.
48. Feng, H., Lu, X., Wang, W., Kang, N.-G., & Mays, J. W. (2017). Block copolymers: Synthesis, self-assembly, and applications. *Polymers, 9*(10), 494.
49. Hawker, C. J., & Wooley, K. L. (2005). The convergence of synthetic organic and polymer chemistries. *Science, 309*(5738), 1200–1205.
50. Matyjaszewski, K., & Spanswick, J. (2005). Controlled/living radical polymerization. *Materials Today, 8*(3), 26–33.
51. Egli, S., Schlaad, H., Bruns, N., & Meier, W. (2011). Functionalization of block copolymer vesicle surfaces. *Polymers, 3*(1), 252–280.
52. Jérôme, C., & Lecomte, P. (2008). Recent advances in the synthesis of aliphatic polyesters by ring-opening polymerization. *Advanced Drug Delivery Reviews, 60*(9), 1056–1076.
53. Kim, J. K., Yang, S. Y., Lee, Y., & Kim, Y. (2010). Functional nanomaterials based on block copolymer self-assembly. *Progress in Polymer Science, 35*(11), 1325–1349.
54. Zhang, G., Zhang, M., He, J., & Ni, P. (2013). Synthesis and characterization of a new multifunctional polymeric prodrug paclitaxel-polyphosphoester-folic acid for targeted drug delivery. *Polymer Chemistry, 4*(16), 4515–4525.
55. Matsumoto, A., Sato, N., Kataoka, K., & Miyahara, Y. (2009). Noninvasive sialic acid detection at cell membrane by using phenylboronic acid modified self-assembled monolayer gold electrode. *Journal of the American Chemical Society, 131*(34), 12022–12023.
56. Liu, S., Jones, L., & Gu, F. X. (2012). Development of Mucoadhesive drug delivery system using Phenylboronic acid functionalized poly (D, L-lactide)-b-dextran nanoparticles. *Macromolecular Bioscience, 12*(12), 1622–1626.

Chapter 24
Application of Nanoparticles in Treating Periodontitis: Preclinical and Clinical Overview

Nathalia Mercado, Priyanka Bhatt, Vijaykumar Sutariya, Fernando Luis Esteban Florez, and Yashwant V Pathak

Abstract Periodontitis involves infection of the gums and other structures such as the periodontal ligaments, and the alveolar bone. The disease results in the formation of periodontal pockets and bone loss, and the tooth may fall out. Nanoparticles have gained a lot of use with various dental applications due to their unique properties that make them suitable for drug delivery. Due to the small size of nanoparticles, they are able to deliver the drug to particular tissues, cells or pathogens in the periodontal pockets. In addition, they display antimicrobial activity by destroying bacterial cell membranes and killing the bacteria. There are several different nanomaterials that are used to treat periodontal disease, such as liposomes, lipid and polymeric nanoparticles, and dendrimers. There has also been development of delivery systems for drugs, proteins, and cells. These include nanocapsules, nanoscaffolds, nanocoatings, and nanoshells. This chapter reviews the use of nanotechnology applications in dentistry and the different types of nanoparticles.

Keywords Chitosan nanoparticle · Drug delivery · Metal nanoparticles Antimicrobial

N. Mercado · P. Bhatt · V. Sutariya
College of Pharmacy, University of South Florida, Tampa, FL, USA

F. L. E. Florez
Division of Dental Biomaterials, Department of Restorative Sciences, University of Oklahoma College of Dentistry, Joint Appointment OUHSC Graduate College, 1201 N. Stonewall Ave., Suite 146B, Oklahoma City, OK 73117, USA

Y. V Pathak (✉)
College of Pharmacy, University of South Florida, Tampa, FL, USA

Adjunct professor at Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia
e-mail: ypathak1@health.usf.edu

Y. V Pathak (ed.), *Surface Modification of Nanoparticles for Targeted Drug Delivery*, https://doi.org/10.1007/978-3-030-06115-9_24

1 Introduction

Owing to the restrictions in dental instruments, nanoparticles have been used to prevent, diagnose, and treat various oral conditions. Additionally, an increase in antibiotic resistance has also led the medical field to use nanoparticles as an alternative. Nanoparticles have a unique combination of properties that make them suitable for various dental applications. They have a high surface area to volume ratio due to their small size, making them easy to manipulate and control on the atomic/molecular scale. They have an enhanced solubility, a quicker onset of therapeutic action and an increased rate of dissolution. Nanoparticles can also be used as drug delivery and ions agents [1]. In addition, they are able to interact closely with microbial membranes in order to treat dental caries and periodontal disease. Metal nanoparticles, such as copper, zinc, titanium, magnesium, silver, and gold, display antimicrobial properties. They can destroy the bacterial cell membrane and kill the bacteria. The antimicrobial activity of nanoparticles correlates with the particle size. Decreasing the size of the nanoparticle is beneficial because it increases their surface area, and allows for a greater contact and interactions with the surface and membranes of pathogenic microorganisms [2]. Particles ranging from 1 to 10 nm in size have shown to have a greater antimicrobial activity than larger particles [3]. Nanoparticles have also been combined with polymers for a variety of potential antimicrobial applications within the oral cavity. Different types of nanoparticles, such as chitosan, polymeric, quantum dot, liposomes, and metal nanoparticles, offer prominent features in the use of dental applications.

2 Periodontitis

Periodontal disease, also known as gum disease, is an infection that damages the soft tissue and structures around the teeth. These structures include the gums, the alveolar bone, and the periodontal ligament. The infection begins when bacteria stick to the surface of the tooth, which leads to the accumulation of bacterial plaque around the tooth. One way that it can lead to periodontitis is that the plaque can harden and form into tartar, or calculus. Plaque can also cause gingivitis, which is inflammation and irritation of the gums. Constant inflammation of the gums can eventually cause pockets to develop between your gums and teeth, which then fill with more bacteria and plaque. Without treatment, one is at high risk of tooth and tissue loss. Red, swollen, or tender gums that bleed easily; painful chewing; loose or shifting of teeth; receding gums; sensitive teeth or gums; and bad breath are major signs and symptoms of periodontitis. Several procedures have been used to treat periodontitis, such as bone grafting, scaling and root planing, use of bone substitutes, and guided tissue regeneration [4]. However, dental materials, instruments, and procedures are not very efficient or precise in targeting microbes; they lack the ability to reach the deep pockets. Nano drug delivery systems offer a more advanced drug delivery approach against periodontitis and are effective against resistant pathogens. Due to their small size they are able to reach sites that are not accessible

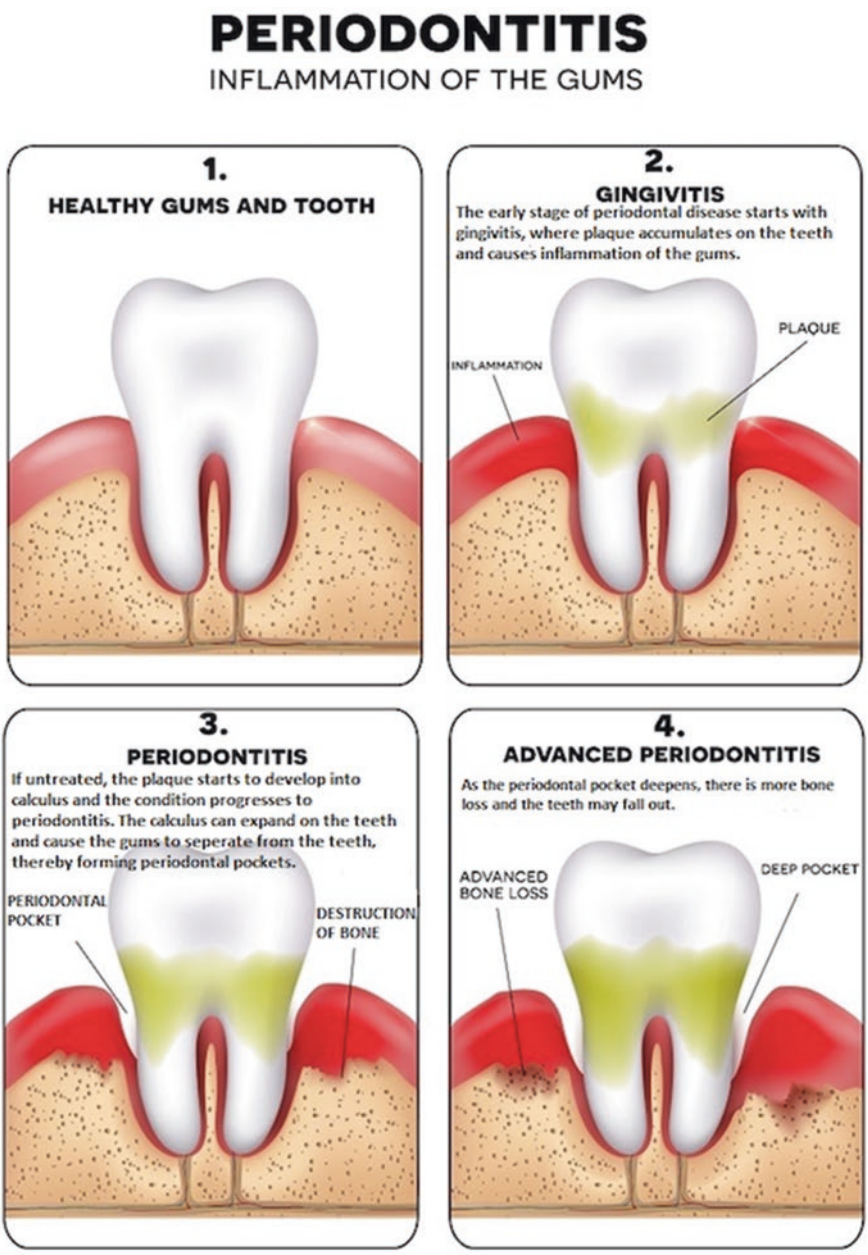


Fig. 24.1 Stages of periodontitis

to other devices, such as periodontal pockets. Additionally, nanoparticles offer a high stability and a controlled release rate. The drug dissolved in the nanoparticle matrix also has the advantage that it does not require a high dosage frequency since the drug is distributed for an extended period of time (Fig. 24.1).

