Nanotechnology in the Life Sciences

Vishnu Kirthi Arivarasan
Karthik Loganathan
Pushpamalar Janarthanan *Editors*

Nanotechnology in Medicine

Springer

Nanotechnology in the Life Sciences

Series Editor

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Nano and biotechnology are two of the 21st century's most promising technologies. Nanotechnology is demarcated as the design, development, and application of materials and devices whose least functional make up is on a nanometer scale (1 to 100 nm). Meanwhile, biotechnology deals with metabolic and other physiological developments of biological subjects including microorganisms. These microbial processes have opened up new opportunities to explore novel applications, for example, the biosynthesis of metal nanomaterials, with the implication that these two technologies (i.e., thus nanobiotechnology) can play a vital role in developing and executing many valuable tools in the study of life. Nanotechnology is very diverse, ranging from extensions of conventional device physics to completely new approaches based upon molecular self-assembly, from developing new materials with dimensions on the nanoscale, to investigating whether we can directly control matters on/in the atomic scale level. This idea entails its application to diverse fields of science such as plant biology, organic chemistry, agriculture, the food industry, and more.

Nanobiotechnology offers a wide range of uses in medicine, agriculture, and the environment. Many diseases that do not have cures today may be cured by nanotechnology in the future. Use of nanotechnology in medical therapeutics needs adequate evaluation of its risk and safety factors. Scientists who are against the use of nanotechnology also agree that advancement in nanotechnology should continue because this field promises great benefits, but testing should be carried out to ensure its safety in people. It is possible that nanomedicine in the future will play a crucial role in the treatment of human and plant diseases, and also in the enhancement of normal human physiology and plant systems, respectively. If everything proceeds as expected, nanobiotechnology will, one day, become an inevitable part of our everyday life and will help save many lives.

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Dream big, Achieve big

—Anonymous

To understand the very large we must understand the very small.

—Democritus, 470–380 BC

The future belongs to those who prepare for it.

—Ralph Waldo Emerson (1803–1882)

Preface

Nanotechnology is a multifaceted scientific field which involves the synthesis and frameworks on the nanometer scale and is presently experiencing a risky advancement on different many fronts. It is relied upon to spark an innovation and play a critical role in different biomedical applications, especially in the drug delivery as is appeared by the abundance of data introduced in this book.

The nanotechnology is rapidly evolving field which is expected to hit a sudden impact on many industries primarily the world of medicine. Therefore, it can said that the union of the nanotechnology and medicine has given birth to nanomedicine world. The biochemical pathways present in a living cell employ the help of the components, which include the water, glucose, antibodies, interferon, signalling molecules, which are all in the scale range of 'nano', thereby to control, guide and to treat these cells. The nano derived compounds/molecules will be utilized in the curing/treating of the cells which can be further used to control and guide for the welfare of the affected patients and also for the welfare of the human world—i.e. to control the bacteria, fungal and other organisms for the production of the compounds for the betterment of the human world. This book is essentially intended to be a reference course book on the application of nanotechnology in the improvement of drug delivery systems and to feature probably the most energizing advancements in this field.

There are many books and journal reviews which provide a detailed discussion of the selective parts of the nanotechnology and medicine together. But there is requirement of a comprehensive book providing a combined knowledge of 'Nanomedicine' and its impact on healthcare. Nanomaterials are structures with characteristic dimensions between 1 and 100 nm; engineered appropriately, they exhibit a variety of unique and tunable chemical and physical properties. These characteristics have made engineered nanoparticles central components in an array of emerging technologies, and many new companies have been formed to commercialize products.

The '*Nanotechnology in Medicine'* covers the wide extent of this field. Beginning with the nuts and bolts, the subject is created to likely clinical applications, huge numbers of which are still at a trial stage. The prefix '*nano'* is utilized generously

and shows the nanodimension of existing scientific fields and clinical fortes. This book will be providing a coordinated depiction of nanomedicine and its new age tools for the diagnostics and treatment alike for the complete service of the human being.

Mumbai, Maharashtra, India — Vishnu Kirthi Arivarasan
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Selangor, Malaysia — Pushpamalar Janarthanan

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Chapter 1
Nanomedicine: General Introduction from A to Z

Shaik Mohammed Ghouse and Ilangovan Pugazhenthi

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1.1 Introduction

Materials whose dimensions are in nanoscale generally taken as being 100 nm or less are called nanomaterials. One millionth of a millimetre is called as nanometre. They are existing in different forms like rod, fibres, particles, tubes etc. chemical composition in bulk form may have different physico-chemical properties compared to the same in nanoscale. i.e. The properties and functions of nanomaterials are totally different to that of in bulk. The nanomaterials are having interesting optical, magnetic and electrical properties which are having significant effects in the fields of electronic medicine. Nanomaterials have been found naturally as well has

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in engineered commercial products like cosmetics, sunscreen materials and some sports goods (Jacobs et al. 2010).

1.1.1 Classifications of Nanomaterials

Based on the composition, nanomaterials are classified as inorganic metal, metal oxide, organic and carbon-based nanomaterials. Metals and metal oxide nanoparticles were obtained from metals like copper (Cu), Cobalt (Co), iron (Fe), Zinc (Zn) etc. (Chavali and Nikolova 2019). These nanoparticles have been prepared with different shapes, surface area and densities which are reflected into their mechanical, electrical, and other properties. Further the efficiency and reactivity of metal nanoparticles may be enhanced by preparation of respective nano sized metal oxide. Some of nano sized organic biocompatible polymer materials have reported for drug delivery applications because of their nontoxicity (Ali and Ahmed 2018). At present carbon-based nano particles like graphene, reduced graphene, carbon nanotube (CNT) have been reported for biomedical applications (Jeong et al. 2018).

1.1.2 Different Methods Used for Preparation for Nanomaterials

(a) **Sol-gel method**

In the sol-gel technique, nanoparticles have obtained through the generation of colloidal suspension followed by gelation. Generally, metals or metalloid elements with various active ligands have been used as a precursor for the preparation of the colloids (Zeng et al. 2016). Initially, the precursor is reacted to generate dispersible oxide and it gives a sol in the presence of water or dilute acid. Later on, the sol has kept on evaporation to yield gel in which the size and shape of particles are controlled to form. Finally, the obtained gel is kept for calcination at various temperature which produces metal oxide nanoparticles. The final size and shape of the nanoparticles may be varied with calcination temperature ranges.

We can explain these techniques by taking preparation of SiO_2 nanoparticles in which $Si(OEt)_4$ (tetraethyl orthosilicate, or TEOS) (Azlina et al. 2016). Has taken as precursor. The sol-gel technique involves hydrolysis followed by condensation of metal alkoxides which has explained as below:

$$\mathrm{MOR} + \mathrm{H_2O} \rightarrow \mathrm{MOH} + \mathrm{ROH}\ \left(\text{hydrolysis}\right)$$

$$\mathrm{MOH} + \mathrm{ROM} \rightarrow \mathrm{M\ \ O\ \ M} + \mathrm{ROH}\left(\text{condensation}\right)$$

(b) **Gas Phase synthesis of nanomaterials**
This synthetic method allowing a simple way to prepare nanomaterials with specific size and chemical composition. It is noteworthy to discuss about homogeneous or heterogenous reactions which are involved in this conventional chemical vapour deposition (CVD) (Kim et al. 2004). In homogenous CVD, nanoparticles have generated in gas-phase and they have deposited on surface of low temperature substrate which can be scrapped into nano-powders. In the case of heterogenous CVD, nanoparticles are formed on the surface of the substrate which generates a dense film. Generally, this method controls the size, shape and crystallinity of materials into required form. Materials which we have prepared by this method are pure and the mechanism involved is known.

(c) **Chemical vapour condensation**
It is a coating process in which chemical reactions occurred on the substrate at particular temperature. Reagents and precursors were supplied in gaseous form. Solid nanoparticles are obtained by pyrolysis of gaseous precursors at decomposition temperature. For example, Alumina nanoparticles were synthesized by CVC method in which aluminium chloride have been used as a precursor where carbon-dioxide and hydrogen were used for decomposition reaction (Lee et al. 2005).
Alumina may be deposited by the reaction:

$$Al_2Cl_6 + 3CO_2 + 3H_2 = Al_2O_3\ (\text{solid}) + 3CO\ (\text{gas}) + 6HCl\ (\text{gas})$$

The advantages of this method include the uniform coating of the nanoparticles or nano film. However, this process has limitations including the higher temperatures required, and it is difficult to scaleup.

(d) **Thermolysis**
It is an easiest method in which desired nano materials are produced in heat resistant crucible containing required precursors. This method can be applied only those materials which has high vapour pressure at high temperatures (Ranjbar et al. 2011). This can be explained by taking simple example of preparation of lithium nanoparticles from lithium azide which decomposes at 370 °C under nitrogen atmosphere (Tanaka et al. 2016).

(e) **Flame assisted ultrasonic spray pyrolysis**
In this process precursors are heated to burnt unwanted components to generate required nano materials. SiO_2 nanoparticles were obtained by heating silica tetrachloride in an oxy-hydrogen flame. The white particles are having sizes ranging from 7 to 40 nm (Jang 2001). Acetylene and oxygen gases are supplied to initiate the pyrolysis of precursor compounds.

(f) **Hydrothermal/solvothermal Method**
In this method, the precursors are taken into sealed vessel containing solvents and reacted under reflux temperature around their critical points with high pressure. If water is used as a solvent then it is called hydrothermal whereas organic solvent is used means that is called as solvothermal process (Nunes et al. 2018). Mostly the reactants have taken in the form of solutions or suspension. With

these organic and inorganic additives are added to increase the homogenous dispersion and to achieve crystalline morphology.

1.1.3 Characterization of Nanomaterials

(a) **X-ray diffraction (XRD)**
Each and every solid material are having unique diffraction patterns which has been used to identify and characterize the particular nanomaterial. This unique pattern is called characteristic finger print region. The principle behind this analysis is diffraction of incident X rays by sample. The diffraction takes place at different directions. Generally, X-ray diffractometer with X-ray diffractometer with Cu-Ka (1.5418 Å) is used to generate X-rays.
The X-ray source is kept as stable and the sample to be analysed is kept as mobile which moves by angle theta, the detector moves by 2 theta. The rotation rate is kept at 1°/min and the sample is scanned for 10°–80° scan. The sample is loaded on a soda glass substrate (West et al. 2015).

(b) **Scanning Electron Microscope (SEM)**
The scanning Electron Microscope technique has been used to analyse surface morphology, chemical composition, size and crystalline structure of the nanomaterials. In this technique electron beams are allowed to fall on the nanomaterials to generate different signals which are characteristics of the surface of the solid nanomaterials. The signal which have been received are more informative which gives more details of the nanomaterials.
The signals consist of secondary electrons, backscattered electrons and diffracted backscattered electrons. To imagine the sample, secondary and backscattered electrons are needed. Morphology and topography of nanomaterials have been informed by mostly secondary electrons (Goldstein et al. 2017).

(c) **Transmission electron microscopy (TEM)**
TEM is a technique in which high energy electron beam is focused through a sample where the interaction between atoms and electrons taken place. The interaction could be useful to know the features such as crystalline structure, grain boundaries etc. This technique is used to find composition, defects. Transmitted portion has to strike phosphor screen where the image is generated. Darker areas of the image represent the transmission of less electrons through while the lighter areas represent transmission of many electrons (Su 2017).

1.1.4 Medicinal Properties of Nanomaterials

(a) **Size and Surface Area of the Particle on Toxicity**
Nanomaterials with decreased size leads to exponential increase in surface are which plays vital role on their applications in various fields. Oral toxicity of

nanoparticles is directly related to size of nanoparticles (Gatoo et al. 2014). It was observed that copper nanoparticles with decreased size are having low toxicity compared to larger particles which are having moderate toxicity. Nano particles less than 50 nm have diffused quickly to all part of the tissues compared to large particles. Most of the studies on toxicity of nanoparticles have showed that nanoparticles with less than 100 nm causes adverse respiratory health effects compared to larger particles (Chen et al. 2006).

(b) **Effect of particle shape**
The studies on toxicity of nanoparticles like gold, nickel and CNT have reported based on their shape. It has reported that the nano particle with spherical shape are very easy and fast in endocytosis process (Champion and Mitragotri 2006). The rod or fibre nano particles shows very less effect in the studies. Compared to other shapes spherical nanoparticles are more toxicity even they are in homogenous or heterogenous phase.

(c) **Effect of Surface Charge**
Charge on the surface of nanoparticles has significant effect on their toxicity on biological systems on various aspects like colloidal behaviour, plasma protein binding and blood brain barrier integrity (Pietroiusti et al. 2011). It is apparent form the reports, that negatively and neutral charged nanoparticles are showing very less cellular uptake compared to positive charged nanoparticles on opsonization by plasma proteins.

1.1.5 Role of Nanotechnology in Medical Field

The recent development of nano technology has applied to various field. Mostly in the case of medicinal field it has been showing significant impact on oncology and cardiovascular medicine (Fymat 2016). Furthermore, Molecular diagnostics, drug discovery and delivery applications has marked the importance of nano materials. Nanotechnology and nano materials play vital role in the development of cancer research. They have been employed to deliver drugs and some particles have been engineered to attract infected cells to remove.

Recently, Worcester polytechnic institute have showed that the CNT act as carrier for antibodies in the form of chips to find infected cancer cells in blood at earlier stage itself (Loeian et al. 2019). In this method, functionalized gold nano rods have engineered to bind to the damaged protein cells where the shift in colour of nano rod is observed. This method is designed to find damaged cells at earlier stage.

The University of Wisconsin designed a bandage which act as nanogenerators to apply electrical pulses on wounds to cure (Long et al. 2018). Chase from Western Reserve University has designed artificial platelets from the bio-compatible polymeric materials to reduce blood loss during surgery or trauma. To repair and treat diseased cells, nanorobots have been designed and programmed to antibodies. As nanotechnology opens new avenues in the field of medicinal research which would be developed even in poor countries for their future economic and social welfare.

1.1.6 What Is Mean by Nanomedicine?

Application of nanotechnology to treat diseases is called nanomedicine. The properties of nanomaterials have been used to treat infected cells. It involves the utilization of bio-compatible nano rods, nanoparticles and nanorobots.

The nanomaterials which induce desired physiological responses with the target cells are called biocompatible materials. The special properties of nanomaterials with less undesirable effect have been utilized to treat infected cells or tissue. It may include nanodevices to manipulate target cell and nano sensors to give information on physiological functions of target tissues.

The biocompatibility of nanomaterials mostly depends on the zeta potential of nanoparticles. The charge, less than 30 mV may helpful to reduce the aggregation of nanoparticles. But maximum surface charge may affects the interaction of nanoparticles.

Further the biocompatibility has enhanced by modification of surface of nanoparticles with poly(ethylene glycol) PEG which would increase the biocompatibility by removing phagocytosis. To increase the bio-compatibility of polyethersulfon as hemodialysis, its surface has to be modified by polyvinyl pyrrodone (Hayama et al. 2004). Various methods to modify the surface of nanoparticles have been reported, but the modification process should have best effect on biocompatibility with biological stream.

1.1.7 Need for Nanomedicine

Nanoparticles have been engineered to carry some particular drug molecules to specific tumour cells. The drug molecules can be bound with the nanoparticles by surface modification so that drug molecules could be delivered at the target cancer cell (Hayama et al. 2004).

To enter the blood stream, drug molecules should be soluble. We can explain this by taking an example of the drug paclitaxel which is not soluble in blood stream which causes severe allergic conditions (El-Readi and Althubiti 2019). This problem has been overcome by the development of biocompatible nanoparticles out of the naturally occurring albumin proteins which carried and dissolved a paclitaxel in bloodstream. Generally, tumour cells are surrounded by leaky blood vessels (Ma and Mumper 2013). The intaking of chemotherapy medicine are very small in size and it could be diffused into the vessels and they have not able to reach target cell instead of this they may attack normal adjacent tissues. This problem may be overcome by application of nanoparticles whose size are larger in size than the chemotherapy drugs. These nanoparticles not only act as a carrier and also streams effectively the path of the arrival of drug into target tumour cell.

Objectives of Nanomedicine

- The main objective of nanomedicine can be defined as to control, construct and improve all the human biological systems by molecular level engineered devices and nanostructures.
- Active components whose size could be varied from 1 to 100 nm has to be taken to achieve medical benefit.
- The aim of nano interactions should be taken within a subcellular or cellular system.
- To develop a new regulatory guideline to ensure safe and reliable medical treatment.
- To develop innovative therapeutics to enable tissue regeneration and repair.

1.1.8 Applications of Nanomedicine

1.1.8.1 Cancer Treatment and Chemotherapy

Cancer is one of the complicated health problems to people to die every year globally. This severe disease has been controlled by applying Radiotherapy and chemotherapy to exterminate solid tumours. Chemotherapy is better when compare to radiotherapy which causes destruction of normal cells. The development of chemotherapy to treat damaged cancer cells have become more attractive field of research. The direct administration of chemicals are drugs can cause lot of disadvantages and side effects so it is important to modify the mode of treatment.

Nowadays the application of nanomaterials to treat tumour cells have been reported. High surface area to volume ratio of nanomaterials have functionalized to bind with some drugs which could be released exactly at targeted tumour cells. Mostly, nanoparticles with different shape, size can be engineered to treat targeted cells or tissue. Nanoparticles prevent the decomposition of attached drugs and increases the absorption of maximum intake of drugs by epithelial diffusion.

The new way of treatment has developed by University of Michigan in which the semiconducting nano materials have been used to destroy the tumour cell by making short-circuiting tumor cell metabolism. An anticancer drug has synthesised when light is illuminated by semiconducting nanoparticle attached with platinum electrode (Kotov et al. 2009).

1.1.8.2 Treatment for Lung Cancer Chronic Obstructive Pulmonary Disease

Nanosized particles have been used to carry chemotherapeutic drugs as inhalers or spray materials for lung cancer. In this treatment, drugs are misted with nanosized fine particles which directly delvers the drug into all parts of the lungs for quick medical effect (Fymat 2016). Recently, some smart nanoparticles employ as carrier

to deliver therapeutic drugs at tumor cells present in the deepest part of lungs. These nanoparticles are processed by using their magnetic properties. Every day, we have inhale a air with no of bacteria and viruses.

Chronic obstructive pulmonary disease (COPD) causes irreversible obstruction of lung airways causing which can be treated only by nanomedicine due to potential penetration of drugs by nano sized carrier. These diseases have caused by infection of lungs by some viruses and bacteria. These virus outbreak some of airborne deadliest epidemic diseases like influenza and pneumonia (Kotov et al. 2009). Currently, the Covid-19 outbreak effects lungs very severely in which nasal administration nanomedicine would be very useful to treat. Nanomedicine can be penetrated very easily to the virus surface where it stops their RNA replications.