3 Applications of Nanoparticles in Dentistry

The antibacterial activity of nanoparticles is directly proportional to the release of ions. With that said, the greater the concentration of nanoparticles, the more ions that can be released, and the higher toxicity to the bacterial cells. Other mechanisms also exist in the destruction of nanoparticles. One mechanism is through the binding of electrostatic forces. Once the nanoparticle binds to the bacterial cell membrane, it causes alterations in bacterial cell functions that subsequently lead to bacterial cell death [5]. The second mechanism involves the production of oxygen free-radicals that can hinder protein function and result in excess radical production (Fig. 24.2).

3.1 In Diagnosis

The use of nanodevices provides higher accuracy when performing diagnostic tests in the mouth by marking specific bacteria. Once marked, the bacteria can be easily identified and removed. There are several different tools that help detect and diagnose oral conditions. Cantilevers are tools that can attach to carcinogenic molecules, or altered DNA sequences. Nanopores can also detect carcinogenic molecules. Nanotubes can be used for analyzing dentin collagen network, dentin pores and their effects on tooth hypersensitivity, the surface of dental implants, and colonies formed on tooth surfaces. Nanowire sensors were developed to detect proteins or viruses in saliva samples [6]. Nanosystems, such as the oral fluid nanosensor test,

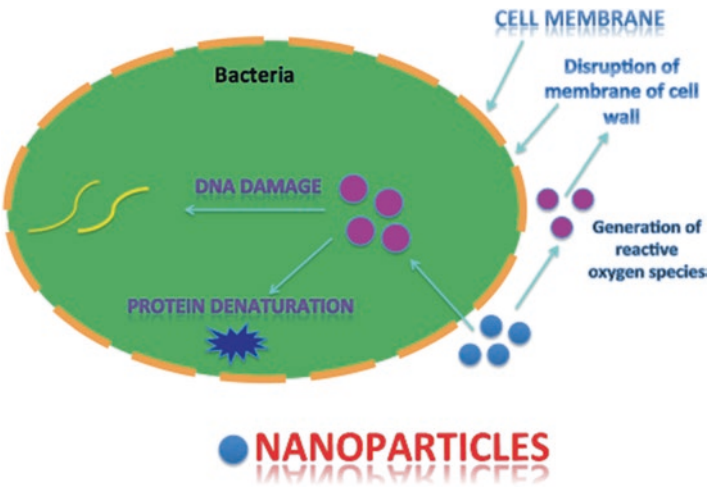


Fig. 24.2 Mechanism of interaction of nanoparticle with bacterial cell

are effective in detection of salivary proteomic biomarkers and nucleic acids specific for oral cancer. Several other tests, such as the oral fluid nanosensor test and the optical nanobiosensor test, are also useful for diagnosis of oral cancer.

3.2 In Treatment

The use of nanomaterials has allowed new treatment opportunities to arise in dentistry. These opportunities include local anesthesia, dentition renaturalization, a cure for hypersensitivity, orthodontic realignment in just one visit to the office, and more [4].

Several nanoparticles, such as zinc oxide and silver, are being used to inhibit bacterial growth by incorporating the nanoparticles into dental composites or dental adhesives [7]. For example, resin composites containing silver ion-implanted fillers have shown to be effective against oral streptococci [8]. Due to their large surface-to-volume ratio, they have better interaction with the bacterial cell membrane, thereby destroying it. Other ways they can inhibit bacterial growth is by blocking the transport and metabolism of sugars, by producing reactive oxygen species, inhibiting the electron transportation across the bacterial membrane, and blocking DNA replication [2].

Some nanodevices can be used for both diagnostic and therapeutic purposes. Dendrimers, for example, are synthetic polymers that have the ability to simultaneously carry one molecule for the removal of cancer cells in the oral cavity and one molecule for diagnosis. Research has also proven that solutions of chlorhexidine mixed with hexametaphosphate (HMP) nanoparticles are significantly effective in inhibiting fungal infestation by blocking the metabolic activity of *Candida albicans* [9].

For orthodontic treatment, brackets can be coated with CuO (copper oxide) and ZnO (zinc oxide) nanoparticles in order to inhibit the growth of *S. mutans* [10]. Coating orthodontic wires with inactive fullerene-like tungsten disulfide nanoparticles can reduce friction that may be caused when sliding a tooth along an arch wire [11]. When straightening or rotating a tooth, orthodontic nanorobots can be used for a painless correction of misaligned teeth [12].

Dental implants have failed due to a lack of bone formation around the implant. However, there have been improvements by the addition of nanoscale deposits of hydroxyapatite crystals and calcium phosphate, which has created a better implant surface for osteoblast formation [13].

When administering local anesthesia, nanotechnology solution can be used to administer anesthesia without pain. This method works by filling the patient's gingiva with micron-sized analgesic dental robots. The nanorobots would reach the dentin and move toward the pulp, and once they have reached the pulp, the nanorobots would block all sensation in the tooth [2].

Nanorobots have also been incorporated in toothpastes and mouthwashes in order to prevent the accumulation of calculus (Tables 24.1, 24.2 and 24.3).

Table 24.1 Delivery systems used for drugs, proteins, and cells [4]

Delivery systems	Description
Nanocapsules	Encapsulation of drugs into nanoparticle shells allows a sustained release of drugs once the capsule disintegrates at specific locations
Nanoscaffolds	These materials also deliver drugs to the target sites. They have the advantage of disintegrating without triggering an immune response in the body. They are used in dentistry for, regeneration of the alveolar bone, periodontal ligament, dental pulp, and enamel
Nanocoatings	These materials can be used to combat endodontic and periodontal diseases that are caused by pathogenic microorganisms. They may be used in combination with other treatments to lower the sub gingival microbial count
Nanoshells	When infrared light is applied to these materials, they produce heat and can destroy the bacteria, cancer cells, and ligature of the vessels. They can be loaded with antibodies, or other proteins and used for drug delivery

Table 24.2 Applications of nanoparticles in dentistry

Antimicrobial nanoparticles	Therapeutic nanodevices
Chitosan	Nanopores
Copper based nanoparticle-metal nanoparticle	Dendrimers
Zinc based nanoparticle-metal nanoparticle	Nanotubes
Silver nanoparticle-metal nanoparticle	Nanoshells
Titanium oxide-metal nanoparticle	Quantum dots

Table 24.3 Incorporation of nanoparticles into dental materials

Nanoparticles	Dental material
SiO2, ZrO2-SiO2	Resin-based composites
ZnO	Cements
Ag-nanoparticles	Adhesives
Apatite and Ti nanoparticles	On dental implants

4 Types of Nanoparticles

4.1 Chitosan Nanoparticles

Chitosan is the second most abundant biopolymer that can be obtained through partial deacetylation of chitin. It is composed of glucosamine units and *N*-acetylglucosamine. Chitosan exhibits antibacterial properties due to its positive charge, making it an ideal candidate for use in root canal treatments. It has various unique properties for drug delivery applications, such as mucus adhesion, hydrophilicity, biocompatibility, biodegradability, healing wounds quickly and a broad antibacterial spectrum. Owing to the many favorable properties of chitosan, it has been used as a suitable material in dental applications.

4.2 Polymeric Nanoparticles

Poly(lactic-co-glycolic acid) is copolymer of poly-lactic acid (PLA) and poly-glycolic acid (PGA). In the presence of water, PLGA undergoes hydrolysis of its ester linkages, resulting in the monomers lactic acid and glycolic acid, which are by-products of different metabolic pathways in the body [14]. The production of PLGA nanoparticles has been beneficial in various fields of dentistry, such as endodontic therapy, dental caries, dental surgery, dental implants, or periodontology. For periodontal treatment, PLGA implants, disks, and dental films are beneficial in local administration of antibiotics. They also reduce the systemic side effects of general antibiotic delivery [15].

4.3 Quantum Dot

Quantum dots are made of semiconductor materials with fluorescent properties and can be used as photosensitizers and carriers. Quantum dots are beneficial in diagnosing oral cancer by, binding to cancer cells. Once they bind, UV light is radiated to them and starts UV light emission, making it easier to detect oral cancers. They can also be used as drug or gene carriers. Thus, quantum dots are useful in prevention of oral cancer.

4.4 Metal Nanoparticles

Metal nanoparticles have large surface area to volume ratios and good antibacterial properties, thereby making them ideal candidates in deterring growth of various microorganisms. Metal nanoparticles are beneficial because they can act on a wide range of microorganisms. For example, silver nanoparticles have a greater affinity for gram-negative and anaerobic bacteria. Various others metal nanoparticles, such as copper oxide, also displays unique properties that make them suitable in the use of dental applications.

4.5 Silver Nanoparticles

Silver was incorporated into dental composites in order to introduce antimicrobial properties and improve biocompatibility of the composites [16]. Silver is very favorable because it displays a strong antibacterial activity and it has anti-inflammatory effects. Due to its large surface area, the silver nanoparticles have better contact with microorganisms, thereby making their antimicrobial activity more efficient.

Silver is very reactive in its ionized form. The silver ions interact with the peptidoglycan cell wall, the plasma membrane, bacterial DNA, and bacterial proteins [16]. That interaction ultimately causes structural changes in bacterial cells, which leads to cell death.

4.6 Copper Oxide Nanoparticles

Copper nanoparticles have antibacterial activity, along with antifungal activity and are beneficial in controlling biofilm formation within the oral cavity. It has been demonstrated that copper oxide nanoparticles can be beneficial in dental coating to inhibit dental infections. Researchers reported that copper oxide nanoparticles inhibit bacterial growth by the process of passing through nanometric pores on the cellular membranes of most bacteria. Their size, stability, and concentration influence the bactericidal activity of the copper oxide nanoparticles.

4.7 Liposomes

Liposomes are lipid-based nanoparticles composed of amphipathic phospholipids that are made up mainly of phosphatidylcholines. Liposomes can be categorized into cationic, anionic, and neutral nanoparticles. They have unique advantages, such as nontoxic, nonimmunogenic nature, protecting drugs or siRNA-based therapeutic agents from degradation, and offering passive and active delivery of genes, proteins, peptides, and various other substances [17], [18]. Additionally, they can increase the half-life of various therapeutic agents. Liposomes are widely used to encapsulate drugs for use against various periodontal pathogens.

4.8 Solid Lipids

Solid lipid nanoparticles have some excellent properties, such as a high drug loading capacity, a large surface area, and immediate biodegradability. Solid lipid nanoparticles are made from physiological lipids, thereby diminishing the danger of toxicity [19].

4.9 Hydrogels

Hydrogels are natural or synthetic polymers with various favorable properties. These properties include a high water-absorbing capacity, ability to mimic natural living tissue, and a high porosity. The porous structure of hydrogels makes them suitable for carrying and releasing drugs (Fig. 24.3 and Table 24.4).

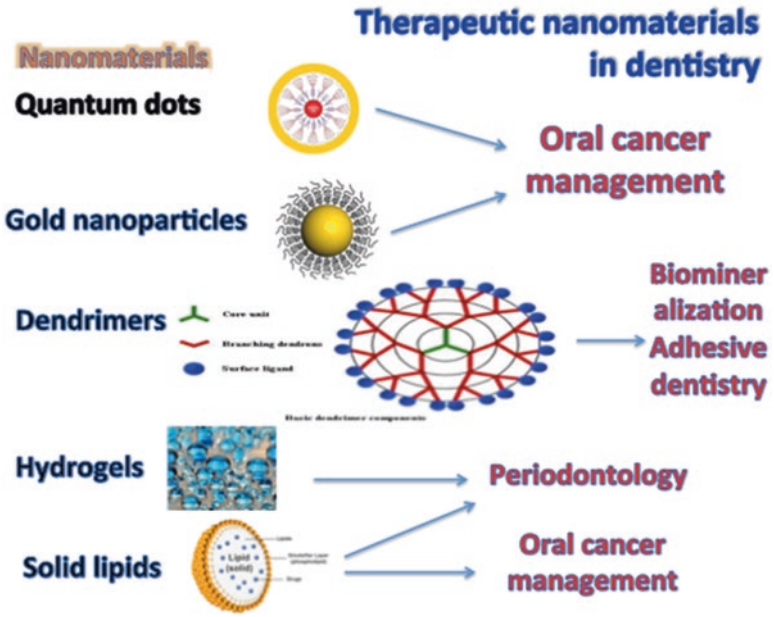


Fig. 24.3 Various types of nanoparticles and their use in dentistry

Table 24.4 Nanoparticles in dentistry

Nanoparticles	Drug	Dental application	Description	References
Chitosan	Triclosan	Local drug delivery for treatment of periodontal diseases	Local therapeutic application in periodontal diseases	[20]
PAA-poly(acrylic acid)	Penicillin		Antibacterial activity against penicillin-resistant bacteria is enhanced	[21]
PLGA	Nanohydroxyapatite and nanocollagen	To treat periodontal disease	Bone regeneration and tissue collagen regeneration	[21]
Chitosan	Silver nanoparticles		Used for infected areas, such as open wounds	[21]
Chitosan	Metronidazole	Local drug delivery for treatment of periodontal diseases	Effective against anaerobic organisms by disrupting bacterial DNA synthesis	[21]
PLGA	Methylene blue	Antimicrobial endodontic treatment	Display significant killing of *Enterococcus faecalis* biofilm species that infect root canals	[22]
PLGA	Minocycline	Drug delivery for treatment of periodontitis	To improve drug loading and treat periodontitis	[15]

5 Properties of Chitosan Nanoparticles

5.1 Antimicrobial Properties

Chitosan nanoparticles have antimicrobial activity against gram-negative and gram-positive bacteria. The molecular weight of chitosan influences its solubility and antibacterial activity. In small molecular weight, chitosan nanoparticles are successful in hindering the colonization of bacteria (such as *Streptococcus mutans*) on the surface of teeth while still preserving the normal oral flora. Chitosan nanoparticles of small molecular weight are able to damage the physiological activities of bacteria by penetrating the bacteria. In contrast, chitosan nanoparticles of high molecular weight have the ability of blocking the entry of nutrients by forming a film around the bacterial cells. Chitosan possesses a positive charge that interacts with the anionic groups on the bacterial cell wall, thereby causing damage to the cell wall. That interaction blocks the exchange of nutrients between the bacterial cell and the extracellular matrix. The electrostatic charges compete for calcium for specific sites in the membrane, which then leads to the leakage of cell contents and cell death. Chitosan is effective in controlling plaque in vitro by obstructing dental plaque pathogens such as *Actinobacillus actinomycetemcomitans, P. gingivalis* and *S. mutans*.

There are four main factors that influence the antimicrobial action of chitosan [23]:

- The microbial species.
- The molecular weight of chitosan, the density of the positive charge, and the hydrophobicity and hydrophilicity of chitosan.
- The soluble and solid state.
- Environmental factors such as pH, ionic forces, temperature, and time.

5.2 Mucoadhesive Properties

Chitosan mucoadhesion is due to the interaction between the positive charge of chitosan and the negative charge of mucous membranes. That interaction increases the adhesion to the mucosa and the time of contact for penetration of drug molecules through it. It also provides a controlled rate drug release in order to improve therapy. Additional advantages of using mucoadhesive chitosan drug delivery systems are reduced administration frequencies and a constant mucoadhesiveness. Other possible factors that play a role in chitosan mucoadhesion are, its wettability, entanglement, Van der Waal's forces, hydrogen bonding, and hydrophobic interactions.

5.3 Drug Delivery Properties

Chitosan nanoparticles can be loaded with various antibiotics, such as metronidazole, chlorhexidine, and nystatin, to distribute to periodontal tissues and treat against fungal infections and oral mucositis. Due to their high surface area and reactivity, the drug is released with more ease. Chitosan is an efficient drug delivery system due to its stability, solubility, and bioavailability, and its ability to deliver the drug to a specific site. Another advantage of using chitosan as a drug carrier is its easy elimination by metabolic degradation in the body. However, this is only applicable for chitosan nanoparticles of small molecular weight. Enzymes must degrade the large molecular weight chitosan nanoparticles. There are various mechanisms for releasing the drugs. The loaded drug can be released from chitosan by diffusion, erosion, or degradation of the chitosan nanoparticle.