1.1.8.3 Pancreatic Cancer

One of the most life threating diseases is pancreatic cancer which can't be controlled due to lack of diagnosis and some disadvantages in pharmaceutical treatment. Targeted tumour cells produce resistance against anticancer drugs and leads to critical conditions. Now some of nano technology-based carriers have been used for both diagnosis and treatment. Nanosized drug delivery process resists the tumour cells while decreasing toxicity. The nano-drug combined formulations which consist of liposomes polymeric nanomaterials, CNT, hybrid nanooptics and quantum dots, have developed to treat pancreatic cancer (Sielaff and Mousa 2018). Some chitosan functionalized poly(ethylenimine) with amphiphilic poly(allylamine) nano formulations have been used to carry hydrophobic drugs to target cells. Recently it is reported that curcumin filled polymeric nanoparticles have reduced the growth of primary tumour (Manzur et al. 2017; Rebelo et al. 2017).

1.1.8.4 Diabetes

The rate of diabetes is increasing day by day and it affects all the people irrespective of the age group. The application of nanotechnology becomes very significant in the management of diabetes (Gupta 2017). Normally, the oral administration of insulin has destroyed by acid present in the stomach and it makes the objective of the treatment useless. In order to deliver the insulin directly into blood stream, nanotechnology approach has to be used. In this, the insulin molecules are bound to colloidal nanoparticle which protects the insulin form gastrointestinal tract and transports into systematic circulation. Hydrogels, antiproteases, cyclodextrins are used to encapsulate insulin molecules and the residence duration of insulin has been increased in the vicinity of intestinal cells for successful absorption.

The most effective biocompatible polymeric nanoparticles are being used as a carrier for insulin. Polymeric nanomaterials like N,N-dimethylaminoethyl methacrylate, polyanhydrides, polyurethanes, polyacrylic acids and polyacrylamide have been reported as very good insulin carriers. These polymers are pH sensitive and it

releases the loaded insulin when a desirable pH is achieved (Harsoliya et al. 2012; Cui et al. 2009).

1.1.8.5 Skin Diseases Therapies

Generally, the skin inflammation is a common among people. These inflammations are caused by exposure of skin in UV light. Now a day's nanotechnology is applied to treat skin related problems. Nano emulsions Nano capsules, nanoliposomes, and nanoparticles are commonly formulated into cosmetic products and body lotions (Basavaraj 2012). These materials diffuse the stratum corneum part of the skin. Currently, sunscreen cosmetic materials have formulated with insoluble titanium dioxide or zinc oxide nanoparticles, which are colourless and reflect/scatter ultraviolet more efficiently than larger particles (Nohynek et al. 2007). Lipid nanoparticles are one of the ingredients is added to enhance the film forming ability of cosmetic products and also to hydrate the dry skin.

1.1.8.6 Cardiovascular Diseases

Hypertension and hypercholesterolemia are two main risk factors lead to cardiovascular diseases like thrombosis, infarction and stroke. Multiple drug therapy has given for treatment however it may show adverse effects. Now the applications of nanotechnology play protective. Carrier to deliver the diversity of active ingredients (Janko et al. 2013). The gold and silica nanoparticles have designed to deliver nitric oxide in the treatment of hypertension where the lowest concentration of nitric oxide has to be increased. It has reported that intravenous injection of CeO_2NP with highest antioxidant property, deceases the microvascular dysfunction and hypertension. Size of CeO_2NPs should be handled carefully because small variations in its dimension may become toxicity (Minarchick et al. 2015). Blood clots which is formed at the blood vessels are called as thrombosis which leads to block the blood circulation and it makes the patient to get cardiac attack. To treat this, nanoparticle is loaded with tissue plasminogen activator (tPA) which is directed to thrombus site and it removes the blood clots and makes free to blood circulation (Cicha 2015; Torchilin 2014).

1.1.8.7 Antimicrobial Activity of Nanomedicines

Day by day the reports on increased antibiotic resistance becomes challenging to human health. The poor solubility, chemical stability and enhanced side effects are decreasing the efficiency of currently using antibacterial drugs. To overcome this, researchers are using nanomedicines. Natural or synthetic polymers with silver nanoparticles composite materials have been used as effective antibacterial agents since decades.

Silver incorporated silver sulfadiazine has been treated for a decade. Silver sulfadiazine is active when it was used at higher concentrations only (Ullah et al. 2019). The antibacterial agent is more active when silver nanoparticles are incorporated by electrospinning method. The antibacterial activity of polyvinyl alcohol and chitosan is enhanced by incorporation of silver nitrate by electrospinning method. Wide antibacterial applications of silver nanoparticles are increased when they have incorporated with zein, polymethyl methacrylate, chitosan, polyvinylpyrrolidone, polyacrylonitrile (PAN), and other polymers. Recently, CNT and GM are more active than silver nanoparticles against bacteria (GhavamiNejad et al. 2015; Yang et al. 2016; Maharjan et al. 2017).

Another major challenge for public health is fungal infection which develops substantial resistance against most of the drugs. The resistance has overcome by using nanoparticles as related to increased drug efflux from microbial cells, bio film formation. Nanoparticles deliver the required does of drug at infected site in that way it decreases resistance of microbes with less adverse effect. The required drug has entrapped or encapsulated in to the nanoparticle matrix and they are by the drug has reserved until it reaches the infected site or tissue. Because of the small dimensions it can be easily diffused in to the target cells to deliver active drug into the sites where it has supposed to be released.

The existence of the unique physiochemical and biological properties of nanostructures makes them compatible material for biomedical applications. Encapsulation of drugs into some of polymer nanoparticles has been done during their polymerization reaction. The oral administration of the drug would be reserved for long duration at gastrointestinal pathway for complete absorption. Poly-ε-caprolactone (Sinha et al. 2004), polyacrylamide (Sana et al. 2019), polyacrylate (Bilensoy et al. 2009), DNA (Bai et al. 2007), chitosan (Turos et al. 2007; Mao et al. 2001; Rejinold et al. 2011), and gelatin are some of the polymer nanoparticles in which drug has encapsulated during their polymerization reaction.

Antifungal drug delivery system of carbon nanotubes, MNPs, and silica NPs has been reported. Benincasa et al. (2011) showed that AmB conjugated to carbon nanotubes presented an excellent activity against clinical isolates of *Candida* spp. The antimicrobial activity against bacteria and fungi (*C. albicans*) was also demonstrated by scanning electron microscopy, showing that microbial cells were wrapped or entrapped by carbon nanotube networks (Olivi et al. 2013). The reduced graphene oxide nanosheets have antifungal activity against *Aspergillus niger*, *A. oryzae*, and *Fusarium oxysporum* (Sawangphruk et al. 2012). In 2014, Cui et al. (2014) showed graphene oxide as a novel two-dimensional nanomaterial for applications in health biomedical with antifungal properties and low cost. Also, Hussein-Al-Ali et al. (2014) demonstrated the antimicrobial activity of MNPs loaded with ampicillin to form a nanocomposite decreases the activity of *C. albicans*. Niemirowicz et al. (2015) also reported an inhibition of the growth of *C. albicans* by using MNPs that can be removed from human plasma, blood, serum, and abdominal and cerebrospinal fluids.

1.1.9 Nanomedicine and Tissue Engineering

Tissue engineering is the branch of science, which studies the development of new tissue and organs starting from a base of cells and scaffolds. Factors that influences the growth of the cell is introduced into the scaffolds to achieve completely functional organs or tissues for implantation. In this field nanoparticles have been used for control drug delivery, DNA probing, for controlled drug delivery (Wilson et al. 2010; Shi et al. 2010), imaging of specific sites, probing of DNA structures (Mironov et al. 2008; Koo et al. 2005), biomolecular sensing, gene delivery, photothermal ablation of cells (Prasad 2009) and, most recently, (Basarkar and Singh 2009; Wang et al. 2008). Additionally, many therapies utilize nanoparticles for the treatment of cancer, diabetes, allergy, infection and inflammation (Panyam and Labhasetwar 2003; Brigger et al. 2012; Kataoka et al. 2012). The nanoparticles plays vital role to improve the biological, electrical and mechanical properties of gene delivery, DNA transfection and viral transduction. Significantly, GNPs and TiO_2 nanoparticles have applied to increase the rates of cell proliferation for bone and cardiac tissue reformation. The contribution of GNPs enhances the osteoclast (bone resorbing cell) generation form hematopoietic cells in bone TE. Gene delivery for matured cells or stem cells has become significant field of research in TE. Human mesenchymal stem cells are multipotent cells that show immunosuppressive properties and have an intrinsic capacity to differentiate into various types of cells, including chondrocytes, osteoblasts, myocytes and adipocytes.

While nanoparticles have demonstrated promising potential in TE applications such as enhancement of biological, mechanical and electrical properties; antimicrobial effects; gene delivery and construction of engineered tissues, many challenges still lie ahead to introduce them into widespread clinical applications. For example, a compelling need exists, at first, for better assessment tools and methods of nanoparticle toxicity, carcinogenicity and teratogenicity. Second, the toxicity, carcinogenicity and teratogenicity of nanoparticles are all highly dose-dependent and exposure-dependent. In many applications, the nanoparticles are used below their threshold concentrations at which they are considered not harmful. However, bioaccumulation of nanoparticles inside the body over a large period of time is well known. Thus, any nanoparticle used in the human body has the potential to accumulate over a long period of time to reach a concentration that can cause toxicity to cells, cancers or harmful effects on reproductive systems and fetuses before their birth. In addition, even though there are numerous products containing nanoparticles/nanomaterials already in the market there are still some scientific and methodological gaps in the knowledge on specific hazards of nanomaterials. Currently, to the best of our knowledge, there are no international standards yet for nano-specific risk assessments, including specific data requirements and testing strategies. The risk assessments of nanomaterials are laborious and costly. Currently, manufacturers are committed to assess the safety of their nanoparticle-based products and to implement the necessary safety measures (self-supervision). To date, the regulatory tools are not nano-specific; e.g., the data requirements for notification of chemicals,

criteria for classification and labeling requirements for safety data sheets are still not widely available. Thus, there is a need for precautionary measures for applications of nanoparticles wherever there is a possibility of chronic bioaccumulation.

1.2 Conclusion

Nanoparticles exhibit superior biocompatibility and well-established strategies for surface modification, which have made them highly effective in numerous biomedical applications. The electric coupling between decellularized cells and proliferation rates upon several tissues have also been enhanced using nanoparticles. The validity of nanoparticles, when it comes to antibacterial growth, has also been studied with much promise. These nanoparticles have been deposited on biocomposite scaffolds, thus regulating bacterial infection during reconstructive bone surgery. Induction of cell mechanotransduction, which is responsible for many physiological processes in the body, was also stimulated by remotely controlled nanoparticles. This review has mentioned a new method for gene delivery. Specifically, magnetofection, which was accomplished through the use of plasmid DNA cationic lipids with complexes of DNA as they interacted through a magnetic force, thus increasing transfection efficiency. Related to this is the use of nanoparticles for the purpose of cell patterning. Three strategies were investigated for cell patterning: the use of MCLs, RGD motif-containing peptide coupled to the phospholipid of magnetite cationic liposomes and aminosilane modified with PEG and magnetic force.

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Chapter 2
Immunology and Nanotechnology: Effects and Affects

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2.1 Introduction

A body's immune system is functionalized to defend various infections and ailments from external source. Immunity can be tweaked as per the internal and external environ to match up with body's necessities. Immunotherapeutic strategies have been a cost-effective process utilised since ages to augment a body's immune sys-

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tem and identify, target, and abolish toxic cells within the body, thus making it a universal testing strategy for elimination of toxic cellular components (Davis and Brodin 2018). A successful therapy is governed by various factors like the structural intricacy of pathogen, an appropriate delivery system, the route by which it is administered, ability to recognise and attack specific target cells, host immunity, etc. (Witztum and Lichtman 2014). Amongst the bodily preventive barriers, the biological one constitutes of cells, soluble factors, and organs that creates a protective surface restricting the invasion of foreign matter. The immune system includes a complex array of biochemical and cellular responses intricately interlinked with some specific pathways and sporadically can be disconcerted at diverse stages leading to immunosuppression or stimulation. Thus, it is crucial to scrutinize immune interactions with any new chemical or biological entity before it is explored industrially, biomedically or therapeutically. With recent developments in the field of nanotechnology, immunotherapy has traversed in targeting diverse challenging disorders including cancer and autoimmune diseases. Moreover, the progression of several adjuvanted or inactivated vaccines to boost immune response, has led to the boom of nano-based self-adjuvanted particles (Shah et al. 2014). Nano sized materials and have been custom-made to either target or avoid interactions with the immune system and fit into various biological and medical arenas by interacting with the innate and/or acquired immune system for successful and efficient prophylaxis. The immediate line of mechanistic nonspecific defence against any infection is conferred by the macrophages and neutrocytes of innate system followed by an immunological memory of the lymphocytic cells developed by adaptive immunity to track down the infection and recognize similar pathogenic counterparts in future (Jones 2005; Mogensen 2009). Both these immune systems function absolutely dynamically in destroying an antigenic molecule in a salubrious body. Hence, nanoscaled particles are now being used to reorient and alter the specific immune responses for preventative and curative outcomes. Existing naturally or architecturally improved, these nanostructured materials include nanoparticles, nanoemulsions, nanotubules, liposomes, fullerenes, virus like particles, immune-stimulating complexes, etc. that provides a cutting-edge stratagem for substantial improvisations and modulations in the immune system to treat various diseases and thus embraces to develop newer immunomodulatory agents, that could effectually orchestrate or deliver immunologically active agents to specific target sites (Goldberg 2015; Dacoba et al. 2017). In this chapter, we enumerate the physical, chemical and biological properties of some nano-structured materials (vaccines, nanotubes, nanoemulsions, dendrimers, polymeric complexes, liposomes, virus like particles, etc.) on the immune response and the role of nanotechnology in modifying and tuning such properties for varied end applications. Immunostimulation and immunosuppression caused by nanomaterials and their mechanistic approach to interact with the immune cells without any unwanted immunotoxicity has also been highlighted. Therefore, our interest lies in exploring the “effects” of nanosized entities in immunological applications and also the potential challenges of how it “affects” the overall process of immune system.

2.1.1 Vaccines

In current times, many disease definite vaccines are in either in ready use or being developed rapidly. Newer progressions in immunological and molecular paradigm have led to the exploration of vaccine materials that are host and disease precise with immunological memory for better targeting and response over an extended period of time. A vaccine should effectually be able to trigger the T helper type-1 (Th-1) and T helper type-2 (Th-2) cells which further induces antigen-specific T and B lymphocytes for recognition and response to antigenic determinant (Guimaraes-Walker et al. 2008; Heffernan et al. 2011). Traditional vaccines used since twentieth century comprise of live, attenuated, killed, toxoid, conjugate, naked, lysates that are whole organism ones and also components like polysaccharides and bacterial toxins. Alternatively, new age vaccines focus on nucleotide differences and antigenic expression *via* sophisticated techniques like recombinant DNA technology and reverse transcription polymerase chain reaction, one among them being the DNA vaccine immunotherapy (Martin et al. 2008; Beckett et al. 2011). **"An overall effective vaccine"** balances and condenses the percentage transmission rate of a disease in an immunized/vaccinated population cluster in comparison to the unvaccinated ones under a given set of conditions (Haber et al. 1991; Halloran et al. 2010). Vaccine effectivity is reliant on the antigenic immunogenicity and can be improved by incorporating adjuvants that activate the humoral, cellular and mucosal immune responses against foreign antigens. Most nanoscale materials enhance the adjuvant potency by targeting its direct delivery to the immune system or increase the effect of innate immunity (Fifis et al. 2004; Chadwick et al. 2010) and system is depicted in Fig. 2.1. Some of the nanotechnological approaches like adjuvants and carriers that have been explored to improve the clinical effectivity of vaccine are reviewed here.

2.1.1.1 Virus Like Particle (VLP) Adjuvants for Vaccines

These virus-like particles ranging from 15 to 100 nm are highly diverse adjuvants and carrier systems comprising of either naturally found viral protein subunits formed by bioconjugation of viral capsid with antigens/ligands or synthetically engineered multidimensional scaffolds with surface presentation of antigens on them. Additionally, integrating ligands and mediators might as well make a vaccine more efficacious immunologically. Studies showed that VLP's incorporated with CpG oligodeoxynucleotides and protein Melan-A (melanoma antigen recognized by T cells-1) tend to activate tumour necrosis factor-α (TNF-α) and interlukin-2 (IL-2) inducing T_{CM} cells (central memory T cells) and T_C cells (cytolytic T lymphocytes) (Ghasparian et al. 2011). Their nano-size, uniform and symmetrical structure and stable conformation enables the favourable uptake of antigens by the antigen producing cells (APC's). The smaller the particle, the better is its penetration through the tissues and rapid its circulation in the lymphatic system leading to

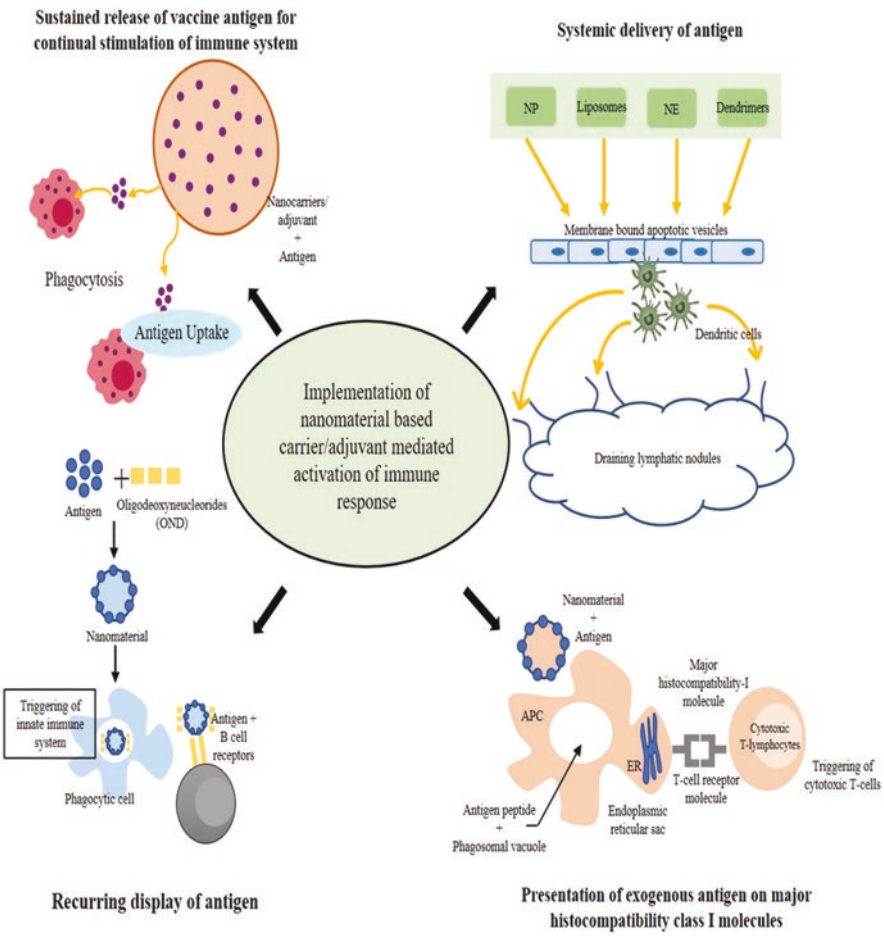

Fig. 2.1 Implementation of nanomaterial based carrier/adjuvant mediated activation of immune response

proficient triggering of acquired immune response as compared to large VLP's (Reddy et al. 2007; Manolova et al. 2008). Dendritic cell specific ICAM3-grabbing non-integrin monoclonal antibodies coated virus like particles also often illustrate better uptake and processing of extracellular proteins *via* the histocompatibility complex class II pathway stimulating the dendritic cell initiation and antigen targeting on both histocompatibility complex class molecules (Schirmbeck et al. 1996). Besides, it also briefs the CD4+ and CD8+ T-lymphocytes for antigen destruction (Kaba et al. 2012) mechanism. Two of the well-known VPL based vaccines, hepatitis B and human papillomavirus virus vaccines have been the oldest and first generation VLPs that was amassed by recombinant protein engineering in mass scale (No et al. 2011). Nevertheless, these had certain limitations with patients having

immunocompromised immune systems and geriatric population. Also, drawbacks of adjuvanted vaccines take in the probable risks of rare and severe immune response in individuals after a time duration. Therefore, it is crucial to investigate further on these lines for to modify vaccination efficacy (Martinez-Sernandez and Figueiras 2013). Nano sized VLPs thus offer improved and varied initiation of humoral and cellular responses for safeguarding against chronic influenzas and HIV contagions and attesting its importance to public health.