5.4 Dental Applications of Chitosan Materials

Chitosan possesses unique properties that have enabled it to emerge as a material for a wide range of dental applications. Due to its nontoxicity, biodegradability, hydrophilicity, biocompatibility, and compatibility to mix with other materials, Chitosan is a favorable material for periodontal tissue regeneration because it can deliver the drug to the periodontal pocket and sustain and/or control the concentration of the drug. It has also been beneficial in modifications of dentifrices, enamel repair, adhesion and dentine bonding; for coating dental implants; and for modification of dental restorative materials [24]. Due to its ability to support viable osteoblasts, chitosan can be used as a template for bone defect restorations [25]. Furthermore, chitosan nanoparticles have been incorporated into root canal sealers in order to inhibit the penetration of microbes and to reduce biofilm formation at the dentin–root filling interface [26].

Chitosan has also been combined with various polymeric and organic substances for bone regeneration purposes. For example, chitosan–gold nanoparticles have shown to enhance osseointegration of dental implants [26]. Chitosan–gold nanoparticles conjugated with PPAR have also been used to modify dental implants, and this has led to the formation of new bone with improved mineral density and a reduction in inflammation.

In prosthetic dentistry, chitosan has also been utilized to modify glass ionomer restoratives. Other uses include antibacterial activity of dental adhesive, and antibacterial activity of composites [23].

In endodontics, chitosan has several useful applications. In vitro, chitosan was shown to improve the stability of dentin collagen, provide a sustained release of calcium ions in the root canal system, and regulate stem cell differentiation from apical papilla [23] (Table 24.5).

Table 24.5 Results of clinical study

Clinical trial phase	Treatment	Indication	Patients in trials	Response in trials and clinical status	References
IV	Scaling and systemic moxifloxacin	Aggressive periodontitis	40	Completed	NCT02125812
IV	Combination of metronidazole and amoxicillin in type 2 diabetes patients with periodontitis	Periodontitis	58	Completed	NCT02135952
II and III	Compare the efficacy of locally delivery of 1% metformin (MF), 1.2% simvastatin (SMV)	Chronic periodontitis	98	Greater pocket probing reduction (PPD) and clinical attachment level (CAL) in SMV group than MF	NCT02372656
I and II	Gingiva mesenchymal stem cell therapy	Periodontitis	30	Completed	NCT03137979
I and II	*Effectiveness of probiotic Saccharomyces boulardii*	Chronic periodontitis	31	Completed	NCT03516370
II	Effectiveness of azithromycin	Chronic periodontitis	80	Completed	NCT01921738
N/A	Evaluation of nanocrystalline hydroxyapatite silica gel	Chronic periodontitis	30	Recruiting	NCT02507596
N/A	Tooth brushing with dentifrice, and turmeric massaging	Chronic periodontitis	91	Completed	NCT02890771
II	*Nigella sativa* (Kalonji) oil	Chronic periodontitis	25	Not yet recruiting	NCT03270280
N/A	Drug delivery of *Garcinia mangostana* gel	Chronic periodontitis	50	Completed	NCT02880397

6 Future Perspectives of Nanoparticles

With nanoparticles gaining more and more popularity, it is expected to find its use in all the specializations of dentistry. In the future, there will be improvements in the diagnosis and treatment of oral cancers, in the production of nanorobotic dentifrices delivered by toothpaste or mouthwash, local anesthesia, in hypersensitivity cure and complete orthodontic realignments [27]. Nanotweezers are currently under development for the future, which can be used for cell surgery.

In future, it is believed that all dental procedures will be performed using nanorobots.

7 Conclusion

The emergence of nanotechnology in the dental field has improved dental materials and devices for oral health care. Different nanoparticles are used to prevent the attachment of biofilm to the tooth surface, which is the main cause for the development of plaque and calculus. Nanomaterials are also used in dental fillings, and as implant materials. Nanoparticles have excellent properties and some display antimicrobial properties that allow it to interact with bacteria and kill it. Nanotechnology is quickly evolving and there will be more improvements in the near future.

References

1. Camacho-Flores, B. A., Martínez-Álvarez, O., et al. (2015). Copper: Synthesis techniques in Nanoscale and powerful application as an antimicrobial agent. *Journal of Nanomaterials, 2015*, 10.
2. Bhardwaj, A., Bhardwaj, A., Misuriya, A., Maroli, S., Manjula, S., & Singh, A. K. (2014, February 26). Nanotechnology in dentistry: Present and future. *Journal of International Oral Health: JIOH, 6*(1), 121–126. PubMed PMID: PMC3959150. Received October 10, Accepted December 11.
3. Sánchez-Sanhueza, G., Fuentes-Rodríguez, D., & Bello-Toledo, H. (2016). Copper nanoparticles as potential antimicrobial agent in disinfecting root canals: A systematic review. *International Journal of Odontostomatology, 10*, 547–554.
4. Abiodun-Solanke, I. M. F., Ajayi, D. M., & Arigbede, A. O.. (2014, September–October). Nanotechnology and its application in dentistry. *Annals of Medical and Health Sciences Research, 4*(Suppl. 3), S171–S177. PubMed PMID: PMC4212373.
5. Ibrahim, A. I. O., Moodley, D. S., Petrik, L., & Patel, N. (2017). Use of antibacterial nanoparticles in Endodontics. *South African Dental Journal, 72*, 105–112.
6. Mitra, S. B., Wu, D., & Holmes, B. N. (2003, October 1). An application of nanotechnology in advanced dental materials. *The Journal of the American Dental Association, 134*(10), 1382–1390.
7. Abou Neel, E. A., Bozec, L., Perez, R. A., Kim, H. W., & Knowles, J. C. (2015). Nanotechnology in dentistry: Prevention, diagnosis, and therapy. *International Journal of Nanomedicine, 10*:6371–6394. PubMed PMID: 26504385. Pubmed Central PMCID: PMC4605240. Epub 2015/10/28. eng.
8. AlKahtani, R. N. (2018, April 1). The implications and applications of nanotechnology in dentistry: A review. *The Saudi Dental Journal, 30*(2), 107–116.
9. Schmalz, G., Hickel, R., van Landuyt, K. L., & Reichl, F.-X. (2017, November 11). Nanoparticles in dentistry. *Dental Materials, 33*(11), 1298–1314.
10. Wang, L., Hu, C., & Shao, L. (2017). The antimicrobial activity of nanoparticles: Present situation and prospects for the future. *International Journal of Nanomedicine, 12*, 1227–1249. PubMed PMID: 28243086. Pubmed Central PMCID: PMC5317269. Epub 2017/03/01. eng.
11. Sivaramakrishnan, S. M., & Neelakantan, P. (2014). Nanotechnology in dentistry—What does the future hold in store? *Dentistry, 4*, 198. https://doi.org/10.4172/2161-11221000198.
12. Shetty, N. J., Swati, P., & David, K. (2013, January 17). Nanorobots: Future in dentistry. *The Saudi Dental Journal, 25*(2), 49–52. PubMed PMID: PMC3723292. Received August 9, Revised November 20, Accepted December 17.
13. Verma, S. K., & Chauhan, R. (2014, August 1). Nanorobotics in dentistry—A review. *Indian Journal of Dentistry, 5*, 62–70.

14. Patel, J., Amrutiya, J., Bhatt, P., Javia, A., Jain, M., & Misra, A. (2018, March). Targeted delivery of monoclonal antibody conjugated docetaxel loaded PLGA nanoparticles into EGFR overexpressed lung tumour cells. *Journal of Microencapsulation, 35*(2), 204–217. PubMed PMID: 29542378. Epub 2018/03/16.eng.
15. Kashi, T. S., Eskandarion, S., Esfandyari-Manesh, M., Marashi, S. M., Samadi, N., Fatemi, S. M., et al. (2012). Improved drug loading and antibacterial activity of minocycline-loaded PLGA nanoparticles prepared by solid/oil/water ion pairing method. *International Journal of Nanomedicine, 7*, 221–234. PubMed PMID: 22275837. Pubmed Central PMCID: PMC3263414. Epub 2012/01/26.eng.
16. Mohamed, H. I. (2012, May). Current perspectives of nanoparticles in medical and dental biomaterials. *Journal of Biomedical Research, 26*(3), 143–151. PubMed PMID: 23554743. Pubmed Central PMCID: PMC3596063. Epub 2013/04/05.eng.
17. Bhatt, P., Lalani, R., Vhora, I., Patil, S., Amrutiya, J., Misra, A., et al. (2018, January 30). Liposomes encapsulating native and cyclodextrin enclosed paclitaxel: Enhanced loading efficiency and its pharmacokinetic evaluation. *International Journal of Pharmaceutics, 536*(1), 95–107.
18. Bhatt, P., Lalani, R., Mashru, R., & Misra, A. (2016). Abstract 2065: Anti-FSHR antibody Fab' fragment conjugated immunoliposomes loaded with cyclodextrin-paclitaxel complex for improved in vitro efficacy on ovarian cancer cells. *Cancer Research, 76*(14 Supplement), 2065.
19. Jain, S., Jain, S., Khare, P., Gulbake, A., Bansal, D., & Jain, S. K. (2010, August). Design and development of solid lipid nanoparticles for topical delivery of an anti-fungal agent. *Drug Delivery,17*(6), 443–451. PubMed PMID: 20486871. Epub 2010/05/22.eng.
20. Pragati, S., Ashok, S., & Kuldeep, S. (2011). Recent advances in periodontal drug delivery systems. *International Journal of Drug Delivery, 1*(1), 1–14. Epub 2011-04-20.
21. Sneha, B. (2014, January). Applications of nanotechnology in dentistry. *Research Journal of Pharmacy and Technology, 7*(1).
22. Pagonis, T. C., Chen, J., Fontana, C. R., Devalapally, H., Ruggiero, K., Song, X., et al. (2010, December 16). Nanoparticle-based endodontic antimicrobial photodynamic therapy. *Journal of Endodontics, 36*(2), 322. PubMed PMID: PMC2818330.
23. Kmiec, M. P. L., Tedesco, M. F., et al. (2017). Chitosan-properties and applications in dentistry. *Advances in Tissue Engineering and Regenerative Medicine: Open Access, 2*(4), 205–211. https://doi.org/10.15406/atroa.2017.02.00035.
24. Husain, S., Al-Samadani, K. H., Najeeb, S., Zafar, M. S., Khurshid, Z., Zohaib, S., et al. (2017, May 31). Chitosan biomaterials for current and potential dental applications. *Materials, 10*(6), 602. PubMed PMID: PMC5553419. Received May 1, Accepted May 27.
25. Jazayeri, H. E., Fahmy, M. D., Razavi, M., Stein, B. E., Nowman, A., Masri, R. M., et al. (2016, August) Dental applications of natural-origin polymers in hard and soft tissue engineering. *Journal of Prosthodontics,25*(6), 510–517. PubMed PMID: 27003096. Epub 2016/03/24.eng.
26. Virlan, M. J., Miricescu, D., Radulescu, R., Sabliov, C. M., Totan, A., Calenic, B., et al. (2016, February 9). Organic nanomaterials and their applications in the treatment of oral diseases. *Molecules, 21*(2). PubMed PMID: 26867191. Epub 2016/02/13.eng.
27. Narang, R. S., & Narang, J. K. (2015, July–September). Nanomedicines for dental applications-scope and future perspective. *International Journal of Pharmaceutical Investigation, 5*(3), 121–123. PubMed PMID: 26258052. Pubmed Central PMCID: PMC4522860. Epub 2015/08/11.eng.

Chapter 25
Surface Modification of Nanoparticles for Ocular Drug Delivery

Kathleen Halasz and Yashwant V Pathak

Abstract The eye is a crucial sensory organ and is responsible not only for sight but also for maintaining balance. Ocular diseases are categorized as being either anterior or posterior segment diseases based on the affected segment of the eye. Anterior segment diseases affect the cornea, iris, pupil, ciliary body and/or conjunctiva. Posterior segment diseases affect the sclera, choroid, fovea, optic nerve, retina, and/or vitreous humor. The anterior segment of the eye can be treated via topical administration such as eye drops, while the posterior segment requires either an intravitreal or subconjunctival injection. Surface-modified nanoparticles have made significant headway in the treatment of various diseases. Research has proven that the VEGF pathway may be targeted for treatment in various diseases, such as those affecting the eye. Although receptor-targeted drug delivery shows many advantageous in the treatment of various diseases there are also several challenges that must be overcome, such as proper receptor identification as well as carrier formulation. Additionally, each patient undergoing receptor-targeted treatment must be evaluated to determine target expression, distribution pattern, and the density of receptors on the surface of the same factors undoubtedly prove the potential of nanoparticle surface modifying techniques in the treatment of various ocular diseases.

Keywords Angiogenesis · Endothelial growth factor receptor(s) · Nanoparticle(s) · Ligand(s)

K. Halasz
Department of Pharmaceutical Sciences, College of Pharmacy, University of South Florida, Tampa, FL, USA

Y. V Pathak (✉)
College of Pharmacy, University of South Florida, Tampa, FL, USA

Adjunct professor at Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia
e-mail: ypathak1@health.usf.edu

Y. V Pathak (ed.), *Surface Modification of Nanoparticles for Targeted Drug Delivery*, https://doi.org/10.1007/978-3-030-06115-9_25

1 Introduction

The eye is a crucial sensory organ and is responsible not only for sight but also for maintaining balance [21]. Ocular diseases are categorized as being either anterior or posterior segment diseases based on the affected segment of the eye (Fig. 25.1). Anterior segment diseases affect the cornea, iris, pupil, ciliary body, and/or conjunctiva. Posterior segment diseases affect the sclera, choroid, fovea, optic nerve, retina, and/or vitreous humor. The anterior segment of the eye can be treated via topical administration such as eye drops, while the posterior segment requires either an intravitreal or subconjunctival injection (Fig. 25.2) (Joseph and Venkatraman 2017).

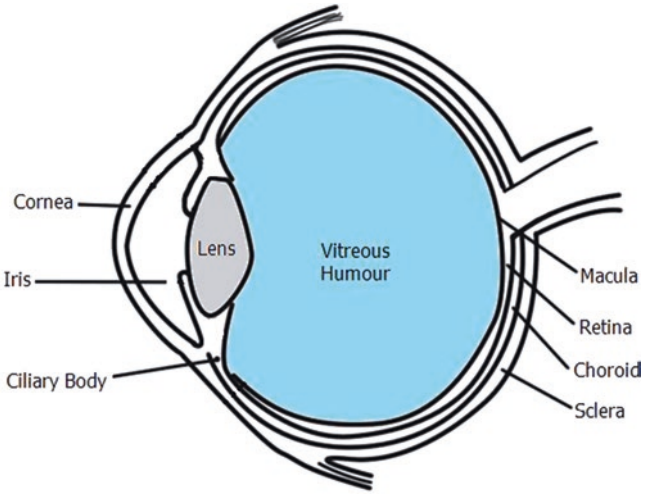


Fig. 25.1 Labeled image of prominent sites within the eye

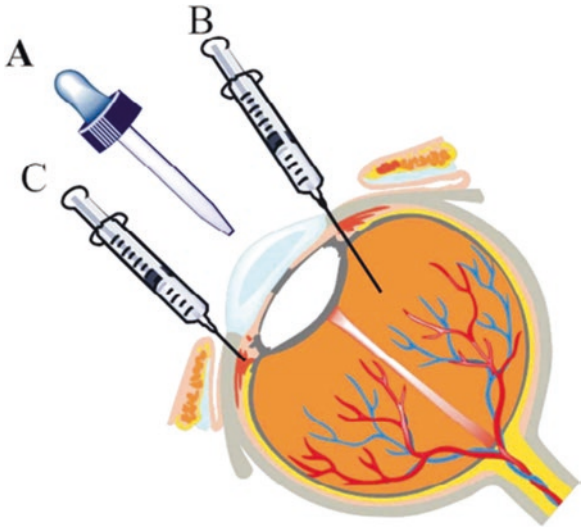


Fig. 25.2 Labeled image of various ocular administration routes: (**a**) Topical, (**b**) intravitreal injection, and (**c**) subconjunctival injection

However, the posterior segment of the eye possesses several barriers, such as the blood–retinal barrier (BRB) and the retinal pigment epithelium (RPE) as well as clearance mechanisms that limit treatment access (Fig. 25.3). Therefore, it is essential that researchers discover an improved method of drug delivery for various ocular diseases.