Chemically developed lipopeptide based **synthetic VLPs** are much more attractive option in assembling the nanoparticle and stabilization of antigen structures (Boato et al. 2007) to boost neutralizing antibodies against severe chronic infections like HIV-1. Synthetic VLPs have frequently been plied to demonstrate envelope glycoprotein GP120 based protein epitope mimetics (PEMs) (Riedel et al. 2011) which are recognised as new primes for vaccine research.

Since these synthetically derived VLPs do not require recombinant technology or monomeric peptide expressions from viral producer cells, these can easily incorporate MHC molecule bound T cell epitopes and palmitoyl-cysteine lipopeptide to activate Toll like receptor-2 (Ghasparian et al. 2011). The induction of immune response becomes much easier if the structural orientation, the antigenic design and presentation is done using the nanoparticle-based vaccine approaches that provide swift responses to the non-accessible and preserved neutralizing epitopes than the readily accessible ones (Mascola and Montefiori 2010; McBurney and Ross 2009) in order to control enormous rates of genetic alteration and variation during infection. Therefore, implementing nanosized viral particle-based adjuvants is the need of the hour for better projection of cell mediated immune responses, cytotoxic T-lymphocyte antigenic determinants and antibodies to restraint an array of opportunistic viral infections (Sanou et al. 2012).

2.1.1.2 Nanoparticle-Based Carriers for Vaccines

Another way of increasing the vaccine efficacy is the use of nanoparticle carriers as such or in combination with other immuno-mediators or human dendritic cell specific antibodies for enhanced antigen delivery and presentation (Look et al. 2010). Among them, is a biocompatible and biodegradable copolymer; poly (lactic-co-glycolic acid, PLG) used as a condensed carrier for hepatitis B surface antigen that aids quick absorption and endocytic organelle localization of vaccine antigens in dendritic cells as well as produces high concentrations of antibodies definite antigens (Bharali et al. 2008). PLGs also serve as good matrices supporting sheathing, co-delivery of active drugs in animal models (Mundargi et al. 2008). Other VLP carriers include nanogels, cationic liposomes, pullulan derivatives and several polymeric nanocapsules and nanospheres. Studies on the use of PLG hydrogels in mice models have revealed these carriers to simultaneously attain hypodermal delivery of hepatitis B antigens and sustained release of colony stimulating factor-2, an essential cytokine for segregating and maturing the dendritic cells (Chou et al. 2010). Such carriers efficiently cause the maturation and migration of the alpha component

of integrins to the draining lymphatic vessels induces hepatitis B antigen-specific antibody even at low antigen titre values. It is relevant to state here that these biodegradable virus like particle carriers are successful candidates for vaccine delivery daises as they not only integrate immunogenic antigens for immunocompromised systems but also balances the nascent structure and steady continual release of the biological antigenic intermediaries over a time frame (Mundargi et al. 2008). Another kind is the self-assembling peptide nanoparticle (SAPNs) vaccine carriers utilized for recurring antigen display. Customized through recombination techniques, SAPNs inculcate in them distinct properties of certain pathogens and VLPs and show improved immunogenic responses to antigens. Due to the icosahedral structure with coiled-coil repeated pattern of hydrophobic and charged amino acid residues, SAPN are a repetitive scaffolding motif that lead to antigenic display, cellular stimulation, increased production of high concentration-high affinity antibodies against phylogenetic antigenic determinants (Raman et al. 2006). SAPNs when tailored with the coiled protein trimer epitopes of severe acute respiratory syndrome coronavirus produces virus specific deactivating antibodies post vaccination (Negahdaripour et al. 2017). Upon immunization of chicken with SAPNs and merger of an immunogenic protein epitope into the extracellular domain of matrix protein-2 of influenza A virus showed better exhibition of antigen determinant oligomers in their nascent spatial arrangement and led to the reduced shedding H5N2 virus subtype in chickens (Li et al. 2018). Further, inserting both B and T cell epitopes of *Plasmodium* species sporozoite surface proteins and modifying it into the SAPNs led to the expression of high titre antibodies and memory T cells (producing interferon-γ) and offered a protection to the mice against the surface protein of malarial parasite *Plasmodium berghei* (Seth et al. 2017).

2.1.2 Nanoemulsions

Typically, two phase immiscible colloids with a dispersed and a dispersion phase, stabilized together using a surfactant modifier defined as nanoemulsions. Emulsions can be either oil in water or water in oil type. These systems are kinetically and to a large extent thermodynamically stable upon dilution. Emulsions can be termed as ‘nano’ or ‘micro’ depending on their component assemblage and the overall stability it confers to a system (Tayeb and Sainsbury 2018). The use of adjuvants dated back to 1990s when alum was the only ideal adjuvant used for most of the human use vaccine because of its safety and effectivity. It was only after this when emulsion adjuvants for vaccines re-emerged with the manufacture of **MF59**. A mixture of squalene oil with polyoxyethylene sorbitan monooleate and sorbitan ester as surfactants, MF59 adjuvanted vaccines have been successfully administered for influenza virus (Domnich et al. 2017). This adjuvanted vaccine works by improving cellular uptake of antigens and heightening the release of chemokines along with site accumulation of different white blood cells upon injection which further upregulates and matures the C-C motif chemokine receptor-7 expressed in semi-mature/

mature dendritic cells to then migrate to the draining lymphatic nodules (Calabro et al. 2011; Lin et al. 2020). In comparison to aluminium salts-based adjuvants, MF59 are potentially superior in inducing both humoral and TH-1 cell mediated immunity and providing antiviral immune responses even at very small doses of viral antigen unlike aluminium adjuvants that confer inconsistent antiviral immune response (Cioncada et al. 2017; Shah et al. 2019). Initial clinical investigation on MF59 vaccine as potent applicants for herpes simplex virus, human immunodeficiency virus, cytomegalovirus, hepatitis B and C virus, human papillomavirus etc. are currently being explored (Patel et al. 2019). Though being one of the most promising and effective adjuvanted vaccine, MF59 suffers a major drawback of being unstable and temperature sensitive in nature. Reactivity upsurge, muscular inflammation and pain at the injection site also limits its use (Schultze et al. 2008). **W805EC**, a *Glycine max* oil based nanoemulsion mucosal adjuvant has been investigated in animals and human model to exhibit enhanced mucosal, humoral and cellular immunity upon intranasal delivery. Its mechanism of action involves retaining the emulsion droplet structure which then binds to the negatively charged proteins. W805EC's micro size and positive zeta potential favour its smooth diffusion to the mucosa and cellular binding/uptake to the plasmalemma causing the initiation of innate and acquired immune response (Stanberry et al. 2012; Myc et al. 2013; Kim et al. 2014). In addition, the epithelial cells of mucosal membrane of the nasal cavity secretes cytokine that then activates the endocytic receptor DEC 205 to the lymph nodes. Nanoemulsion adjuvants also act as cell death inducers by releasing 'calreticulin', an immunological cell death signal and enable phagocytosis of antigen-loaded dendritic cells in tissues (Makidon et al. 2012; Bielinska et al. 2014). Emulsions inherently have the capacity to impede microbes and exhibit adjuvanted action and thus are used as multifaceted pharmacological agents for vaccinia, syncytial and influenza viruses, etc., are considerably less toxic with no antagonistic effects on the various models tested upon (Kaurav et al. 2018; Morcol et al. 2019).

2.1.3 Liposomes

Regardless of the availability of a number of nano adjuvants and carriers being used to deliver vaccines, traditionally, liposomes serve as excellent vaccine carriers for targeted delivery. Liposomes favour the encapsulation of a diverse group of antigens, polar and nonpolar drugs and exhibits increased immunogenicity. These are comparatively safe on the body without the emergence of any adverse immune reactions. Some of the commercially marketed liposomal carriers are mainly of PEGylation type, such as Doxil®, Ambisome®, Myocet®, etc. (Marasini et al. 2017; Joshi et al. 2019). The positive charge on lipid structure and its overall spatial arrangement enables liposomes to competently absorb and preserve antigens to nanostructures, retain it at the site of administration, augment better immunogenicity with stimulation of innate and T helper type cell responses that lead to the triggering of antigen presenting cells (Henriksen Lacey et al. 2010; Tandrup Schmidt

et al. 2016; Marasini et al. 2017). Improved systemic adjuvant action, antibody mediated immunity for various applications and reduced toxicity at multiple sites of monophosphoryl lipid A and Toll-like receptors in the body are some of the characteristics of cationic liposomes (Bal et al. 2011). Reports on amended constancy and immunity against tuberculosis causing *Mycobacterium* has been elucidated (Mohammed et al. 2010). Liposomal adjuvants such as virosomes, archeosomes, etc. have also been seen to have immunostimulatory effect on several cellular mediators. In conglomeration with vesicles of cell membrane, these assist the appropriate targeted antigen delivery as vehicles (Yu et al. 2019). Mechanistically, the size of the liposomal entities decides their method uptake *viz.*, smaller sizes mimic the viral uptake whereas bacterial uptake pathway is followed with increase in size (Zahednezhad et al. 2019). Hydrodynamic size, two-dimensional surface electric charge, physiochemical features makes liposomes suitable for its use as a whole vaccine soon that could surpass the current cationic lipid particulate vaccines such as quaternary amines, imidazole, cholesterol and amidine-based compounds for treatment of several infectious diseases.

2.1.4 Immunostimulatory Complexes (ISCOMs)

Lipophilic antigenic adjuvanted nanocarriers used for vaccine delivery, ISCOMs are a conglomeration of phosphatides, aglycone carbohydrate moieties, cholesterol and antigens forming open sphere like structural lattices of the size of viruses which allows their incorporation into the cell membrane and enables dendritic cell mediated antigenic endocytosis (Barr and Mitchell 1996). ISCOMs can suitably be used for oral, intranasal, parenteral delivery of antigenic epitopes to induce immune response of mucosal sites (Rhee 2020) and their connotation with intracellular lipid bilayers enables their entrapment within the cytosol present in antigen producing cells. Both antibody and cytokine-mediated immunity against a range of antigenic determinants as well as T helper type-1 and 2 pathways are actuated by these immune-stimulatory carriers (Cibulski et al. 2016). Besides the presentation of major histopathology complex-I (MHC-I) protein on antigen processing cell surfaces with its exposure to the transmembrane glycoprotein CD8, ISCOMs also assist the cross presentation of external antigens in the endogenous pathway, proving their exclusivity which is not seen in most other nanocarriers (Cibulski et al. 2016; Morelli and Maraskovsky 2017). The mechanism trails around with the transportation of antigen from the endosome into the cytosolic matrix of the antigen producing cells resulting in its proteasomal deprivation and demonstration to the T lymphocytes through MHC-I pathway. This process is initiated and progressed *via* the C-type multi-lectins expressed on the dendritic cell exterior and bound to the carbohydrate moiety of saponins. This characteristic of ISCOMs is advantageous in the targeted delivery of vaccines at mucosal sites (Corthésy and Bioley 2018). Research on vaccine delivery by these immune-stimulating complex adjuvants to oral and nasopharyngeal sites have also gained importance (Mowat et al. 1999; Hu

et al. 2001). Studies to boost the mucosal and systemic adjuvant function of B-cell targeted fusion protein cholera toxin A-1in has shown that ISCOMs preserve this fusion protein and avert its enzymatic disruption in the digestive tract (Helgeby et al. 2006; Harandi and Lycke 2017). In fact, the correlation of cholera toxin A-1 protein with ISCOMs leads to highly immunogenic entity which on administering in nano quantities are well tolerated in the systemic mucosal routes (Mowat et al. 1999). McEntee et al. (2015) demonstrated high CD4+ T lymphocyte-immunoglobulin A/G immune responses in mice models upon administration with the ISCOM-cholera toxin A-1 amalgam and also highlighted their substantial stability upon freeze drying and lyophilization (McEntee et al. 2015). Recently designed ISCOMATRIX™ therapeutic adjuvants have improved antigenic and immunomodulatory properties in comparison to ISCOMs and have much wider applications. Devoid of an antigen, ISCOMATRIX™ vaccines contain a semi-purifiable Quil A extract which has an additional therapeutic and prophylactic impact on a diverse group of bacterial, viral, cancer antigens (Skwarczynski and Toth 2016). These exhibits humoral as well as cellular immunity and can modulate both the immune response systems by both the major histocompatibility complex pathways. By directing the distinctive cytotoxic T-cells, T-helper cells and immunoglobulins towards the mucosa, these antigen devoid adjuvants have been used to treat a variety of infectious diseases. Likewise, another cationic variant of ISCOMATRIX, the PLUSCOMs are much more competent in conferring effectual antigenic exhibition and amplified T-cell immune response against very small doses of antigens as compared to the conventional ISCOMs (Pham et al. 2009). However, ISCOMs require a lipophilic antigen because of the open spherical structure they possess, thus limiting their use in the other antigen types (Sun et al. 2009). ISCOMs could be an exhilarating method for mucosal vaccination in the times to come.

2.1.5 Polymeric Micelles, Dendrimers and Carbon Nanotubes

Strategically oriented nano assemblage of synthetic polymers, polymeric micelles are amphipathic in nature. Their less energy bonds help them dissociate easily and hence these are considered as a suitable candidates for varied applications. Studies depict that a 30S peptide micellar vaccine of these polymeric micelles can induce antigen-defined antibodies for pneumonia (Morein et al. 1983). With high levels of Immunoglobulin/A and hemagglutination inhibition titres, these micellar structures modified as per their hydrophilic and hydrophobic units can serve well for H5N1 avian flu (Prabakaran et al. 2010). Their substantial stability and alterable physicochemical properties have made them suitable as nanocarriers in vaccine delivery. Dendrimer molecules are structurally alike the micelles are covalently linked, making them much tougher and indissociable. These homogenously branched polymers are highly compatible for a number of industrial and biomedical applications. A derived dendrimer, known as the multiple antigenic peptide (MAP) are currently in use as nanocarriers for vaccine delivery and have shown promising results with the

intramuscular malarial vaccines (Kim et al. 2018). Carbon embedded nanosized tubes also are effective in initiating immune response. Carbon nanotubes have branched carbon atoms linked together to form a closed structure permitting them to be excellent immunogenic carriers for antigen-antibody response.

2.2 Modulation of Immune System by Nanoscale Materials

Mutual conglomeration of Nanotechnology and Immunology have shown promising effects in therapy and treatment of diverse array of ailments. The interaction of nanomaterial with the immune system sometimes can persuade either a beneficial or detrimental outcome on the immune system. This immune based stimulation or suppression is dependent on a number of factors like the antigenic structural conformation, process end products, the stability and reactivity of the material. As a result, this immunomodulation affects the immunogenicity, adjuvanticity, inflammatory and recognition mechanisms of the immune system, diminution in the cellular components with decreased stimulus, incapacity to deal with infection and other malignancies, allergies, etc. (Dacoba et al. 2017). Some of the immunostimulatory and immunosuppressive effects of nanomaterial-based therapeutics are conversed here. And schematic is represented in Fig. 2.2.

2.2.1 Immuno-Stimulation

The overexpression of the immune-stimulators by the activation of certain cell components can lead to a number of consequences cellular immunogenicity and cell sequestration. Therapeutic nanomaterials may generate a specific antigen-antibody response for recognition of both the nanoscale material and body's own jots as seen in most of the biologically utilized prophylactics (Chamberlain and Mire-Sluis 2003). A research data on polyamidoamine dendrimers revealed covert antigenic response of 3, 5 and 7th amino group of dendrimers (Roberts et al. 1996). A parallel study on the cross reaction between the C_{60} and C_{70} fullerenes yielded specific polyclonal antibodies, but did not produce anti- nanogold particle specific antibodies (Chen et al. 1998). This disruption in the process of systemic antigenic exhibition could possibly be due to the varied surface properties and functional assemblages of these particles, reactivity to a number of moieties and their predisposition genetically. Adjuvanticity is another aspect of immune-stimulation. Most of the nanoparticles are used as effective vaccine adjuvants for an upsurge in immune response. One such example is the HIV2 viral vaccine that upon reaction with nanomethylacrylate particles exhibited much higher volumes of specific antibodies in mice in contrast to conventional aluminium based adjuvants (Saravanan et al. 2018). A substantial titre of Immunoglobulin G was produced by liposomal nanoparticles in purified rabies virus glycoprotein immunized mice (Ertl 2019). Dykman et al.