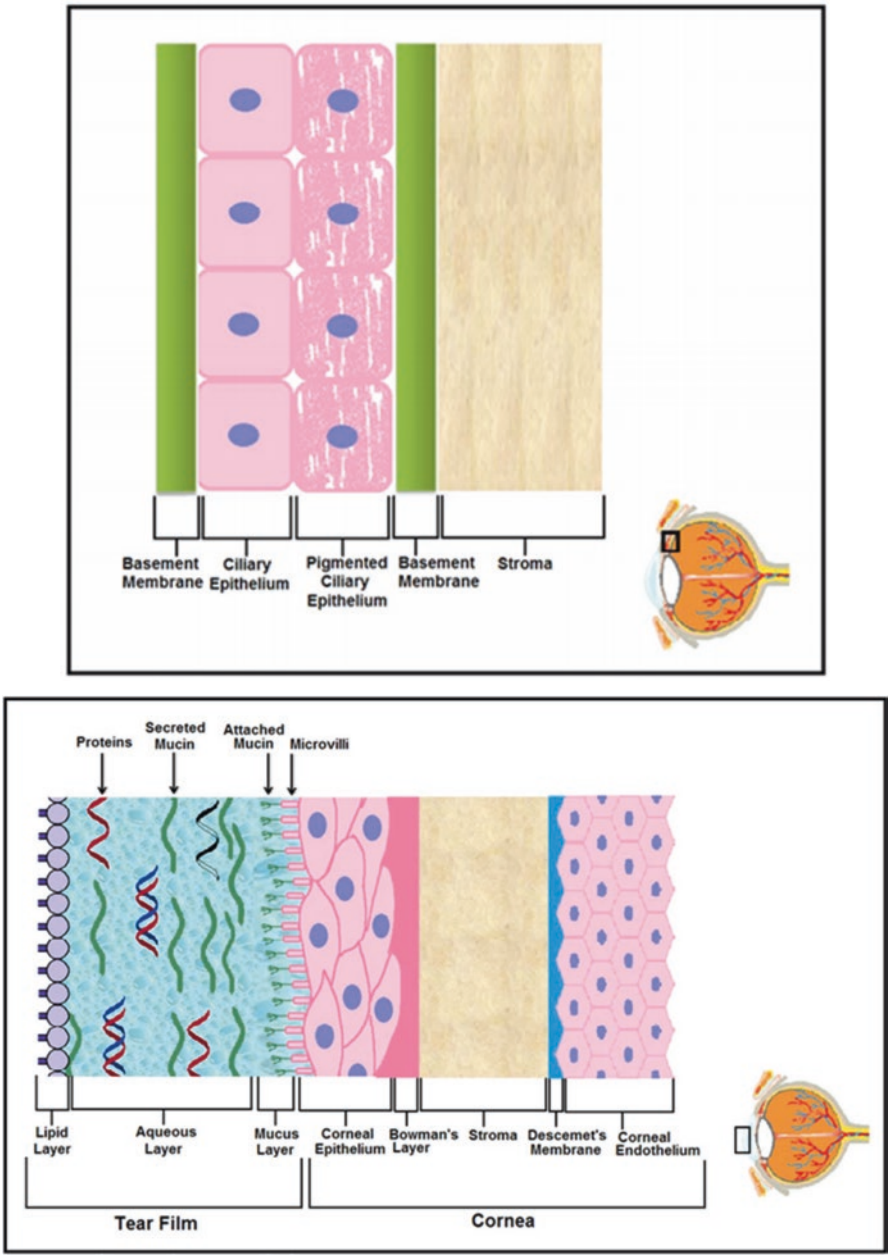


Fig. 25.3 Labeled image of prominent barriers within the eye: (**a**) Blood–aqueous barrier, (**b**) cornea and tear film, (**c**) conjunctiva, and (**d**) retinal barriers

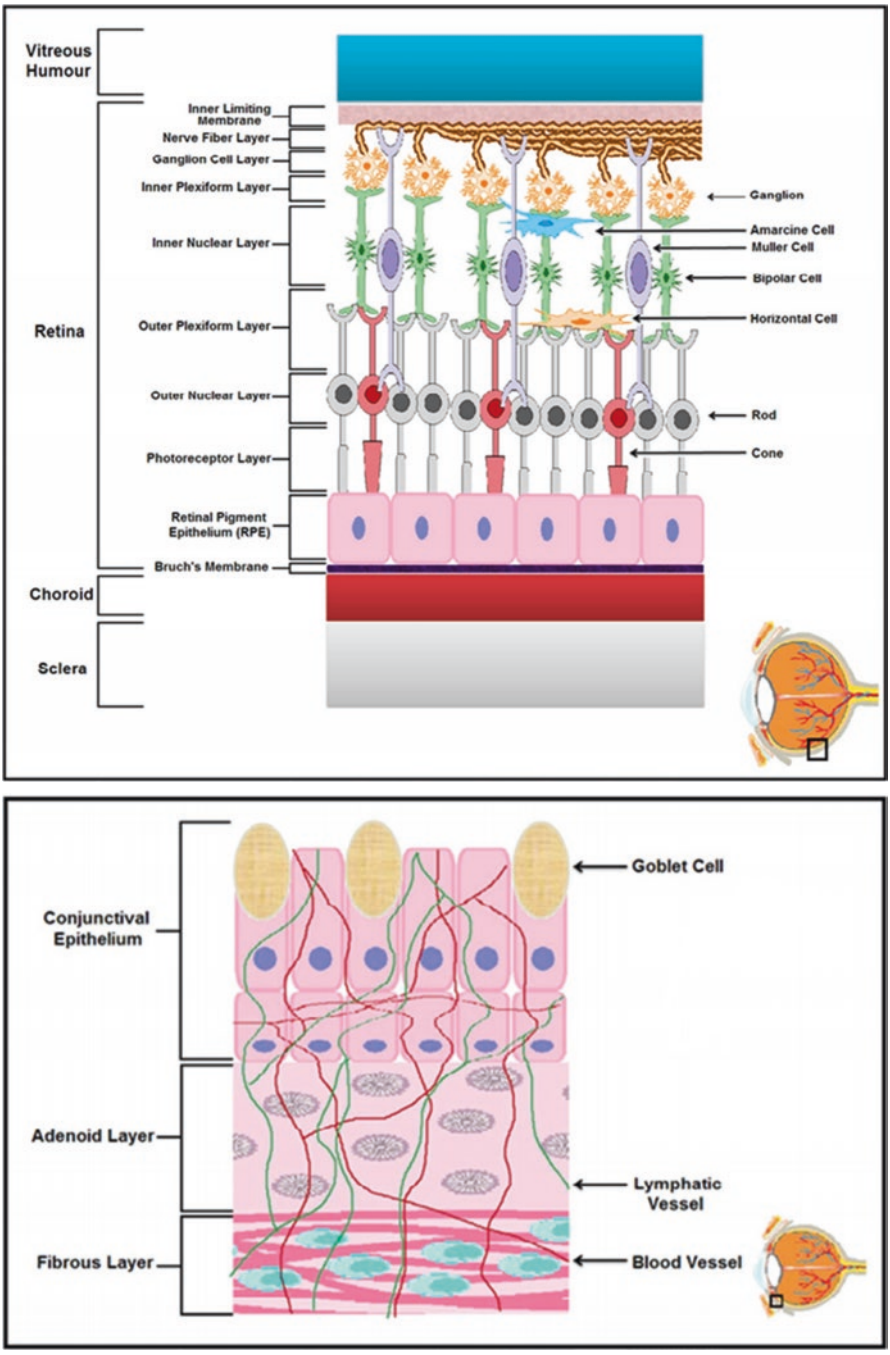


Fig. 25.3 (continued)

One method in obtaining this improved drug delivery system is by entrapping drugs in polymeric nanoparticles (NPs) and modifying their surface [22]. NPs have the unique ability to be altered to meet particular needs depending on its application. Nanostructures are made up of atoms or molecules with various sizes and morphologies and can be applied to the field of pharmaceutics by encapsulating drugs with an improvement in sustainability, efficacy, distribution, biocompatibility, and reduced toxicity [23]. The utilization of modifying the surface of these NPs opens the door for selective drug targeting, which was first introduced by Paul Ehrlich over a century ago [1]. Targeting can occur either passively via the enhanced permeability and retention (EPR) effect or actively through utilization of surface modification with functional ligands.

2 Pathogenesis of Ocular Diseases

There are numerous angiogenic factors that have been known to contribute to the progression of ocular diseases. Therefore, diseases correlated with neovascularization can be combated with various antiangiogenic therapies targeting these distinctive factors [24]. Vascular endothelial growth factor (VEGF) plays a vital role in mediating vascular permeability and angiogenesis. The occurrence of raised VEGF levels has been noted in the neovascular membranes of some diseases (age-related macular degeneration (AMD), retinal vein occlusion (RVO), and diabetic retinopathy (DR)) and its intraocular levels demonstrate a strong correlation with the neovascularization that is correlated with the varying disease pathologies [25]. The endothelial growth factor receptor (EGFR) family, including VEGFRs, are located on the surfaces of cells to initiate a cascade of signals for the synthesis of DNA as well as cell proliferation, adhesion, and migration. EGFRs possess the unique ability to internalize into the nucleus of the cell, thus escaping the lysosomal pathway and making it more advantageous than receptors that lack the ability of translocation [2]. Additionally, it has been reported that hypoxia inducible-factor 1α (HIF-1α) results in the overexpression of VEGF as well as other proangiogenic factors that are prominent components of many ocular diseases (Fig. 6a). Therefore, the suppression of HIF-1α may prove to be another beneficial therapeutic target for varying ocular diseases characterized by an overexpression of VEGF (AMD, RVO, DME, DR, etc.) [3].

Current research in the field of nanomedicine utilizes conventional anti-VEGF drugs encapsulated within an NP. However, several of these drugs have also been tested for the treatment of various ocular diseases, thus confirming the therapeutic, anti-VEGF similiarities between ocular disease and cancer (Table 25.1).