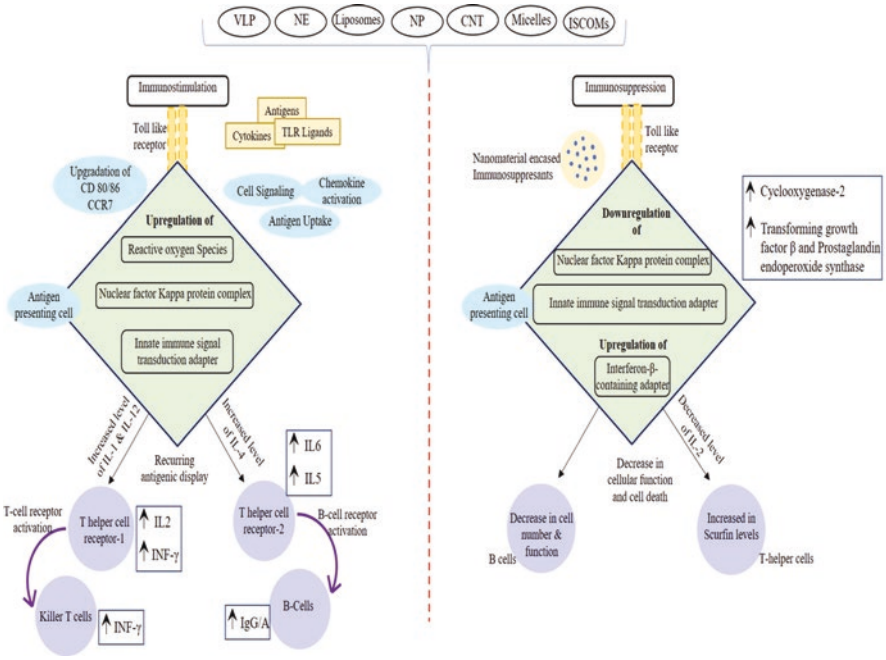

Fig. 2.2 Nanomaterial instigated immunomodulation of the immune system

(2004) demonstrated the agglomeration of gold nanoparticles with the whole surface bound half-antigen conjugates shot up the specific immunoglobulin levels in immunized animals. Further clinical investigation on hypersensitivity reactions of nanocarriers and also the antigenic structure-activity association needs to be dealt with. Inflammation is a result of the interaction of nanoparticle-immune response thus modulates the cells of immune system. Inflammation is a preliminary detection phase where upon recognising a foreign entity, immediate activation of cytokines and chemokines comes into play, disrupting the foreign particle. Nevertheless, the exact molecular mechanism of by which the immune cells trigger an inflammatory response when in contact with different types nanomaterials is still inadequately perceived. A better understanding of the structural composition the surface charges, the conformational motifs might throw light on the mechanistic functioning of inflammatory reactions. Positively charged Liposomal carriers are much better inflammation reaction inducers than the negatively charged ones. Cationic liposomes combined with polyamidoamine dendrimers were responsible for increased secretion of IL-2, Type-II Interferon and cachexin upon interaction with the human WBCs (Hattori 2016). Also, an upgraded expression of CD80/86 and maturation of dendritic cells was reported when combined with bacterial DNA by Cui and workers (2005). Liposomes have proved to be beneficial in cancer therapy as they possess anti-tumour properties. Another integral part of inflammatory responses is the balance between Type 1and 2 immunity. T helper cells 1 and 2 is triggered by

inflammation, which then stimulates the cell mediated and humoral immune response primarily shielding against anaphylactic or antibody dependent immune reactions (Bal et al. 2011; Marasini et al. 2017).

Amid the nanocarrier and adjuvants used for vaccination, nanoparticles can influence both the T-helper cell type responses depending on their relative sizes. Nanoemulsions, dendrosomes and smaller particles in the range of 100–400 nm exhibits only type-1 immune response (Manolova et al. 2008). Consequently, it is extremely vital to have a detailed scrutiny on how the material configuration and its activity could influence the immune response and prevent adverse cellular reactions. A more comprehensible approach on the mechanism of nanomaterial-modulation mechanism and its effect on the immune system will help attain efficient and improve designs for vaccines.

2.2.2 Immunosuppression

Suppression of immune response by the nanomaterials might be advantageous in preventing harmful aversions, autoimmune diseases, infections, etc. Nanomaterial mediated down-regulation of immune system could help reduce severe inflammatory disorders, a few of which has been emphasized here. Dendritic polymers merged with glucosamine molecules served as an inhibitory 'go-between' in induction of cytokines in human phagocytic and dendritic cells that were exposed to bacterial lipoglycans (Kim et al. 2018). Lipid based engineered nanoparticles favourably conglomerate with cell adhesion molecules on the inflammation induced endothelium, thus weakening inflammatory and hyperallergic reactions in the bronchioles by depriving the lymphocytic cells to attach the adhesion molecules, thereby decreasing the local inflammatory response at the site (Mitsui et al. 2016). Likewise, solid lipid nanoparticle and butyric acid cholesteryl ester conjugates diminished the binding of lymphocytes to inflamed endothelial cells making its place into colon cancer prophylaxis (Dianzani et al. 2006). Nanosized lactic-glycolic acid polymers embedded into celestone facilitated the reduction of inflammation in arthritic rat models (Kumar et al. 2017). Buckyball's ability to absorb electrons onto their ellipsoid surface, thus acting as scavenging moieties to reactive oxygen species enables them to diminish reactive species generation and immunoglobulin E arbitrated signalling (Mitsui et al. 2016). When single walled carbon nanotubes were exposed to mice with pharyngeal infection, amplified activation of dendritic cells followed by phagocytic cells of the alveolus and lymphocytes were triggered to reduce the inflammation in alveolus (Nahle et al. 2020). Fascinatingly, multi-dimensional nanotubes mediate the function of T lymphocytes can therefore modulate the T cell proliferation and suppress T cell antibodies. CNT also causes the upregulation of the expression of cylocoxygenase-2 and prostaglandin endoperoxide synthase enzymes in the mice spleen. These enzymes are modulators of cellular proliferation and cell death in tumours and other forms of cancer. Transforming growth factor also play a role in immunosuppression and controls its release in the phagocytes of

alveoli. Nanoemulsions can as well have immunosuppressive responses while delivering auto-antigens in the body. $W_{80}5EC$ in combination with thyroglobulin, an auto-antigenic dimeric glycoprotein, exhibited abridged T helper cell-1 and 2 responses with upregulation of enzymes like scurfin and transforming growth factor-beta and improved T cell regulation. The nanoemulsion can therefore stimulate Toll like receptors-2 and 4 (that contains myeloid differentiation primary response gene-88 and Interferon-β containing adapters which induce pro-inflammatory cytokines), control targeted antigenic delivery and modulate the immune system (Bielinska et al. 2016). Extensive research conducted on nano sized material based immunostimulation has led to the invention of various bioengineered remediation for inflammation that could be tailor made to suit a particular application. Uninvited immune suppressing effect, nevertheless needs detailed inspection to understand how a nanomaterial could mechanistically confine with the body's immunity concurrently, while conferring protection against various malignancies and infections. Therefore, scrutinizing a material for its suitability, reactivity, stability, activity, toxicity is of utmost importance to help restrict immunosuppression and deliver better biological therapeutics.

2.3 Conclusion

The merger of immunology and nanotechnology in current times have proved to be a boon to immunotherapy based preventive healthcare arena and regenerative medicine. Nanotechnology derived prophylaxis has revealed propitious outcomes in the form of vaccines, nanocarriers, nanoemulsions, liposomes, immunostimulatory complexes, nanotubules, dendrimers, etc. for targeting specific aliments. Nanomaterials with varied dimensions, structural orientation, charge, porosity, reactivity and stability exhibit immuno-compatibility that helps in designing custom made immunological responses to target a particular disease/disease. Exploring the use of ligand molecules with nano-adjuvants and carriers have considerably modulated the immune cells to identify and trigger target specific receptors. For example, the absorption process of nanoparticles with different cytokines and chemokines, that is used as vaccine carriers (to activate innate and acquired immune systems) have rendered protection against many epidemics and viral diseases. Nowadays, antibody mediated immunological replacement therapy is finding its way where nanomaterial conglomerated immunoglobulin moieties could possibly modulate and manipulate the immune system. Also, immunomodulatory stratagems for preclinical experiments has led to the successful development and commercialization quite a handful of nano-preparations. Nanotechnology has also contributed significantly in managing advanced aliments like HIV, neuroendocrine tumours, tuberculosis, and many more. Hence it is crucial to understand the underlying immunological mechanisms to further improve the strategy and engineering of nano based formulations for better treatment exposition.

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Chapter 3
Role of Nanocomposites in Sensors and Medical Devices

Jeyachandran Sivakamavalli, Kiyun Park, and Ihn-Sil Kwak

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3.1 Introduction

Nanoparticles could be helpful to crosslink the hydrogel or microspheres, to entrapping property to the hydrogel network (Thaya et al. 2016). Because most of the nanoparticles and composites possess the electrochemical properties mechanical, optical, thermal, barrier, sound, magnetic, electric stimulation, etc. (Sivakamavalli et al. 2014). Due to the presence of these features these nanomaterials are used in catalysis, separation devices, drug delivery, and many other biotechnological areas such as antimicrobial activity and biofilm inhibition studies (Thaya et al. 2018). When combined with few polymers which provide the suitable nanostructures for instance clay minerals and forms the intercalated nanocomposite. Monomer units are combined together and forms the polymer chains is inserted with clay minerals resulting in a well ordered multilayer stacking morphology. Another one

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nanocomposite if produced through exfoliation or deamination, here clay platelets are uniformly dispersed in a continuous polymer matrix. However, it should be noted that in most cases the cluster (so-called partially exfoliated) nanocomposite (III) is common in polymer nanocomposites (Fig. 3.1).

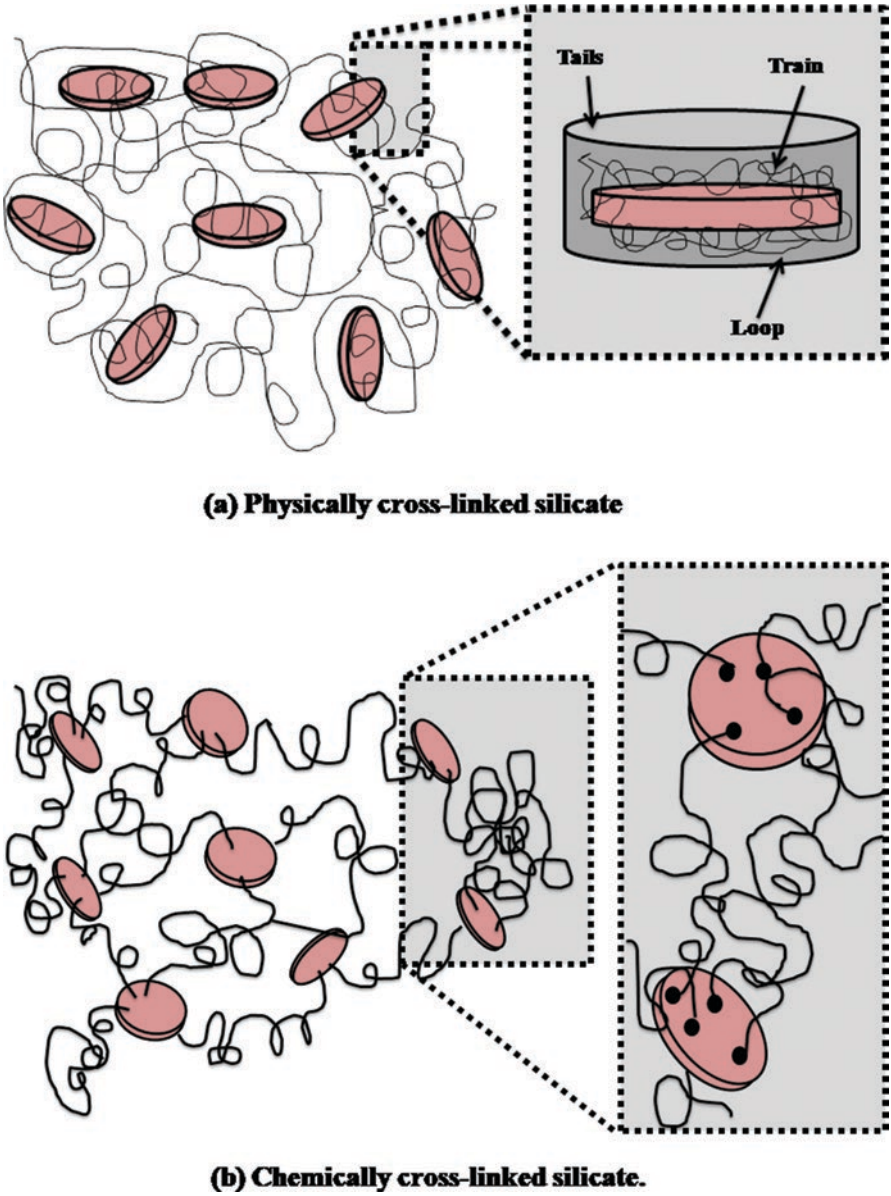


Fig. 3.1 Nanocomposite polymer hydrogels (**a**) Physically cross-linked silicate and (**b**) Chemically cross-linked silicate. Clay based polymer composites

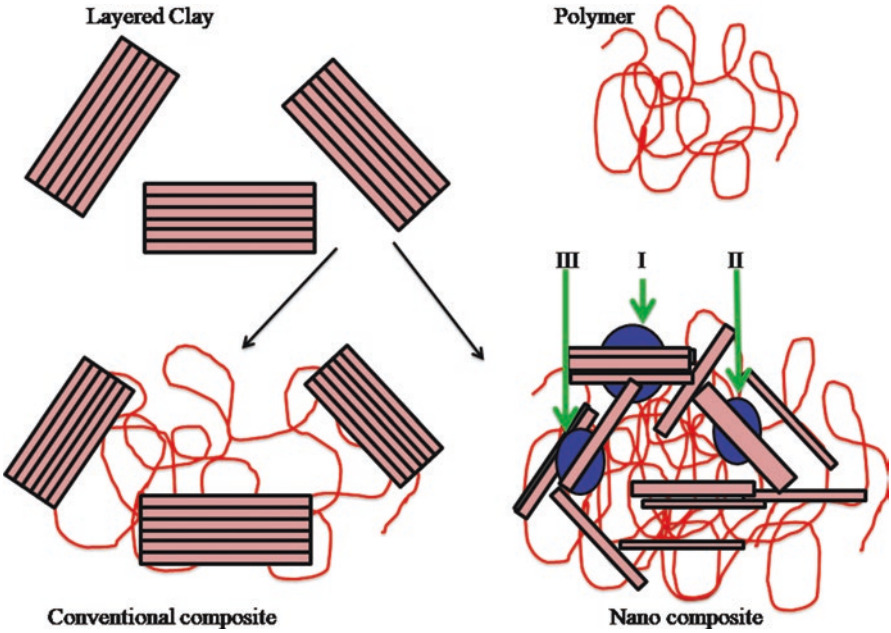


Fig. 3.1 (continued)

3.1.1 Processing Techniques

Three methods were used to prepare the clay-based polymer nanocomposites, including *in-situ* polymerization, solution exfoliation and melt intercalation. As shown in Fig. 3.2, each technique consists of sequential steps to get polymer nanocomposites and begin with organoclays or sometimes pristine clays. *In-situ* polymerization, monomers are intercalated into layered clays and consequently polymerized within the gallery via heat, radiation, pre-intercalated initiators or catalysts. In solution exfoliation, coated clays are exfoliated into single platelets using a solvent polymer should be soluble one. The polymer suspended with clay and adsorbed onto the platelets, at last the solvent is eliminated from the clay polymer complex through evaporation. In melt intercalation, coated clays are directly mixed with polymer in the molten state.

3.1.2 Properties of Nanocomposites

Polymer nanocomposites exhibit extremely improved features and performance as compared to other polymers. Such improvement is attained without the increase of polymer density and the loss of its optical properties and recycling. For example, a

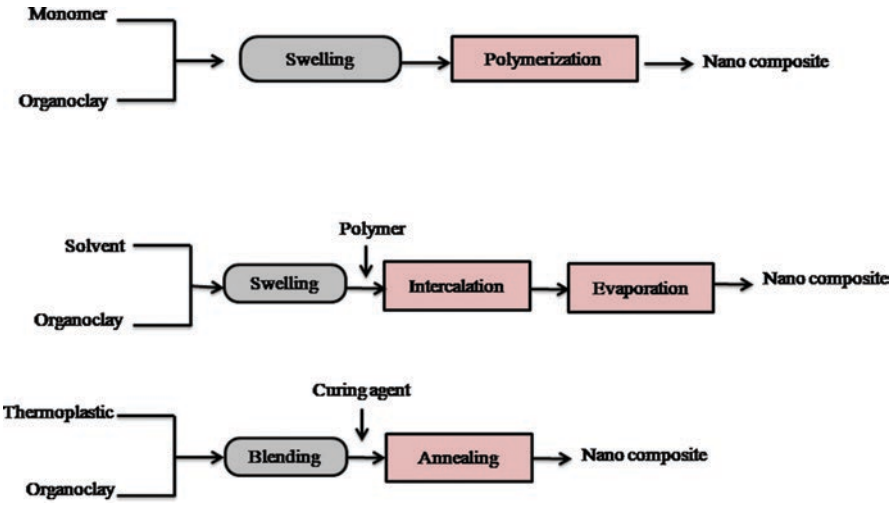


Fig. 3.2 Flowchart for three processing techniques for clay-based polymer nanocomposites

polymer nanocomposite has 2–8% of clay which gives the enhanced mechanical (stress, strain) properties together with the thermal (dimensional) stability; these materials reduce the gas and liquid permeability. Moreover, improve the flame retardancy while retaining optical clarity of pure polymer. Finally, this can display interesting conductivity and improved biodegradability.

3.1.2.1 Mechanical Properties

The development in mechanical features of polymer nanocomposites consist high rigidity and good affinity between polymer and organo clay materials. For instance, stronger edge connections considerably decrease the pressure absorption point upon frequent distortion which simply take place in conventional composites reinforced by glass fibers and thus guide to weak fatigue strength. The unique mechanical properties of nylon-6 clay nanocomposite synthesized by *in-situ* polymerization be first established by researchers at the Toyota Central Research Laboratories. Those type of nanocomposites possess strength and modulus, namely, 40% in tensile, 60% in flexural strength, 68% in tensile and 126% in flexural modulus. RTP company have reported nylon-6 clay nanocomposites synthesized from direct melt intercalation contains the similar properties like above materials (Sherman 1999). The increase in modulus is believed to be directly related to the high aspect ratio of clay layers as well as the ultimate nanostructure. Moreover, a dramatic increase was also observed in exfoliated nanostructures such as montmorillonite (MMT) based thermoset amine-cured epoxy nanocomposite (Wang and Pinnavaia 1998; Zilg et al. 1999).

3.1.2.2 Thermal Properties

Generally thermal stability of polymer nanocomposites produce the more volume of volatile products because of its easily heat nature. This thermal stability is enhanced using clay platelets that hinder the diffusion of volatiles and assist the formation of char after thermal decomposition. Blumstein (1965) evidenced that the thermal stability development in polymethyl methacrylate (PMMA) nanocomposite, showed that intercalated PMMA have 10% clay degraded at about 40–50 °C higher than unfilled PMMA. Followed by Lim et al. (2002) reported that improved thermal stability of nanocomposites made with various organoclays and polymer matrix. For instance, the upgrading in thermal stability is account for cross-linked polydimethylsiloxane exfoliated with 10% of orange montmorillonite (Burnside and Giannelis 1995) and intercalated nanocomposites made from the polymerization of methyl methacrylate, styrene (Noh and Lee 1999; Akelah and Moet 1996; Doh and Cho 1998) and epoxy precursors. Another important thermal behavior through heat resistance upon external load which can be measured from the heat distortion temperature (HDT).The HDT of nylon-6 nanocomposites stated by Toyota researchers which increased from 65 °C of pristine nylon to clay minerals. Moreover, the aspect ratio of clay platelets was observed to affect greatly the 145 °C. The increase in HDT has also been observed in clay-based nanocomposites for other polymer systems such as polypropylene (PP) and polylactic acid (PLA). Such kind of increment in HDT is very difficult to achieve in conventional polymer composites reinforced by micro-particles. Finally, flame retardancy and mechanical properties are both better in clay-based polymer nanocomposites while the mechanical features are degraded in polymer composites with conventional flame retardants The fire resistance of polymer nanocomposites is ascribed to the carbonaceous char layers formed when burnt and the structure of clay minerals. The multilayered clay structure act as an outstanding insulator and mass transport barrier. Char development and clay structure impede the escape of the decomposed volatiles from the interior of a polymer matrix (Gilman et al. 1998).

3.1.2.3 Barrier Properties of Nanocomposites

Polymer nanocomposites have admirable barrier nature against gases (e.g., oxygen, nitrogen and carbon dioxide), water and hydrocarbons. Many reports evidenced that the decrease in permeability strongly depends on the aspect ratio of clay platelets, with high ratios dramatically enhancing gaseous barrier properties. The best gas barrier ability seen in polymer nanocomposites with fully exfoliated relative permeability coefficient for polyimide (PI) filled with 2% of organ clay. The permeability to water vapor of exfoliated poly (caprolactone) (PCL) nanocomposites has also been examine a dramatic reduction in the relative permeability with the increase of nanometer clay platelets (Messersmith and Giannelis 1995). In addition, polymer nanocomposites have also shown improved barrier properties against organic solvents alcohol, toluene and chloroform.

3.1.2.4 Electrical Conductivity of Nanocomposites

Clay natural resources display sole electrical properties, particularly ionic conductivity, although the clay layers can be regarded as insulators, the hydrated interlayer caution and their mobility ensure a important ionic conductivity. Furthermore, the intercalation of neutral type might affect the hydration shells of interlayer captions and so appreciably modify the ion mobility, electrical conductivity and other electrical parameters. The ionic conductivity of crown ether-clay was accounted to be several orders of magnitude higher than corresponding clay (Ruiz-Hitzky et al. 1995; Aranda et al. 1992). In addition, it increases temperature up to a maximum value depending on the nature of the intercalated crown ether. Further development in conductivity is predictable by intercalating electroactive polymers into clay minerals. The ionic conductivity of the polyethylene oxide (PEO)-clay nanocomposites avoid the movement of anions (negatively charged clay layers), the anion-complexed cation interactions, PEO-clay nanocomposites show higher ionic conductivities than clays, temperature maximum at around 600 K. Moreover, the maximum conductivity in the direction parallel to clay layer 10^{-5} to 10^{-4} S/cm range, alike nanocomposite reported by direct melt intercalation of PEO (40%) in to Li-MMT (Vaia et al. 1995).

3.1.2.5 Biodegradability of Nanocomposites

Another attractive and stimulating property considerably improved biodegradable nature of nanocomposites made from organoclay and biodegradable polymers. Ratto et al. (1999) first reported biodegradability of PCL nanocomposites showed an improved biodegradability than pure PCL. Lee et al. (2002) proved biodegradation of aliphatic polyester-based nanocomposites displayed the retardation of biodegradation improved the barrier properties of aliphatic polyester nanocomposite clay. Ray et al. (2003a, b) showed PLA nanocomposites prepared with organoclay enhance the biodegradation, which helps for the terminal hydroxylation in the clay layers. A respirometric test demonstrated the degradation of PLA matrix in a compost environment around 58 °C. Conversely the weight loss reflects the structural changes; CO_2 evolution provides the ultimate biodegradability of PLA in nanocomposites.

3.1.3 Applications and Commercial Development of Nanocomposites

Polymer nanocomposites exemplify an exciting and promising alternative to conventional composites owing to the dispersion of nanometer clay platelets and their markedly improved performance in mechanical, thermal, barrier, optical, electrical,

and other physical and chemical properties. Thus, many companies have taken a strong attention and invested in increasing nanoclays and polymer nanocomposites for commercial products.

3.1.3.1 Applications in Automotive Components

Nowadays usage of automotive vehicles and industries increasing rapidly hence the components to build the vehicles and other things also essentially needed, so there is a prerequisites to need for the polymer nanocomposites also increasing hugely in automotive and other industries. Nevertheless, such composites are made-up of large amounts of micro-particles, thermal stabilizers, chemical resistant and flame resistant additives into the polymer matrix. Consequently, their improved performance often comes with the increase in materials density and low fuel efficiency. In disparity, polymer nanocomposites offer elevated performance with noteworthy weight fall and reasonable resources for transfer industries such as automotive and aerospace. Toyota Motors in early 1990s (Okada et al. 1991) produced the nylon 6 nanocomposites for the usage of timing-belt cover, exhibits good rigidity, excellent thermal stability and no wrap and also saved the weight 25%. The weight advantage of polymer nanocomposites could have a significant impact on environmental defense and material recycling.

3.1.3.2 Packaging Materials

The outstanding barrier properties of clay-based polymer nanocomposites would consequence in substantial improvement of shelf-life of packaged foodstuff. In the meantime, the optical lucidity of polymer nanocomposite layer is normally alike pristine foil, which is impossible for conservative polymer composites. Hence, the advantages would build acceptable extensively in wrapping industries as packaging films and beverage containers, such as processed meats, cheese, confectionery, cereals, fruit juice and dairy products, beer and carbonated drinks bottles. For instance, Bayer developed new plastic films for food packaging made of nylon-6 exfoliated nanocomposites (Scherer et al. 1999). A variety of eco-friendly polymers and polymer nanocomposites reported including PLA, poly (butylene-succinate) (PBS), PCL, unsaturated polyester, polyhydroxy butyrate, aliphatic polyester. Amongst, PLA reflects more commercial attention due to its renewable property and readily biodegradable.

3.1.3.3 Nanocomposite Coatings and Pigments

Novel nanocomposite additives have been developed by TNO materials based on a combination of layered natural or synthetic clays and block-co-polymers or surfactants. The excellent adhesion connecting the clay layers and polymer matrix

encourage amazing enhancement in material. The plant colors or nano pigments made from clays and organic dyes are supposed as possible environment-friendly substitute for toxic cadmium (Cd) and palladium (Pd) pigments. The plant colors dispersed on a nanoscale in bulk polymers and coatings. Various colors of pigments are perhaps fashioned by choose appropriate dyes from a wealth of organic dyes. In addition, materials dyed with plant colors stay totally translucent as the dimension of these pigments is lesser than the wavelength of light. An improved oxygen, ultraviolet (UV) and temperature stability joint with high brilliance and color effectiveness displayed huge surface area of the nanopigments and their enhanced interaction with light.

3.1.3.4 Nanocomposites in Electro Materials

Polymer nanocomposites are promising route to novel organic–inorganic materials with peculiar electrochemical behavior of conducting polymers associated with clay minerals attracts potential applications such as modified electrodes, biosensors, solid-state batteries, smart windows and other electrochemical devices. For example, polypyrrole (PPR) nanocomposites could develop modified electrodes used as sensors or as devices for electrocatalysis. PEO nanocomposites might be novel electrolyte materials because of their relatively higher ambient conductivity and weak temperature reliance over conventional $LiBF_4$/PEO electrolytes as well as their single ionic transference character. In addition, such a nanocomposite is an outstanding model system to probe and understand the structure and dynamics at the interface.

3.1.3.5 Nanocomposites for Drug Delivery

Giannelis and co-workers (Cypes et al. 2003) have reported that Polymer nanocomposites used in controlled drug delivery, in addition organoclays not only reduced the rate of drug (i.e., examethasone) release from the biocompatible poly (ethylene-co-vinyl acetate) matrix but also increased the Young's modulus as compare to the pure polymer. Lee et al. (Lee and Fu 2003; Lee and Jou 2004) fabricated a sequence of clay-based poly (N-isopropyl acrylamide) nanocomposite hydrogels explore swelling and drug release behaviors. Which increase in the content of either intercalated quaternary ammoniums in organoclays or clay in nanocomposites lead to the reduce in swelling ratio of nanocomposite hydrogels but increase the gel strength. Drug release nature of clay-based poly (N-isopropyl acrylamide) nanocomposite hydrogels was examined for a few model drugs (i.e., neutral caffeine, cationic crystal violet and phenol red). Drug release generally depends on the factors such as content of clay and its intercalated agents, the charge of drug solute, interaction between the gel and drug solute, and ionic strength of the medium. Lin et al. (2002), attempted to expand a composite as drug carrier and *in-situ* release for colorectal

cancer therapy. Such a composite was made by intercalating 5-fluorouracil into the interlayer space of montmorillonite (MMT).

3.1.3.6 Applications in Sensors and Medical Devices

The possibility to improve the properties of hydrogels apart from drug delivery when the polymer is incorporated with clay platelets. Especially in thermal responsivity, swelling-deswelling rate and molecular diffusion, is predictable to extend clay-based nanocomposites to such applications as artificial muscles and rapid actuators in biomedical. Poly(urethane urea)s (PUU) are used in number of biomedical applications such as blood sacs in ventricular assist devices and total artificial hearts. Though, these kinds of elastomers have relatively high permeability to air and water vapor, to modify the chemistry of polymer leads to the reduced permeability. Xu et al. (2001, 2003) reported a more well-organized approach in which clay platelets were dispersed into polymer. The resulting polymer nanocomposites demonstrate significantly reduced (fivefold) gas permeability and mechanical properties (e.g., stiffness, modulus).

3.1.3.7 Nanocomposite Hydrogels for Tissue Engineering and Regenerative Medicine

Smart, stimuli-responsive polymers were initially used for drug delivery, tissue engineering and regenerative medicine, sensors and actuators. Some of polymeric systems, their applications and modes of action reviewed by Kohane and Langer (2008) and by Mano (2008). Polymeric hydrogels are based on both natural and synthetic polymer materials. Natural products used are collagen, hyaluronan, chitosan, alginate, chondrocyte sulfate, fibrin, and silk. Amongst the synthetic polymers are polyethylene oxide (PEO), polyethylene glycol (PEG), polyethylene glycol diacrylate (PEGDA), polyvinyl alcohol (PVA), polyacrylic acid (PAA), polyhydroxy ethyl methacrylate (PHEMA), poly (acrylamidomethyl sulfonic acid) (PAMPS), poly (propylene fumarate-co-ethylene glycol) [PPFEG], and pluronics. Viscoelastic properties of hyaluronan (HA) have been utilized for ocular surgery and, together with chondroitin sulfate (CS), for cartilage engineering. Modification of CS by incorporation of glycidyl methacrylate was reported by Li et al. (2004). It may be photo polymerized and converted into a homogenous hydrogel and found to be non-toxic for cartilage replacement.

3.1.3.8 Nanocomposites for Proteins and DNA Release

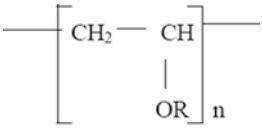
Encapsulation of proteins and DNA into hydrogels through its hydrophilic and biocompatible is particularly attractive on comparison with hydrophobic matrices, might be absorb and denaturate biological materials. Sustained delivery of active

proteins and DNA by a variety of hydrogels has been extensively investigated (Shi et al. 2008) the pH-sensitive release of lysozyme, as a model protein, by the poly (N-vinyl formamide) ethoxy propane.

3.1.4 Polyvinyl Alcohol (PVA)

Polyvinyl alcohol (PVA) pays the more attention in numerous areas biomedical and pharmaceutical applications because of excellent biomaterials, including its non-toxic, noncarcinogenic, bio-adhesive, good strength, low fouling potential, temperature, and pH stability and processing behavior (Eliassaf 1972; Peppas and Merrill 1977; Peppas and Korsmeyer 1987; Tamura et al. 1986; Tanigami et al. 1995). Further, it has a simple chemical structure and modifications are possible by chemical reactions. Moreover, PVA gels exhibit a high degree of swelling in water as well as in biological fluids and those are rubbery/elastic in nature, making it employable as a biomaterial for various applications, including contact lenses, lining for artificial hearts, skin replacement, artificial cartilage replacement, and drug delivery (Eliassaf 1972; Peppas and Merrill 1977; Peppas and Korsmeyer 1987; Tamura et al. 1986; Tanigami et al. 1995; Bajpai and Singh 2004).

3.1.4.1 Chemical Characterization



where R = H or $COCH_3$

3.1.4.2 Analytical Methods

Various methods have been described for the detection of PVA; unfortunately no quantitative method is available for the determination of PVA. Potassium iodide and iodine solutions were used to measure the PVA concentration in wastewater for instance in the filter paper possess 1000–20,000 mg/l. The green color complex produced when PVA react with boric acid also used to detect small amounts in polyvinyl chloride resins (Melo-Júnior et al. 2008).

3.1.4.3 Phosphorylated PVA

PVA is a commonly known as hydrophilic, biodegradable, and commercially obtainable linear polymer. By virtue of the outstanding hydrophilic uniqueness and gelling capability, PVA has been extensively used as water-soluble matrix polymer

for synthesizing IPN and semi-IPN hydrogels (Zhang et al. 2005). However, integration of PVA into superabsorbent network able to improve the water absorbency through strong hydrogen bonding interaction with gel network. Additionally, modification of PVA properties enhance the superabsorbent nature and phosphorylation is an effective method for modifying multihydroxyl polymer (Finch 1996). PVA easily phosphorylated like polysaccharide due to the number of OH groups on its chains and phosphorylated PVA (P-PVA) exhibits improved hydrophilicity, anionic properties and molecular electrostatic repulsion, and retain the bulk morphological features.

3.1.4.4 Functional Uses

Technological Uses

Polyvinyl alcohol used in a variety of applications in food industries act as a binding and coating agent, foil film coating agent particularly in function wherever moisture barrier/protection are necessary. As a part of tablet covering formulations planned for foodstuffs as well as food supplement tablets, PVA shields the active ingredients from moisture, oxygen and other environmental components, while concurrently masking their taste and odor. It permits easy handling of finished product and facilitates ingestion and swallowing. The thickness (viscosity) of PVA allow for the application of the PVA coating agents to tablets, capsules and other forms to which film coatings are naturally functional at moderately high solids stuffing.

Food Categories Application

Polyvinyl alcohol utilized in high moisture foods in order to retain the overall satisfactory taste, texture and quality of the foods. Confectionery products might be consist PVA to preserve the integrity of the moisture sensitive constituents (Table 3.1).

Table 3.1 PVA applications in various food industries

Uses of polyvinyl alcohol (PVA)		
Food industry	Food use	PVA usage level (%)
Dairy based desserts	Ice cream and frozen yogurt preparations	0.2
Confectionery	Multicomponent chocolate bars	1.5
Cereals and cereal products	Readymade breakfast cereals with dry fruits	0.5
Food supplements	Food supplement tablets	1.8
Readymade savories	Nuts and fruit mixtures	1.5

Paper Coating Application

Poly (vinyl alcohol) is extensively used as a paper coating fabric since its good quality chemical resistance, film forming capability, water solubility (Finch 1973; Marchessault and Skaar 1967). In addition, TiO_2 is used on paper as its skill to develop the pastiness of the paper substrate without touching the adherence between the PVA layer and paper surface (Wypych 2001). Also, its anti-bacterial nature by UV activated photo catalysis (Tamada et al. 1986) can be utilized in disposable sheets and wipes preparation. An interesting option is usages in traditional bulk coating would be nanofiber coating with PVA fibers contains well-dispersed TiO_2 nanoparticles. Especially in electro spinning, PVA/nanoTiO_2 fibers could make very lightweight coatings, because the solvent evaporates during the spinning process, with proper process parameters a dry, thin mat can be deposited on a substrate.

3.1.4.5 PVA Based Tissue Engineering Application

Because of the elasticity of polymer scaffold, it provides the mechanical strength and flexibility to the heart valve and PVA-based hydrogel scaffolds for valve tissue engineering. Generally hydrogels has high water content and tissue-like elasticity. In addition, the abundant hydroxyl groups on PVA can be readily modified to attach growth factors, adhesion proteins, or other molecules of biological significance (Nuttelman et al. 2001). Despite the potential advantages of PVA scaffolds, the ability of cells to attach to hydrogels is limited. Previously, some evidences shown that the attaching important cell adhesion proteins fibronectin, onto the surface of glutaraldehyde-crosslinked PVA hydrogels promotes the attachment of cells. Hydrophilicity relates to the inability of cells to attach to hydrogels, leading to minimal adsorption of cell adhesion proteins on the gel surface. By covalently linking fibronectin onto PVA hydrogel surfaces, fibroblast and valve interstitial cell (VIC), proliferation, and migration were dramatically improved.

3.1.5 Ionomers

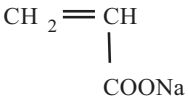
Ionic polymers consisting of a hydrocarbon backbone and pendant carboxylic acid groups which are neutralized either partially or completely with metals, particularly sodium and zinc. Sodium and zinc ionomers are commercially available ionomers reported by Du Pont Company (Inoue & Velde 1995) under the trademark surlyn and because both are important plastics. Ionomers possess the better properties when compare to normal PVA.

- High strength
- Good clarity
- Improved oil resistance.

Ionomers are flexible, tough, truly thermoplastic, melting point similar to low density polyethylene, (150–250 °C) can be extruded into films by blow molding (lay flat tubing, foamed sheet, rod, tubes, etc.). The films and sheets could be formed by various methods into skin and blister packages. An significant new function the toughness and clarity provides the ability to use as surface coating for glass bottles. A thin layer of the ionomer (0.004 in.) is applied to the hot glass bottle from a fluidized bed of the powdered ionomer. The film of plastic forms a continuous bag around the bottle.

3.1.5.1 Sodium Acrylate Ionomers

Sodium acrylate is an anionic monomer CH_3-CH_2-COONa. Acrylate polymers possess an anionic charge, sodium neutralized polyacrylates are the most common form used in industry.