Table 25.1 Drugs utilized to target VEGF in various diseases

Drug	Disease	Phase	Company/Reference
Bevacizumab (Avastin®)	AMD	Marketed off-label	Bascom palmer eye institute
	DME		
	Cancer	Marketed 2004	Genentech [13, 14, 19]
Ranibizumab (Lucentis®)	AMD	Marketed 2006	
	DME		
	RVO		
Doxorubicin	AMD	Preclinical	[3]
	Cancer	Marketed 1995 and 2000	[14, 20]
		Clinical trial Phase III	Celsion corporation [20]
Aflibercept (Eylea®)	AMD	Marketed 2011	[13]
	RVO	Marketed 2012	
	Colorectal Cancer	Marketed 2012	[13]
Pegaptanib (Macugen®)	Ocular: AMD	Marketed 2004	Pfizer [13]

2.1 Ocular Diseases Characterized by VEGF Overexpression

The three most prevalent diseases characterized by VEGF overexpression are AMD, DR, and RVO. These diseases occur in the posterior segment of the eye (Fig. 25.1). AMD is a disease in which the RPE as well as photoreceptors degenerate over time. This disease affects ~two million people in the USA and is expected to reach three million by 2020 [4]. The current leading cause of legal blindness, however, is DR. Out of the 4.2 million diagnosed ~655,000 result in irreversible blindness. DR is a vascular disorder within the retina that results from diabetes mellitus and shares a mutual pathology with AMD [5]. Similar to AMD, RVA displays more prevalence in men and women >65 and is the second most common cause of blindness [6]. Additionally, one form of RVO, known as central retinal vein occlusion (CRVO), has been found to be also associated with diabetes mellitus and other vascular diseases [7].

3 Surface-Modified Nanoparticles to Optimize Ocular Biocompatibility

The ideal characteristics of NPs depend on the specific therapeutic application. Size, zeta potential (i.e., surface charge), and aggregation/binding properties of NPs are generally determined via dynamic light scattering (DLS) as well as various imaging methods. It is imperative that drug-loaded NPs utilized for treatment in vivo are optimized in all of these characteristics to ensure biocompatibility in treatment. For instance, surface charge significantly impacts cellular uptake and may be adjusted via surface modifying techniques. The ideal surface charge for a

nanoparticle in terms of stability is ±25 mV [8]. Repeated reports demonstrate that nanoparticles with a surface charge of approximately −30 mV demonstrate significant antiangiogenic effects on ocular diseases resulting from neovascularization by providing more efficient penetration into the vitreous cavity [9]. For example, the utilization of ultraviolet (UV) irradiation as well as cold-plasma O_2 has demonstrated an ability to modify the surface of a particle from anionic to cationic [10]. On the contrary, utilization of acidic materials in formulation and modification has the potential to decrease surface charge [11].

Like surface charge, aggregation and binding properties of nanoparticles may be optimized via surface modification. For example, Bagwe et al. formulated silica NPs and modified their surface via cohydrolysis in order to reduce both aggregation and nonspecific binding [12]. The results of their study demonstrated minimal nonspecific binding in functionalized nanoparticles when compared to those that were left unmodified. Additionally, Bagwe et al. determined that their technique for surface modification also improved conjugation potential [12].

4 VEGF Targeting Surface-Modified Nanoparticles

One of the most prominent features of ocular diseases is elevated levels of angiogenic factors such as HIF-1 and VEGF. Antiangiogenic treatments for varying diseases typically utilize VEGF as its target and, as stated previously provide a parallel in the treatment methods of various diseases due to the similar overexpression of VEGF. Conventional anti-VEGF drugs that are currently being utilized include doxorubicin (DOX), bevacizumab (Bev), and aflibercept, [13].

Zhu et al. formulated DOX mesoporous silica based inorganic NPs with layered double hydroxide (LDH) (SiO_2@LDH-DOX) to target VEGF [14]. Bev (Avastin®) was utilized to modify SiO_2@LDH-DOX NPs in order to reduce the toxicity of DOX to normal surrounding tissues and structures. Bev was chosen because it is an FDA approved anti-VEGF antibody that inhibits the binding of VEGF to one of its receptors (VEGFR) by forming a protein complex. Therefore, the Bev modified DOX NPs (SiO_2@LDH-Bev-DOX) will not only increase VEGF targeting potential, but will also aid in the antiangiogenic therapeutic effects provided by DOX. The resulting formulation had an average diameter of 253 ± 10 nm and confocal microscopy was utilized to determine successful VEGF targeting by comparing SiO_2@LDH-Bev-DOX with SiO_2@LDH-DOX [14]. As hypothesized, SiO_2@LDH-Bev-DOX was found to accumulate in the nuclei more quickly and at a higher quantity than unmodified NPs, thus confirming its efficiency in accurately targeting VEGF.

Similarly, Goel et al. formulated sunitinib-loaded mesoporous silica NPs to target the VEGF pathway for both diagnostic as well as therapeutic purposes [15]. Sunitinib was chosen due to its unique ability to inhibit receptor kinases such as VEGFR. The particles were then surface-modified with amino groups, chelator conjugation, PEGylation, and, finally, linked with thiolated VEGF and a radioisotope

for labeling. Transmission electron microscopy (TEM) was utilized to determine that there were no morphologic alterations after surface modification of the sunitinib NPs other than an increase in size due to the addition of the hydrated shell. As hypothesized, the thiolated VEGF surface-modified sunitinib NPs provided more efficient targeted when compared with non-surface-modified NPs [15]. Therefore, higher quantity of drug may accumulate within the targeted area with little to no undesirable interaction with surrounding cells and structures.

5 VEGF Receptor-Targeting Surface-Modified Nanoparticles

Receptors expressed on the surface of cells may be utilized to provide targeted treatment for various diseases, such as those affecting the eye, through their endogenous ligands. Certain receptors become overexpressed in affected cells but not normal cells. Therefore, these specific receptors may be utilized to target treatment to a desired site with little to no interaction with surrounding cells. The two prominent forms of receptor-targeted drug delivery are antibody drug conjugates (ADCs) and protein drug conjugates (PDCs) [2].

The utilization of antibodies for disease treatment has been researched for years with little application due to unfavorable immunogenic responses. Therefore, humanized monoclonal antibodies (mAbs) were developed and utilized in both immunodiagnostics as well as immunotherapeutic applications due to their discerning recognition of cell specific antigens. There are >15 antibodies currently approved for clinical use as well as >100 in preclinical and clinical trials [16]. These mAbs may be conjugated to the surface of drug loaded NPs to increase half-life, improve residence time at the targeted site, increase drug dose, control the release of drug, reduce free drug residence, and circumvent multidrug resistance. ADCs are composed of the antibody itself, drug or drug-loaded NP, and a linker molecule. Peptide linkers are the most advantageous when considering ocular drug delivery due to their biocompatibility, as they are degraded gradually by lysosomes. There are currently >25 ADCs undergoing clinical experimentation [16].

VEGFR receptors have the ability to be targeted by various, specific antibodies in order to treat diseases correlated with elevated VEGF levels [16]. In 2004 the FDA approved Bevacizumab (Avastin®), which binds to VEGFR to inhibit angiogenesis, for the treatment of metastatic colorectal cancer. This was expanded in 2014 when the FDA approved its use in combination with chemotherapy (Paclitaxel® (PT), DOX, or Topotecan) for the treatment of recurrent platinum resistant ovarian cancer [16].

Several ADCs have been developed and are currently undergoing clinical trials. For example, SGN-15 (cBR96-DOX-Immunoconjugate) utilizes DOX conjugated with chimeric mAb BR 96. This treatment approach is in Phase I/II of clinical trials and has thus far been successful in slowing the growth of the disease and is well

tolerated by the body [16]. Additionally, lifastuzumab vedotin (DNIB0600A) utilizes an anti-mitotic agent (MMAE) conjugated with humanized IgG1 anti-NaPi2b mAb and is undergoing Phase I/II clinical trials. The results have demonstrated an improved safety profile when compared with conventional treatments and future studies will investigate specific activity post-treatment [16].

Patel et al. formulated cetuximab (Cet), and EGFR mAb, conjugated docetaxel-loaded NPs (DTX NPs) (Cet-DTX-NPs). DTX is currently available as Taxotere® but displays limited solubility as well as associated toxicity [17]. The results demonstrated that Cet-DTX-NPs have highest reduction of proliferation when compared to DXT NPs as well as free drug. Cellular uptake was elevated, thus increasing intracellular drug levels due to the specific binding affinity of Cet with EGFRs. Additionally, Cet-DTX-NPs demonstrated a site-specific sustained drug delivery design when compared to DTX NPs and free drug solution [17]. Therefore, the results demonstrated a reduction in dose-dependent toxicity as well as an increased safety margin for patients.

Shi et al. utilized PT loaded PLGA NPs conjugated with VEGF (VEGF-PT-NPs) in order to bind to VEGFR in human umbilical vein endothelial cells (HUVEC). A covalent coupling method was utilized to provide VEGF-decorated surface modification of the PT NPs, which were found to be 332.2 ± 1.6 nm larger than nonmodified PT NPs but still displayed a uniform, spherical morphology [18]. The conjugation reaction utilizes carboxyl groups on the surface of the NPs and avoids the polymer matrix entirely, leaving the sustained drug release rate unaltered when compared to nonconjugated NPs. The results demonstrated significant interaction between VEGF-PT-NPs with the cell surface as shown by fluorescence microscopy [18]. MTT assay showed there was a decrease in inhibitory concentration (IC_{50}) when compared to PT NPs as well as free drug. Additionally, VEGF-PT-NPs demonstrated a significant increase ($p < 0.05$) in antitumor activity when compared with both PT NPs and free drug solution [18].