3.1.5.2 Applications

- Sequestering agents in detergents. (By binding hard water elements such as calcium and magnesium, the surfactants in detergents work more efficiently.)
- Thickening agents
- Coatings
- Fake snow
- Super absorbent polymers. These cross-linked acrylic polymers are referred to as "Super Absorbents" and "Water Crystals", and are used in baby diapers. Copolymer versions are used in agriculture and other specialty absorbent applications. The origins of super absorbent polymer chemistry trace back to the early 1960s when the U.S. Department of Agriculture developed the first super absorbent polymer materials. This chemical is featured in the Maximum Absorbency Garment used by NASA.
- Sold as an additive for bath water which turns the water into goo. The mixture is then dissolved by the addition of sodium chloride. The nanocomposites were obtained by *in situ* polymerization of acrylic acid (AA) and sodium acrylate (AANa) intercalated into organo-kaolinite, which was obtained by refining and chemically modifying with solution intercalation step in order to increase the basal plane distance of the original clay. The modification was completed by using dimethyl-sulfoxide (DMSO)/methanol and potassium acetate (KAc)/water systems step by step.

3.1.6 Clay

Clays are the materials for ceramics production most abundant material in the surface mineral world (Alemdar et al. 2005) showing the greatest diversity of reactions. Smectite-group clay minerals have large adsorption capacities for polymer molecules due to their unique crystal structure. Montmorillonite is a member of smectite group minerals and has a layered structure. The polymers in montmorillonite dispersions interact with the clay particles, according to their ionic or non-ionic character. The ionic polymers bring electrostatic interactions, but the non-ionic polymers are adsorbed on the surface of clay minerals through steric interactions. Polymer concentration, molecular weight, hydrolyzing groups, size and shape of clay particle, its surface charge, clay concentration in suspension, pH, and temperature all affect the clay/polymer interactions. The adsorption of polymers onto the surfaces of clay particles influences the rheologic and electrokinetic properties (Chang et al. 1992). Clays are common ingredients in pharma industry both excipients and active substances. In the 1960s it was observed that oral absorption of several drugs was reduced by co-administration of clay-based intestinal adsorbents or by the presence of clay stabilizers (e.g., suspending or emulsifying agents) in liquid formulations Polymer/clay nanocomposites are a new class of hybrid systems in which inorganic or organo-clay nanoparticles (often montmorillonites) are dispersed in a polymer matrix. These materials have some interesting advantages compared to the pure polymer, such as enhanced mechanical and rheological properties.

3.1.6.1 Clay Minerals

Layered Structure

Clay minerals used for polymer nanocomposites classified into three groups. 2:1 type, 1:1 type and layered silicic acids. Their structures (Fig. 3.3) are briefly described as follows.

2:1 Type

This type clay belongs to the smectite family with crystal structure consisting of nanometer thick layers (platelets) of aluminium octahedron sheet sandwiched in between two silicon tetrahedron sheets. Tacking of layers leads to a vander waals gap between the layers. Isomorphic substitution of Al with Mg, Fe, Li in the octahedron sheets and/or Si with Al in tetrahedron sheets gives each three-sheet layer an overall negative charge, which is counterbalanced by exchangeable metal cations residing in the interlayer space, such as Na, Ca, Mg, Fe, and Li.

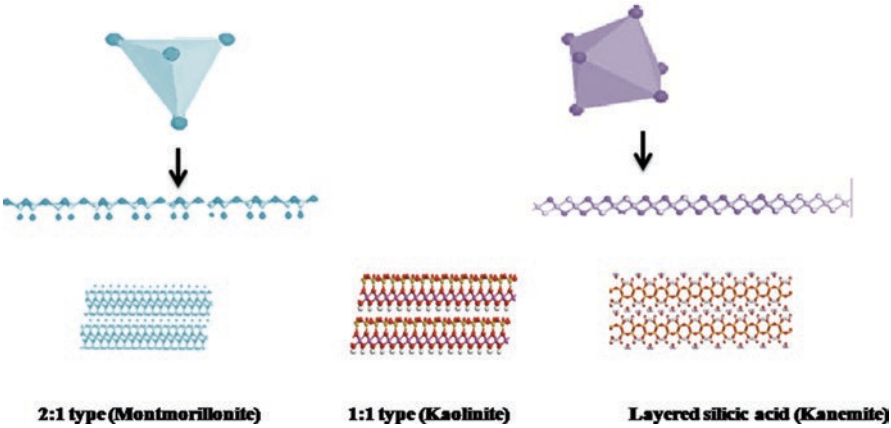


Fig. 3.3 Structure of clay minerals represented by montmorillonite, kaolinite and kanemite. They are built up from combinations of tetrahedral and octahedral sheets whose basic units are usually Si–O tetrahedron and Al–O octahedron, respectively

1:1 Type

The clays consist of layers made up of one aluminium octahedron sheet and one silicon tetrahedron sheet. Each layer bears no charge due to the absence of isomorphic substitution in either octahedron or tetrahedron sheet. Thus, except for water molecules neither cations nor anions occupy the space between the layers, and the layers are held together by hydrogen bonding between hydroxyl groups in the octahedral sheets and oxygen in the tetrahedral sheets of the adjacent layers. The clays consist mainly of silicon tetrahedron sheets with different layer thickness. Their basic structures are composed of layered silicate networks and interlayer hydrated alkali metal cations. The silanol groups in the interlayer regions favor the organic modification by grafting organic functional groups in the interlayer regions. They are natural clay minerals except for octosilicate, but can be synthesized as well. Layered silicic acids are potential candidates for the preparation of polymer nanocomposites because they exhibit similar intercalation chemistry as smectite clays. Besides, they possess high purity and structural properties that are complementary to smectite clays.

3.1.6.2 General Characteristics of Clay Minerals

The important characteristics of clay minerals, richest intercalation chemistry, high strength and stiffness and high aspect ratio of individual platelets, abundance in nature and low cost. First, unique layered structure and high intercalation capabilities allow them to be chemically modified to be compatible with polymers, which make them predominant development of clay-based polymer nanocomposites. In addition, their relatively low layer charge (x = 0.2–0.6) means a relatively weak

force between adjacent layers, making the interlayer cations exchangeable. Therefore, the intercalation of inorganic and organic cations and molecules into the interlayer space are facile, which is an important aspect of their uses in polymer nanocomposite manufacturing. Among the smectite clays, MMT and hectorite are the most commonly used ones while others are sometimes useful depending on the targeted applications. Moreover, although smectite clays are naturally not nanoparticles, these could be exfoliated or delaminated into nanometer platelets with a thickness of about 1 nm and an aspect ratio of 100–1500 and surface areas of 700–800 m^2/g.

3.1.6.3 Surface Modification of Clay Minerals

Clay has many advantages which causes the agglomeration in the polymer matrix and incompatible nature between hydrophilic clay and hydrophobic polymer. Organic treatment reveals the compatible specific polymers like (thermoplastics, thermosets or elastomers). Such modified clays are commonly referred to as organoclays. Surface modification process similar to coupling or chemical bonding with polymers. The first organic molecule reported for this purpose is amino acid adopted in the *in-situ* polymerization of nylon 6 nanocomposites. Though, the most popular alteration for clays is to replace the interlayer inorganic cations (e.g., Na^+, Ca^{2+}) with organic ammonium cations. Apart from this the surface modification is essential for swelling (normally over 20 Å) and hence reduce the layer–layer attraction, which permit a positive dispersion and adjustment of polymer into the interlayer space.

3.1.7 Characterization of Nanocomposite

3.1.7.1 Swelling Ratio and Measurement

The swelling ratios were obtained by weighing the initial and swollen samples at various time intervals and calculated as follows:

Swelling ratio = $(W_t - W_d)/W_d$ Where W_t is the weight of the swollen hydrogel at a given time during swelling and w_d is the weight of dry hydrogel.

3.1.7.2 Drug Loading

Tetracycline HCl is a bacteriostatic agent whose mode of action is inhibition of bacterial protein synthesis; have the capability to attach to the hard tissue walls of the pockets, and thus establish a drug reservoir following sub gingival irrigation with determined solutions. After binding on the root surface, biologically active tetracycline is released over time (Fig. 3.4).

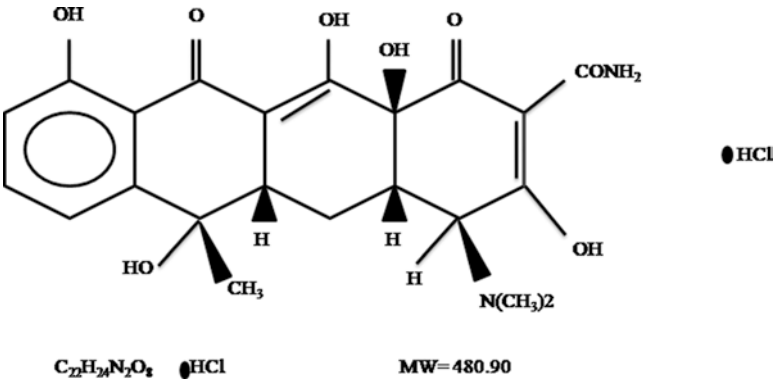


Fig. 3.4 Chemical structure of Tetracycline HCl

Mode of Action

Tetracyclines possess a wide range of antimicrobial activity against gram-positive and gram-negative bacterial strains and the antimicrobial and antibiofilm activity would be enhanced based the concentration of the nanocomposite and particular bacterial strains (Vaseeharan et al. 2013; Thaya et al. 2016). Bacterial ribosomes are the target site for the tetracyclines and its ribosomes of gram-negative bacteria is attain by passive diffusion from side to side hydrophilic pores in the outer cell membrane and consequent reaction through energy-dependent active transport system that pumps all tetracyclines from first to last the inner cytoplasmic membrane. This active transport system may need a periplasmic protein carrier and this drug specifically binds to the 30S ribosomes and appear to inhibit protein synthesis by prevent access of aminoacyl tRNA to the acceptor site on the mRNA–ribosome complex. These inhibitory belongings of the tetracyclines could be reversed by ashing, which suggests that the reversibly bound antibiotic rather than the small portion of irreversibly bound drug is responsible for the antibacterial action (Amsden 1991).

Administration

Generally tetracycline ingestion after the eating followed by 2 h break because tetracycline binds easily with magnesium, aluminium, iron, and calcium, which reduces its ability to be completely immersed by the body. Similarly, dairy products or preparations containing iron are not recommended directly after taking the drug.

3.1.7.3 Design Criteria for Nanocomposite Hydrogels in Drug Delivery Formulations

To use any kind of material in drug delivery system need more criteria to choose the material and network fabrication govern the rate and mode of drug release from hydrogel matrix. Many steps are occurs before we load the drug into the hydrogel or any other carrier molecule, that formulations decides the hydrogel features. The subsequent criteria and variables are more significant for scheming hydrogel to function as drug carriers. The hydrogel delivery formulation is considered with the suitable physical and transport goods, it may immobile not succeed to carry out its therapeutic role when surrounded *in vivo* due to a local inflammatory response. The development of fibrous capsule adjoining the delivery mechanism make supplementary diffusion obstacle that may limit drug release rates while increased proteolytic active may increase rates of matrix and drug degradation. Proper material choice, fabrication procedure, and surface texture of the appliance are thus forever significant in designing biocompatible hydrogel formulations for controlled release (Table 3.2).

3.1.7.4 Drug Loading Methods for Nanocomposites Hydrogels

The incorporation of drugs into nanocomposite hydrogel delivery matrices can be performed by one of the two methods. Those are, In situ loading and Post loading

In Situ Loading

Ligands or Drugs forms the drug-polymer conjugates are suspended in polymer precursor solution and hydrogel network construction and drug encapsulation are accomplished. The release of drugs can be controlled by diffusion, hydrogel

Table 3.2 Designing criteria and variables for the composite preparations

Design criteria	Design variables
Transport properties	Molecular weight and size of protein
Molecule diffusion	Molecular weight of polymer
	Cross linking density
	Polymer-protein interactions
	Hydrogel degradation rate
	Additional functionalities
Physical properties	Polymer/cross linker/initiator concentrations
Gelling mechanism/conditions	Temperature, pH, ionic strength
Structural properties	Molecular weight of polymer
Biodegradability	Mechanical strength
Stimuli-responsiveness	Concentration of degradable groups
	Concentration of responsive groups
Biological properties	Cytotoxicity of the hydrogel
Biocompatibility	Capsule formation

swelling, reversible drug-polymer interactions or degradation of labile covalent bonds.

Post Loading

Absorption of the drugs depends on the hydrogel networks, diffusion is the major driving force for drug uptake and release will be determined by diffusion and gel swelling. Hydrogels possess the ligand binding sites which lead to the drug-polymer interaction and drug diffusion reflect the drug release from hydrogel network.

3.1.7.5 Drug Release Mechanisms from Nanocomposite Hydrogel Devices

The physicochemical properties of hydrogel network and drug-loading method will determine the mechanisms by drug release

1. Diffusion controlled
2. Swelling controlled
3. Chemically controlled

Diffusion controlled defines the flicks law of diffusion with either constant or variable diffusion coefficients is commonly used in modeling diffusion controlled release from hydrogels. Drug diffusivities based on its free volume and hydrodynamic or obstruction based theories (Siepmann and Peppas 2001). Swelling and controlled release occurs when diffusion of drug is faster than hydrogel swelling. The modeling mechanism involves moving boundary conditions where molecules are released at the interface of rubbery and glass phases of swollen hydrogels as shown in Fig. 3.5. Hydrogels may go through a swelling-driven stage transition from a glassy state where entrap molecules stay immobile to a rubbery state where molecules rapidly diffuse. In these system, the rate of particle discharge depends on the rate of gel swelling. One example of swelling-controlled drug delivery systems is hrdroxypropyl methylcellulose (HPMC). Drug overloaded HPMC tablets are

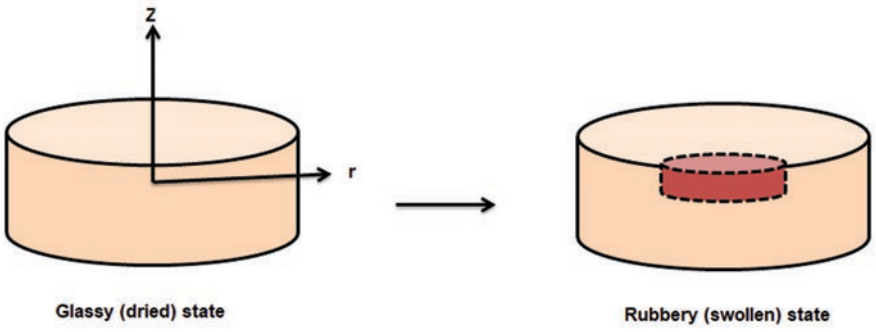


Fig. 3.5 Schematic of HPMC hydrogel tablet in the glassy (left) and rubbery (right) state

three dimensional, hydrophilic matrixes stored in a dry, glassy state. Chemically controlled release is used to explain molecule release determined by reaction occurring within a delivery matrix. Chemically controlled release can be additional classify according to the kind of chemical response occurring throughout drug release. The release of encapsulated drugs can occur through the dreadful conditions of pendant chains or through exterior erosion or bulk-degradation of the polymer backbone.

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Chapter 4
Nanovaccine: A Modern Approach to Vaccinology

Jagannathan Selvaraj, Vijayakumar Rajendran, and Balasubramaniyan Ramalingam

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4.1 Introduction

Infectious diseases are the most significant cause of morbidity and mortality worldwide; pathogenic bacteria are responsible for approximately 50% of this burden. Vaccine development has a proud history as one of the most public health interventions to date.

A vaccine is a live killed, attenuated, inactivated form of pathogenic microbes like as bacterium or virus, or a part of the structure of the pathogenic microbe, that upon administration enhancing antibody production, i.e., cellular immunity against that but is incapable of causing severe infection. A vaccine should be cost-effective to preventing infectious disease, epidemiologically targeted implementation of vaccines has diminished morbidity and mortality from infectious diseases that earlier were causing problems and economic impediment, i.e., diphtheria, measles, polio, pneumococcal infections, and invasive Haemophilus influenza type B. Worldwide vaccination programs as per the guidance of WHO have eradicated smallpox diphtheria, poliomyelitis and the neonatal Tetanus in most of the developed and various developing countries (World Health Organisation 2019).

Vaccine development is moving in the direction of the rational design of new candidate vaccines that may no longer contain live or inactivated whole pathogens. In the delivery of the drugs field, the Nanotechnology has come out as an immense prospective technology to deliver at an appropriate define site. Based on these techniques, the needed drugs, proteins, nucleotides, and vaccines could be delivered more appropriately and to reach the specific targeted site (Mamo and Poland 2012).

Nanotechnology is the new interdisciplinary area to lead the predictable progress in molecular biology, biotechnology, diagnostics, and therapeutics. It gives way for the delivery of antigens, diagnosis of diseases, nanoemulsion etc. (Fig. 4.1) (Storni et al. 2005; Vijayakumar et al. 2011).

4.2 Nanovaccines

Nanoscales technologies are in medicine having more than four decades of age, i.e., Liposomes are nanoparticles with Phospholipids bilayer have been used in the pharmaceutical industry ever since in 1960 (Torchilin 2005). At present, the composition, size, charge, shape, hydrophobicity, and surface property of nano vaccines are undependable and that have been accepted for human use, and now the clinical/pre-clinical numbers are increasing.

The positive approach of nano vaccines, which is not only allowing antigen improvement as well as vaccine stability, immunogenicity, and pertained to discharging delivery of target. Currently, more numbers of prophylactic nano vaccines have been approved for human use, and some of the vaccines are in the phases of clinical/pre-clinical, due to the developments in Nanovaccinology (Kushnir et al. 2012; Plummer and Manchester 2011; Roldao et al. 2010).

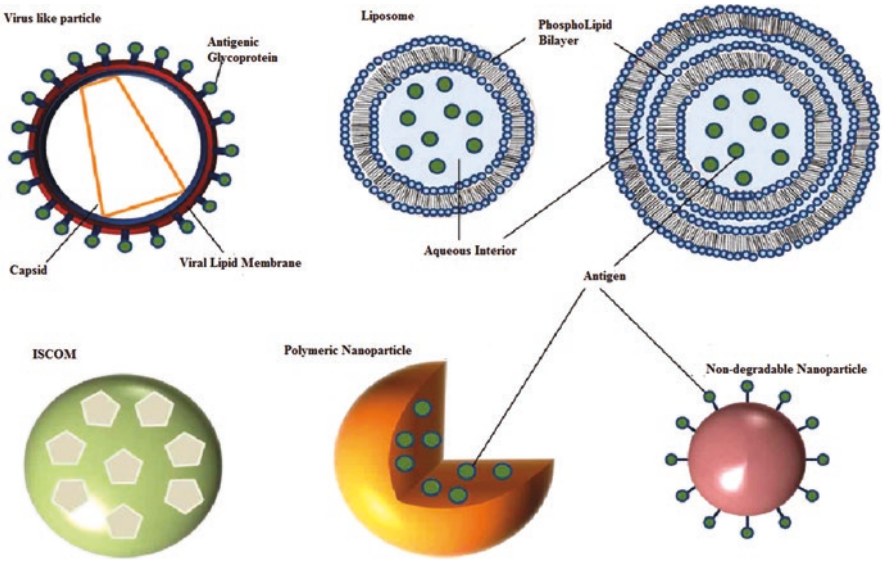

Fig. 4.1 Schematic representation of various Nanoparticle Delivery systems [Virus Like Particle, Liposome, ISCOM, Polymeric Nanoparticle and Non-Degradable Nanoparticle]

In nanotechnology, an additional wide-ranging field is Nanovaccine is the newer technology of vaccination through the target spot of antigen delivery. The Mucosal immune response has more significance in the prevention of infection/spreading of disease, Encapsulation of antigen in suitable animal models with microparticles/nanoparticles is competent in enhancing the immune response.