6 Conclusion and Future Perspectives

Surface-modified nanoparticles have made significant headway in the treatment of various diseases. Research has proven that the VEGF pathway may be targeted for treatment in various diseases, such as those affecting the eye. Although receptor-targeted drug delivery shows many advantages in the treatment of various diseases there are also several challenges that must be overcome, such as proper receptor identification as well as carrier formulation. Additionally, each patient undergoing receptor-targeted treatment must be evaluated to determine target expression, distribution pattern, and the density of receptors on the surface of the cell [2].

Unfortunately, there has been minimal research in the utilization of surface-modified NPs for the treatment of ocular diseases. However, many findings for

diseases also characterized by an overexpression of the same factors undoubtedly prove the potential of nanoparticle surface-modifying techniques in the treatment of various ocular diseases.

Acknowledgments Special thanks to Shannon Kelly, Muhammed Iqbal, and Nikhil Kulkarni.

References

1. Choi, S.-W., Kim, W.-S., & Kim, J.-H. (2003). Surface modification of functional Nanoparticles for controlled drug Deivery. *Journal of Dispersion Science and Technology, 24*(3), 475–487.
2. Vhora, I., Patil, S., Bhatt, P., Gandhi, R., Baradia, D., & Misra, A. (2014). Receptor-targeted drug delivery: Current perspective and challenges. *Therapeutic Delivery, 5*(9), 1007–1024.
3. Kelly, S., Halasz, K., & Sutariya, V. (2017). HIF-1a inhibitors for the treatment of posterior segment ocular diseases. *International Journal of Nanomedicine and Nanosurgery, 3*(1).
4. Friedman, D., O'Colmain, B., Munoz, B., Tomany, S., McCarty, C., de Jong, P., et al. (2011). Prevalence of age-related macular degeneration in the United States. *Archives of Ophthalmology, 129*(9), 1188.
5. Hazare, S., Yang, R., Chavan, S., Menon, M., & Chougule, M. (2016). Aging disorders of the eye: Challenges and approaches for their treatment. In Y. Pathak, V. Sutariya, & A. A. Hirani (Eds.), *Nano-biomaterials for ophthalmic drug delivery*. Switzerland: Springer.
6. Wong, T., & Scott, I. (2010). Retinal-vein occlusion. *New England Journal of Medicine, 363*, 2135–2144.
7. Riaz, S., Khan, M., Qazi, Z.-u.-D., Qadeer, R., & Shaukat, S. (2017). Different patterns of retinal vein occlusion on Fundus Fluorescein angiography. *Ophthalmology, 15*(2), 77–81.
8. Zhang, Y., Yang, M., Park, J., Singelyn, J., Ma, H., Sailor, M., et al. (2009). A surface-charge study on cellular-uptake behavior of F3-peptide-conjugated iron oxide nanoparticles. *Small, 5*(17), 1990–1996.
9. Jo, D., Kim, J., Lee, T., & Kim, J. (2015). Size, surface charge, and shape determine therapeutic effects of nanoparticles on brain and retinal diseases. *Nanomedicine, 11*(7), 1603–1611.
10. Miura, T., Miyake, N., Tanabe, K., & Yoshinari, M. (2011). Change in zeta potential with physicochemical treatment of surface of Anatase-form Titania particles. *Journal of Oral Tissue Engineering, 9*(2), 64–70.
11. Clogston, J., & Patri, A. (2011). Zeta Potential Measurement. In S. Mcneil (Ed.), *Characterization of Nanoparticles intended for drug delivery. Methods in molecular biology* (Vol. 697). New York: Humana Press.
12. Bagwe, R., Hilliard, L., & Tan, W. (2006). Surface modification of silica Nanoparticles to reduce aggregation and nonspecific binding. *Langmuir, 22*(9), 4357–4362.
13. Amadio, M., Govoni, S., & Pascale, A. (2016). Targeting VEGF in eye neovascularization: What's new? A comprehensive review on current therapies and oligonucleotide-based interventions under development. *Pharmacological Research, 103*, 253–269.
14. Zhu, R., Wang, Z., Liang, P., He, X., Zhuang, X., Huang, R., et al. (2017). Efficient VEGF targeting delivery of DOX using Bevacizumab conjugated SiO2@LDH for anti-neuroblastoma therapy. *Acta Biomaterialia, 63*, 163–180.
15. Goel, S., Chen, F., Hong, H., Valdovinos, H., Hernandez, R., Shi, S., et al. (2014). VEGF121-conjugated Mesoporous silica Nanoparticle: A tumor targeted drug delivery system. *ACS Applied Materials and Interfaces, 6*, 21677–21685.
16. Bhatt, P., Vhora, I., Patil, S., Amrutita, J., Bhattacharya, C., Misra, A., et al. (2016). Role of antibodies in diagnosis and treatment of ovarian cancer: Basic approach and clinical status. *Journal of Controlled Release, 226*, 148–167.

17. Patel, J., Amrutiya, J., Bhatt, P., Javia, A., Jain, M., & Misra, A. (2018). Targeted delivery of monoclonal antibody ocnjugated docetaxel loaded PLGA nanoparticles into EGFR overexpressed lung tumour cells. *Journal of Microencapsulation, 35*, 204–217.
18. Shi, Y., Zhou, M., Zhang, J., & Lu, W. (2015). Preparation and cellular targeting study of VEGF-conjugated PLGA nanoparticles. *Journal of Microencapsulation, 32*(7), 699–704.
19. Nagpal, M., Nagpal, K., & Nagpal, P. (2007). A comparative debate on the various anti-vascular endothelial growth factor drugs: Pegaptanib sodium (Macugen), ranibizumab (Lucentis) and bevacizumab (Avastin). *Indian Journal of Ophthalmology, 55*(6), 437–439.
20. Pillai, G. (2014). Nanomedicines for Cancer therapy: An update of FDA approved and those under various stages of development. *SOJ Pharmacy and Pharmaceutical Sciences, 1*(2), 13.
21. Malavade, S. (2016). Challenges in ocular pharmacokinetics and drug delivery. In V. Sutariya, A. Hirani, & Y. Pathak (Eds.), *Nano-biomaterials for ophthalmic drug delivery* (pp. 593–612). Switzerland: Springer.
22. Kompella, U., Bandi, N., & Ayalasomayajula, S. (2003). Subconjunctival nano- and microparticles sustain retinal delivery of budesonide, a corticosteroid capable of inhibiting VEGF expression. *Investigative Ophthalmology and Visual Science, 44*(3), 1192–1201.
23. Hirani, A., & Pathak, Y. (2016). Introduction to nanotechnology with special reference to ophthalmic delivery. In V. Sutariya, A. Hirani, & Y. Pathak (Eds.), *Nano-biomaterials for ophthalmic drug delivery* (pp. 1–8). Switzerland: Springer.
24. Hirani, A., et al. (2014). Triamcinolone acetonide nanoparticles incorporated in thermoreversible gels for age-related macular degeneration. *Pharmaceutical Development and Technology*, 61–66.
25. Kvanta, A., Algvere, P., Berglin, L., & Seregrad, S. (1996). Subfoveal fibrovascular membranes in age-related macular degeneration express vascular endothelial growth factor. *Investigative Ophthalmology and Visual Science, 37*(9), 1929–1934.

Correction to: Successful Delivery of Zidovudine-Loaded Docosanol Nanostructured Lipid Carriers (Docosanol NLCs) into Rat Brain

Tapash Chakraborty, Malay K. Das, Lopamudra Dutta, Biswajit Mukherjee, Sanjoy Das, and Anupam Sarma

Correction to:
Chapter 14 in: Y. V Pathak (ed.), *Surface Modification of Nanoparticles for Targeted Drug Delivery*, https://doi.org/10.1007/978-3-030-06115-9_14

This book was inadvertently published with incorrect affiliation of the authors Lopamudra Dutta and Biswajit Mukherjee in Chapter 14. The correction has been updated in book to reflect the affiliation as Department of Pharmaceutical Technology, Jadavpur University, Kolkata, West Bengal, India.

The updated online version of this chapter can be found at https://doi.org/10.1007/978-3-030-06115-9_14

Y. V Pathak (ed.), *Surface Modification of Nanoparticles for Targeted Drug Delivery*, https://doi.org/10.1007/978-3-030-06115-9_26

Index

Y. V Pathak (ed.), *Surface Modification of Nanoparticles for Targeted Drug Delivery*, https://doi.org/10.1007/978-3-030-06115-9