The PLGA was loaded with Tetramethyl rhodamine-labeled dextran which is a hydrophilic polysaccharide used for microscopic analysis, was prepared by solvent evaporation methods and the resulted materials was administrated to a group of immunopotentiators like Macrophage and dendritic cells, after 24 h the microscopic results revealed that both immune cells are up taking same intensity. A number of nanosize formulated subunit peptide vaccine delivery strategies based on the composition of polymers, peptides, lipids, and inorganic materials have been proposed, and it was inducing a cellular and humoral immune response (Skwarczynski and Toth 2011). As per the research work of Tsai et al., the nano-vaccine bound with the T cell-stimulating molecules and the type 1 diabetes in mice was cured by a nanotech-based vaccine through the boost of the weaker immune cells to prevent from damaging (Tsai et al. 2010).

The nano-vaccines are target-oriented, and it consisted of biocompatible/biodegradable nanoparticle and its emerging field as a novel vaccine; it can able to directly target the disease/origin of infection that were different from the existing drug molecule those which affect all parts of the body. Nanovaccines have the reassurance together the entire body immune system to destroy infections and also prevent further infections as well as spreading of the diseases, and it can able to elicit cell and

humoral immunogenicity. When compared with the DNA based available vaccine, and it is more reasonable than conventional vaccination. Modified adenovirus it contains self-assembled bio-nanoparticles that can able to deliver to the target gene, the immune response is elicited through the cells undergone translation progression for the secretion of the specific protein, the next type of nano-vaccine are, the needed synthesized polysaccharide molecules as a nanoparticle vaccine it can able to targeting the protein molecules and attached with a carbohydrate-binding domain that is special protein module. This methodology only permits the nanometer of the nanoparticles measuring between 50 and 90 nm, and the lesser size is required for the elicit immune primary immune response also decrease the time duration for the propagation of vaccine strains from 60 to 28 days (Pati et al. 2018).

4.2.1 Characterization of Nano Vaccines

To meet the required quality attributes the nanoformulation has to essential to undergone the characterization, i.e., structurally, composition, stability, etc. During the nanoformulation, more chances of dissimilarity occur due to contamination, poly dispersion of Nanoparticles, the accrual of toxic components or due to completion of particle formation to avoid that kind of variation either between or within advanced techniques are available to determine the identification of uniformity within the colloidal solution. Spatial distribution results are much needed in the nanoformulation for identification of antigen is encapsulated/conjugated on to the surface, surface modified and charge influences cellular uptake consequently the size may vary (minimum 20–200 nm maximum 2000 nm) based on the cellular specificity and migration towards the target region and shape of particles determine that the intracellular interaction and antigen-presenting capacity and enhance the immunity (Gao et al. 2018).

The following analytical methods are available for the characterization of Nanoparticles i.e., Electron microscopy [FESEM, SEM, and TEM], Dynamic Light Scattering [DLS, Zeta sizer, Zeta potential], and Density Gradient centrifugation (Caputo et al. 2019).

Hydrophobicity of NPs plays a significant role in the nano-vaccination in the interaction of antigen permeability to intracellular transport and solubilization of protein antigen. The techniques used for the presence/quantification/analysis of needed antigens are Lowry and Bradford assays, ELISA, Dot-blots assay, SDS-PAGE and Western Blot, Density gradient centrifugation.

In some occasion it may be necessary to measure the compositional content of the NP, the QuilA is very significant constituent for ISCOMs, the lesser concentration leads to a hemolytic effect, this deficit can be analysed through Reversed-phase HPLC/and the assay of Rocket Electrophoresis (Kersten and Crommelin 1995).

It is because some reagents lead to toxic when it is a high dose. The concentrations of Phospholipids and Cholesterol are significant components of ISCOMs; these are quantified through GC and Phosphorus assays (Lendemans et al. 2005).

Using with Inductively Coupled Plasma Mass Spectrometry/instrumental Neutron Activation analysis, the metal like gold was a presence in Nanoparticles (Fabricius et al. 2014).

4.3 Nanoparticle Interaction with Antigen-Presenting Cells (APC)

The immune cells like macrophage and dendritic cells have excellent facilities in uptake mechanisms with nanoparticles, and it leads to the development of essential and efficacious nanoparticle vaccines. The dimension, charge, and shape of the nanoparticles play a significant role in the antigen up taking as well the shape of nanoparticle are more stringent for the interaction with antigen-presenting cells (Dobrovolskaia and McNeil 2007; Kumari and Yadav 2011; Zolnik et al. 2010).

Studies of the antigen-presenting cells (APC) with nanoparticles of antigenic components have more attracted, broad significance with a focus on how the antigen to deliver in APC and further induction through cross-presentation and maturation of the Nanoparticle antigen towards the activation of cell-mediated and humoral immune response of CD4 and CD8 stimulated and production of specific antibodies (Babensee 2008; Bachmann and Jennings 2010; Gheibi Hayat and Darroudi 2019; Jones 2008; Reddy et al. 2006; Scheerlinck and Greenwood 2008)

4.4 Liposomes as Vaccine Delivery Vehicles

The Liposome concept was first investigated in the year 1965 (Bangham 2005). It has a structure of spherical lipid bilayer with core rings and aqueous. The diameter sizes are varying from micrometer to nanometre, and as a model for membrane transport through diffusion, at that moment the liposome is checked for the adjuvanticity and using for the different vaccine formulations due to its the biochemical molecules, the predominant of phospholipids in the aqueous core (Hall et al. 2004). The core and phospholipids are amphiphilic in nature, and it includes the tail are in hydrophobic nature, and it consisted of fatty acids are linked to a backbone of glycerol as a head group is a hydrophilic nature. Whilst as aqueous environment condition the polarized structure makes possible of self-assembly into behavior with the fatty acids facing each other due to this the oil like comportment forming among outwards facing phosphate groups, because of this the liposome's are having both charges it leads to adaptable and important towards the antigen carrying. The antigen molecules like proteins/hydrophobic peptides when placed in into liposomal inner hydrophobic center the entire hydrophilic molecules are encapsulated in the vesicles or surface bonding can also happen (de Jonge et al. 2004; Glück et al. 1999; Han et al. 1997; Tiwari et al. 2011).

The antigen will bind in liposome through covalent attachment or electrostatic interactions along with few hydrophobic interactions. The advantages pertaining to liposomes is, it can highly adapt with the properties like physicochemical size, charge, and lamellarity to fine-tune of the liposomes through the altering of lipid composition (Szoka Jr and Papahadjopoulos 1980). As per the studies of Watson et al. (2012) and Giddam et al. (2012) the Liposome's are non-toxic, non-immunogenic and biodegradable, and otherwise, if the composition is derivatives of bacteria or viral membrane that could enhance the immune response (Giddam et al. 2012; Hall et al. 2004; Li et al. 2011; Watson et al. 2012).

4.4.1 Antigen Localization in Liposomal Formulations

There are various ways to integrate antigens into liposomes before administration optimization of liposome as per its enhancement of immune response. While the liposome-encapsulated antigen immune response deficient due to unreachable of the antigen with APC, however when oral administration of the encapsulated antigen leads to enhanced stimulation of local IgA and serum IgG (Fujii et al. 1993; Phillips et al. 1996).

Consequently, the liposome formulation possibly customized for specific requirements and purposes. When the oral route administration of the vaccine can be easily enzymatic degradation, if the same vaccine is in liposome encapsulation, there is not possible of enzymatic degradation. As per the research finding of Wilschut et al. (1994), the immune response was enhanced while the liposomes are administrated before the administration of antigen (Wilschut et al. 1994). Fully encapsulated antigen immune responses are more than surface bounded antigens of liposome's vesicle (Aramaki et al. 1994). The liposome-mediated immune response not only the selection of antigen and lipid composition, but it depends on the virtual magnitude and liposomal localization. This category of inventive methods way to the improvements of potential mucosal immune response based vaccines. The liposomal based vaccine has the additional adjuvant capacity will confirm to be a mucosal vaccine against different infectious diseases. The encapsulated and surface bounded antigenicity of liposomal are triggering the capacity of T and B cell priming (Moon et al. 2011).

4.5 Nanoemulsion

The nanoemulsion is the newer concept in vaccine delivery methods. In Michigan, university research groups have trailed the nanoemulsion in the size of 400 nm containing Hepatitis B antigen and the immune response are in the satisfactory levels (Fig. 4.2). During their research they are prepared the nanoemulsion with the soybean oil as oil phase along with alcohol, detergents, and water, the macro emulsion

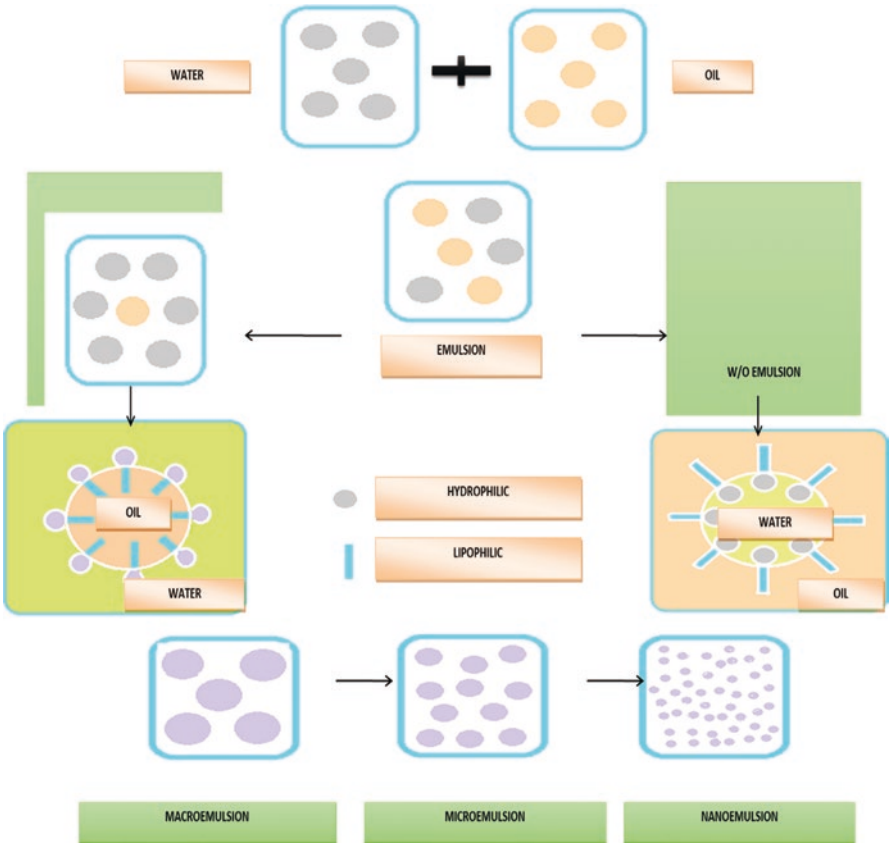

Fig. 4.2 Transition of nanoemulsion

was further treated in ultra Sonicator in optimal temperature and other related parameters reduced to the size of the particles 400 nm, the nanoemulsion was entrapped with infectious antigen and placed in the nose for triggering the immune response instead of needle-based immunization and the immune response (Bielinska et al. 2008).

The vascular endothelium, in various internal organs, is played a vital role for the allocation of nanoparticles, the critical parameters of the nanoparticles like charge, shape, size zeta potential, hydrophobicity are highly influenced with plasma protein, immune cells (Makidon et al. 2008; Sharma et al. 2009). The antigen is not degraded during permeation in the layers of skin and muscle due to protection of the nanoemulsion. Dendritic cells are located in the skin that also as the functioning of antigen-presenting cells, and it is the capacity of most competent migratory capacity, optimum efficiency towards capture and processing of MHC high-level expression, and also exclusion and co-stimulatory. Targeted delivery of protein antigen to dendritic cells was achieved (Banchereau and Steinman 1998; Cruz et al. 2011; Reddy et al. 2006).

4.6 Transdermal Immunization

Transdermal immunization is an innovative approach by which the antigen along with an adjuvant is applied directly on the skin that elicits potent humoral as well as cell-mediated immune responses specific for antigen (Glenn et al. 1999; Mishra et al. 2013; Peachman et al. 2009; Scharton-Kersten et al. 1999). It can significantly assist transdermal macromolecule delivery across intact skin. It generates an ephemeral opening in the skin barrier, enabling macromolecule to reach the systemic circulation. Advantages of transdermal immunization are: (1) it can interact the antigen directly to the antigen-presenting cells those were present in the skin; (2) reduced amount of antigens required for the immunization; (3) sustained release; (4) reduce the frequency of administration; (5) patient compliance; (6) self-administration is possible; (7) eliminate accidental needle-stick; (8) non-invasive zero-order delivery; (9) reduce the overall cost of immunization (Hammond et al. 2000).

For Transdermal immunization, various techniques are available like Iontophoresis, Sonophoresis, Microneedle delivery (Fig. 4.3) to enhance the immunization safe, pain-free, and affordable price. Enhancement of nanoparticles and chemical enhancements are being explored for the Jet injection route of vaccination. The available vaccine is conventional that is lacking in the accurate adjuvants; there is a scope to find suitable, safe, affordable, and effective adjuvants that are most needed in modern vaccinology. To concern with that, the transdermal based immunization has been analyzed by the research and development community. Stratum corneum lies in the layer of skin, and it is densely associated with the antigen-presenting cells (APC), the APC are mainly dendritic and Langerhans cells in the epidermis and dermis region (Kim et al. 2012).

During the Transdermal immunization, first, the strategic targeting to epidermis and dermis, without disrupting the underlying subcutaneous tissue, is a complicated technique that requires only those who are professionally trained healthcare personnel. The microneedle techniques have been a proposal of suitable/potential replace of that skin disrupting issue as well as existing hypodermic syringes.

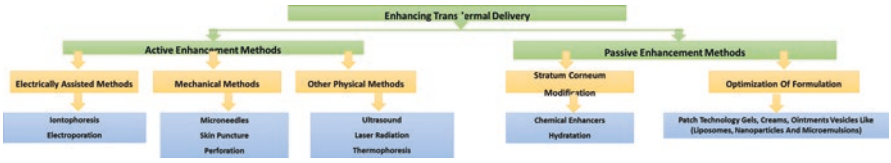

Fig. 4.3 Transdermal delivery pathway techniques

4.7 Microneedles

The skin is firm because of the stratum corneum, the microneedles are solid and the theory of "punch with the piece," the MNs are only in the size of a micrometer. It has the suitable/needed drug or antigen are any nanoformulations are directly pierced through the skin barrier [stratum corneum] in a suitable direction that is horizontal to the smooth of the skin (Fig. 4.4) (Kim et al. 2012).

Because of the micron size, the MNs drug/vaccine tube permitted the material through the skin and no needs of professional training, currently, the existing vaccine injection, that persons/should be professionally trained. In addition, the MNs are pain-free, now the self administrable MNs patches contained an assortment of appropriate vaccine coated that make possible extensive distribution of the vaccine/drug inappropriate and short time of the uncontrolled disease. Dry coated microneedle vaccine formulation in the immunobiological industry will restrict the cold chain cost as well as distribution to remote areas in developing countries. The microneedle based vaccine has dose-sparing quality in which targeting the immune cell productive zones, there are four major types of microneedles in development: solid, coated, dissolving, and hollow microneedles. The microneedle patch vaccine formulations in the pharmaceutical industry will minimize the laydown cost of cold chain processes and improve the distribution of vaccines to rural areas located in developing countries (Arya and Prausnitz 2016).

However, the dose sparing quality is one more extensive improvement MNs whither, the explicit targeting of affluent convolutions of immunogenic APCs, there

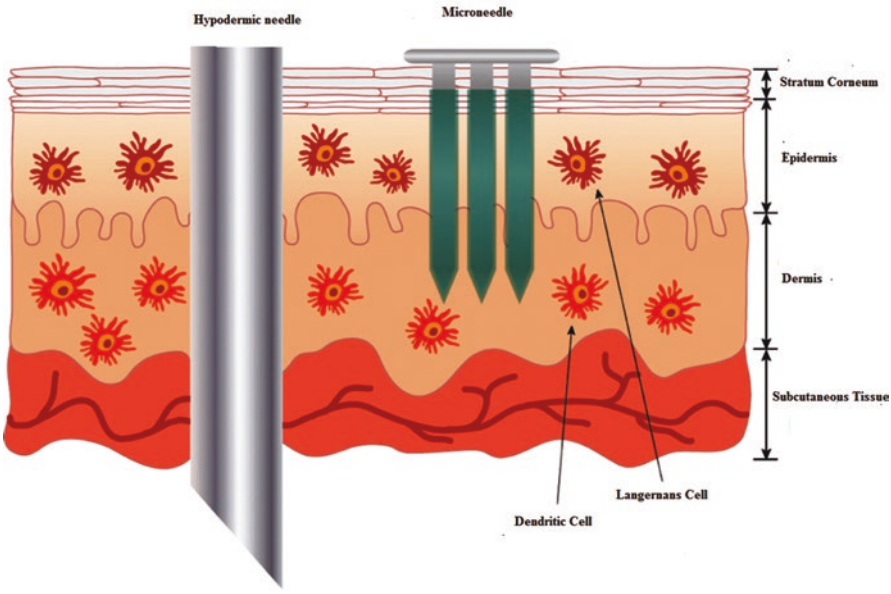

Fig. 4.4 Microneedle

the production of high immunogenicity than the traditional IM route. Currently, lots of research analyses are being organized globally to collectively correlate the efficient immunogenicity induced by MNs immunization as contrary to existing routes of delivery. The studies of Dean et al. (2005) in the Rabies vaccine, the rabies vaccine was injected through 1–3 mm BD Soluvia microneedle syringes to 66 healthy volunteers towards the confirmation of safety and consistency. During immunization using only one-fourth dosage of rabies vaccine, the seroconversion rate of the volunteers has a higher range, than the IM route, in the same time the quantity of the antigen was very lesser (one fourth) and this study clearly indicated that targeting the immune cells, that contains more numbers of immune cell networks (Dean et al. 2005).

The reactions of the small hollow implantable dissolving-type microneedle that formulated with freeze-dried hepatitis B surface antigen along with aluminum hydroxide and lipopolysaccharide as adjuvants, which derived comparable immune responses as the liquid formulation of the vaccine after two immunizations (Hirschberg et al. 2010). The various researchers stated in their reports that affluent stimulation of virus-specific memory B cells and enriched lung clearance in Mice than IM delivery of inactivated seasonal influenza virus vaccines coated on solid metal microneedles indicated that the microneedles hold an encouraging possible as an alternative to conventional vaccine administration methods (Kim et al. 2009, 2010a, b, c; Koutsonanos et al. 2011).

During the coating and drying process, there is a possibility of aggregation of antigen particles to overcome through the addition of trehalose as a stabilizer was added in inactivated influenza virus strain A/PR/8/34 vaccine for stability quantification analysis (Quan et al. 2009). The same virus strain was used for the analysis of its immunogenicity, the virus coated metal MNs was inoculated in Mice and found strong Th1 but not in IM routed vaccine (Kim et al. 2010c). Moreover, Kommareddy et al. (2012) focused on H1N1 research, and further, they fabricated dissolving-type microneedles encapsulated antigen of inactivated influenza and used for immunization in mice and the immunogenicity are allowable in the standard limit (Kommareddy et al. 2012).

A liquefy microneedle patch abide of the biocompatible polymer poly vinyl pyrrolidone and encapsulated with inactivated influenza virus strain A/PR/8/34 vaccines, and it induced robust antibody responses and enhanced cellular immune responses than intramuscular route immunization (Quan et al. 2009). The dose sparing techniques of MNs in pre-clinical assessment of whole inactivated influenza virus vaccine in the laboratory animals of mice affirms that 100-fold dose sparing when the same was administrated through Intradermal route (Alarcon et al. 2007). Detailed studies were also done in MNs that were coated with rotavirus vaccine formulation, and the immunogenicity was in acceptable range (Moon et al. 2013).

The influenza vaccine MNs in mice, they inferred that increasing concentration of cytokines that earlier immune response and the cytokines play an important role in the functioning of dendritic cells, macrophages, and neutrophils (del Pilar Martin et al. 2012). Analysis of low-dose microneedle and low-dose intramuscular routes of the same antigen, the other critical parameters are in same, after the scheduled duration the animals are bleed and the elicited immune response, and that the low-

dose microneedle persuaded higher immune responses that were comparable to the serological antibody titers produced by high-dose intramuscular route (Quan et al. 2010a, b).

4.8 Virus-Like Particles

The virus-like particles are molecules closes as a virus, and it is a collection of self-assembling multi-protein molecules like viral structural proteins, i.e., capsid, envelope, and due to non-availability of viral genetic material, there is no possibility of replication. The outer surface of VLPs serves as immunogenic epitopes, and that elicit strong B and T cell response. Gardasil et al. developed the commercially approved HPV composed with L1 VLP are shown highest virus-neutralizing antibody titers in the laboratory animal [C57BL/6], the L1VLP HPV vaccine was delivered to mice through Microprojection–Nanopatch a densely packed array and the booster with IM route (Corbett et al. 2010).

The influenza virus strain H1N1 A/PR/8/34, the HA subunit along with matrix protein [M1] with the VLP, the formulated VLP vaccine shown higher immune response (Kim et al. 2010c). The VLP vaccine formulated with the sugar glass stabilizer [trehalose] the immune response of the trehalose based VLP influenza have more antibody titer when compare with the without, as well as the stability also more. In the case of the microneedle delivery, the VLP H1N1 with trehalose, the antigen is not destabilization, the immune response was more when compared with conventional IM route of immunization (Kim et al. 2010b; Quan et al. 2009). During the virus challenge method for potency analysis, the VLP H1N1 with HA subunit were coated in microneedle and applied to mice skin through manual, after the elicited the immune response all the mouse is survived that means both conventional IM routes are identical (Song et al. 2010a, b). The H1 (A/PR/8/34) and H5 (A/Vietnam/1203/04) VLP, and they found that the microneedles in human skin shown more morphological changes and cell number in epidermal sheets of Langerhans cells (Pearton et al. 2010).

In addition, a mechanism study examining the effect of microneedles in human skin presented a line of evidence indicating that H1 (A/PR/8/34) and H5 (A/Vietnam/1203/04) VLP vaccines delivered by microneedles stimulated Langerhans cells, which resulted in cell morphology change and a curtailed cell number in epidermal sheets (Pearton et al. 2010). The VLPs are now commercially available; it is self-assembled targeted viral protein, as well as it as an antigen delivery platform (Ionescu et al. 2006; Tissot et al. 2010) Garg et al. (2020), reported that, VLP based vaccine for Zikha virus [ZIKV VLP] after challenged in the small animal model and found that the generated high titer of neutralizing antibodies. The VLP based vaccines are in the trail as well as various phases, the diseases like Chikungunya and Japanese encephalitis and Dengue virus, CHIKV has shown more efficacy in animal model now it is under clinical trial, in case of JEV and DENV the VLP are utilized with prME expression without capsid protein (Akahata et al. 2010; Garg et al. 2020; Wong et al. 2019).

4.9 Advantages of Nanovaccine

The currently available major nano-vaccine is non-invasive, and the route of delivering are oral, nasal, diffusion patch or microneedle array, these techniques are more advantage, i.e., pain-free, multi-dose and needle-based The nanoemulsion preparation of Hepatitis B antigen found to be tolerable and effective and does not require refrigeration and it is effective for a month at 25 °C and for 6 weeks at 40 °C; therefore it facilitates its final distribution in small areas/villages of developing countries (Nandedkar 2009; Vijayakumar et al. 2013).

Currently nano-vaccine against HIV gp120, it is the most important binding protein, and it induces the cellular and mucosal immunity, the immune response of the HIV gp120 is above the acceptable range. The peptide-nano-bead based nano-vaccine against FMDV, influenza, more encouraging results.

4.9.1 Potential Issues with Nanovaccine

Any new medicine is needed their considerable evidence regarding the safety, efficacy, and potency is mandatory prior to small trails with the beginning of human trails, the important worries with nano-vaccine technologies are (1) Biocompatibility with host system; (2) Toxicity variations; (3) Nanoparticle size, charge and shape not in composition; (4) Issues in toxicity, while long term accumulation in vital internal organs; (5) Lacking reproducibility in large scale; and (6) Small nanoparticles are cleared quickly from the body (Luo et al. 2017).

The evaluation of nanoparticles is very tough while during the rapid selection of nanoformulations for vaccine/drugs; the nanoparticles lead to adverse effects on humans are likely exposure (low level) from the long term, which is more complicated and also needs very prolong testing for identification.

The facility towards designing nanomaterials is needed to regulatory guidelines for protecting the safety of nanomaterials in universal and nanomedicines in particular. Undoubtedly it is mandatory to characterize the nanomaterials expected for therapeutic use in both it is manufactured qualities initially and after induced into physiological surroundings (Lin et al. 2014).

4.10 Conclusion

In the coming few decades, Nanotechnology will have a significant impact on all phases of an existing medicine. The application of nanotechnology in vaccines will create them more productive and fewer invasive and may provide opportunities to develop new vaccines against unpreventable, incurable diseases. Perhaps most importantly, nanotechnology will allow vaccine formulation which is stable enough

to be distributed without refrigeration to remote villages in the developing world, where access to medical facilities is minimal, it could save many lives, and slow the spread of HIV, malaria, and other major infectious diseases. Many of the nano vaccines are non-invasive, delivered by the oral or nasal route, diffusion patches, or microneedle arrays, thus allowing pain-free delivery with minimal damage. This is an advantage over conventional vaccines, which are usually multi-injection, multi-dose delivery systems (Kendall 2006).

In the research finding of the biodegradable nanoparticles have enormous possible as transport carriers for mucosal and systemic vaccine delivery system. Generally, the concentric fatty sphere produced by the virus, and it elicits a powerful immune response nanoparticle-based vaccine (Akagi and Akashi 2006).

When the nanoemulsion based vaccine is available in the market, there is not necessary of refrigeration/cold storage for a month at 25 °C, and for 6 weeks at 40 °C and these are applicable in developing countries during the final allocation in remote villages/areas. The MHC based peptide nano vaccines against autoimmune disease with massive potential (Clemente-Casares et al. 2011). The observation of Zhang et al. (2006) observed that when the nanomaterials were stored in long-duration, there are changes in the particle size and shape but not in composition and it leads to toxicity due to the clearance of the particles, hence further the proper evaluation of the nano-vaccine in the aspects of safety, efficacy, and potency (Zhang et al. 2006).

The Nanoparticles delivery systems have been more advantages when compared with the conventional vaccine. The conventional vaccine antigens are poor immunogenicity it needed a proper adjuvant to enhance the immunogenicity, at the same time as in aluminum-based adjuvants have been used, while in immune response there is a shortage as well as reactogenic with the host during immunization, this kind of reactogenic, shortfall of immune response will be alternated with nano-vaccine with the various delivery system. This is not only the delivery of antigen but also host biocompatibility along with the superior immune response (Akagi and Akashi 2006).

One of the ways in which NPs are capable of eliciting different immune responses is through their size; moving into cells passing through the non-classical pathways and then processed as such, delivering antigens in different ways also has a retrospective effect on the resulting immune response, whether the antigen is decorated on the NP surface for presentation to antigen-presenting cells/encapsulated for slow release and prolonged exposure to the immune system. The NPs are also adaptable and can be customized with immunostimulatory compounds to improve the potency of the immune response or with molecules to increase their stability in vivo. While these delivery vehicles may present as an exciting prospect for future vaccination strategies, it is also worth noting their potential drawbacks, particularly those associated with cytotoxicity (Gao et al. 2018).

Ever since the Nanovaccines have only short narration, and it not have a long-standing safety profile used for human use. Further, it needs more studies to be carried for toxicity; once this happens as expected, this may be the improved alternative technique for the vaccine delivery and further licensed for human use. Nanovaccine

based on biomimetic principle consists of distinct impacts like biocompatibility, low toxicity, bioavailability, and targetability undergo as an outstanding agent to cure various diseases (Vijayan et al. 2019).

The upcoming triumph of vaccine shall not only depend on the achievement of scientific advancement, but it needs the association of researchers from interdisciplinary in diverted fields such as physical chemistry, structural biology, epidemiology, bioinformatics and molecular immunology, the success of vaccine will be accomplished through inventive ideas that will lead the basic breakthrough The phenomenal scientific consideration among the technological improvement of in the discipline of immunology collectively with nanomaterials is the key contributor to vaccine advancement (Kim et al. 2019).

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Chapter 5
Nanotechnology in Imaging Applications: An Overview

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5.1 Introduction

Biomedical imaging is one field with widespread interest due to its ability to help with analysis as well as diagnosis through imaging at both the molecular and cellular level (James and Gambhir 2012). Early diagnosis of any disease is crucial and critical in assisting the treatment and therapeutic response. Currently used contrast agents in biomedical imaging can produce undesirable toxicity effects, non-specific distribution in tissues other than the target and so on. Therefore, there is a need to explore other materials which can be used as contrast agents with better properties and biocompatibility.

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5.2 Why Nano in Biomedicine?

In view of their high surface area to volume ratio, nanoparticles possess unique properties which are different from the molecules as well as the bulk (Nune et al. 2009). Precise control over size and shape by fine tuning the synthesis process is one major advantage of exploring the potential applications of nanomaterials in biomedicine. Nanomaterials are becoming indispensable in biomedicine in view of their potential applications in both diagnostics as well as treatment. Due to their smaller size, nanoparticles have significant permeability and retention in tumours can be exploited by imaging techniques.

5.2.1 Imaging Techniques

Some of the advantages of using nanoparticles as imaging agents are: (1) the concentration of the imaging agent in the nanoparticle can be controlled by optimization of the nanoparticle synthesis process, (2) the circulation time of the imaging agent in the system can be fine-tuned by modifying the surface properties of the nanoparticles which have been loaded with the imaging agent (Hashim et al. 2014).

5.2.2 Carrier for Drugs

Nanoparticles are also widely used as platforms to carry drugs into the target tissues for therapeutic purposes. Drugs are encapsulated inside the functionalized nanomaterial which then accumulates in the target tissue delivering the drug. There are currently numerous animal model studies related to the delivery of anti-cancer drugs like doxorubicin using nano-delivery vehicles. When nano vehicles are used for both diagnostics as well as therapy, the field is known as theranostics (Yu et al. 2018). This review will focus on the role of nanoparticles in diagnostic imaging applications.

5.3 Type of Nanoparticles Used for Imaging Applications

Inorganic nanoparticles, polymeric nanoparticles and liposomes, carbon-based nanomaterials, magnetic nanoparticles, metal oxide nanoparticles, dendrimers, quantum dots (Naseri et al. 2018) are some of the types of nanoparticles which are currently being tested for their application in diagnostics.

5.4 Application of Nanoparticles in Imaging

5.4.1 Fluorescence Imaging

Fluorescence imaging is one among the most widely used techniques in imaging applications and is known for its sensitivity (with resolution at the nanometre scale) and versatility. This imaging technique can be applied in two ways for biomedical applications: (1) imaging of intrinsically fluorescent bio-molecules and (2) imaging of cells which have been made fluorescent by addition of fluorophores or fluorescing nanoparticles (Wolfbeis 2015). Nanoparticles when used in fluorescence imaging are more photostable than molecular probes, can be easily internalized into cells and are site specific. Fluorescing nanoparticles have the capability to generate intense fluorescence signals even et low intensity excitation due to the presence of a large number of molecular fluorophores in each nanoparticle (Caponetti et al. 2019). Silica based nanoparticles, noble metal nanoparticles, metal oxide nanoparticles and carbon-based nanomaterials are some of the common groups of nanomaterials used for fluorescence imaging (Wang et al. 2013; Baker and Baker 2010; Boisselier and Astruc 2009). Semiconductor quantum dots for example have molar extinction coefficients 10–50 times more than the traditional organic dyes and can produce a broader absorption spectrum (Yu et al. 2003).

5.4.2 Magnetic Resonance Imaging (MRI)

MRI provides good resolution as well as high contrast in tissues compared to other imaging techniques. This imaging technique utilizes radio frequencies as well as magnetic fields to produce high resolution and good contrast non-invasively. Iron oxide nanoparticles and more specifically super paramagnetic iron oxide nanoparticles (SPIONs) are widely used as contrast agents for MRI imaging (Laurent et al. 2007). The localization of the nanoparticle in the body is dependent on the size of the nanoparticle and therefore process variables for the synthesis can be optimized to produce nanoparticles of a specific size range. Iron oxide nanoparticles with a diameter of less than 4 nm are preferred as T1 contrast agents while the larger particles with diameter greater than 4 nm are preferred as T2 contrast agents (Kim et al. 2011). The mode of administration of SPIONs is either through oral or intravenous administration and are in different stages of clinical trials. Noble metal nanoparticles as such are very reactive and tend to get oxidized quickly and are therefore used in combination with iron oxide nanoparticles to provide better stability and contrast. Various combinations of the metal nanoparticle-iron oxide nanoparticle composites are still in pre-clinical trials and long-term in vivo toxicity needs to be assessed (Estelrich et al. 2015). However, compared to other types of nanoparticles, magnetic nanoparticles hold greater potential in view of their biodegradability and reduced toxicity (Javed et al. 2017).

5.4.3 Computed Tomography (CT)

Computed Tomography is a widely used imaging technique that can be used to visualize cross sectional images of tissues in the body (Naseri et al. 2018). CT has potential advantages such as cost, faster scan time, high resolution, provides quantitative information of contrast agents, compatibility and wider acceptability with patients (Kim et al. 2017; Mahan and Doiron 2018). The sensitivity of CT towards contrast agents is less as compared to techniques such as MRI (Chinen et al. 2015) and therefore dense nanoparticles provide a solution to increase the detection limit of the contrast agents using the technique. Nanoparticles used for CT applications are synthesized to have a core with contrast generating atoms. The surface of the nanoparticles is biofunctionalized with agents such as lipids or proteins to provide biocompatibility as well as ensure biodistribution (Jia et al. 2013; Kim et al. 2017). Au nanoparticles are the most commonly used metal nanoparticles for cell tracking applications using CT in view of their biocompatibility, unique property of Surface Plasmon Resonance, high X ray absorption coefficient and ease of control over shape and size (by fine tuning process of synthesis) (Xi et al. 2012). Higher the atomic number and electron density of the contrast agent, higher is the attenuation coefficient and better is the contrast in CT (Meir and Popovtzer 2017; Popovtzer et al. 2008) and this is another further reason for the more common application of Au nanoparticles in CT (Au has a high atomic number of 79). On an average, 10–100 pg Au/cell is the uptake concentration required to visualize the cells using CT (Meir and Popovtzer 2017).

5.4.4 Ultra Sound (US)

Ultrasound is employed for both diagnostic as well as therapeutic purposes in biomedical field. The technology is cost effective in terms of operative as well as instrument cost and has high tissue penetration capability and is safe. With regard to therapeutic applications, US is used to treat injuries to the soft tissues, to aid in wound healing and also to soften scar tissues. US has also been successfully used to remove kidney stones while US with low intensity is used to stimulate bone growth (Lingeman et al. 2009; Griffin et al. 2012). Sonodynamic therapy is one such technique which uses low intensity US and a sonosensitizer molecule (Chen et al. 2014). In addition to polymeric nanoparticles, metal nanoparticles and inorganic nanocomposites are regarded as promising platforms to carry the sonosensitizer molecules (Canavese et al. 2018). In vitro studies using gold nanoparticles have mainly focused on utilizing US to treat tumour. For example, gold nanoparticles increase the uptake of the sonosensitizer molecules into the cancerous cells (Sazgarnia et al. 2011). Few studies have also explored the use of gold nanoparticles as nanosensitizers which when activated by US act as nucleation sites

(Kosheleva et al. 2016). Functionalized gold nanoparticles also show promise in reducing tumour growth when activated by US by the generation of ROS species (Brazzale et al. 2016).

5.4.5 *Positron Emission Tomography (PET)*

Positron Emission Tomography is another molecular imaging technique with high detection sensitivity, significant penetration depth and extent of quantifiability. PET can assist in earlier detection of lesions, individual patient screening and treatment with optimization of dosage (Cai and Chen 2007). This technique is more commonly used to image and diagnose tumours and in cancer metastasis. Nanoparticles when used in PET imaging can function as amplifiers enabling enhanced contrast and sensitivity (Goel et al. 2017). Silica based nanoparticles, Au nanoparticles, ultra-small nanoparticles such as nanodots, zinc oxide nanoparticles, iron oxide nanoparticles and carbon dots are some of the most commonly used nanomaterials for PET imaging (Abadjian et al. 2016). Incorporation of the radionuclide into the nanoparticle core during the process of synthesis is one way to achieve the amplification of signal required (Goel et al. 2014). In one related study, mesoporous silica nanoparticles coated with PEG and DBCO injected into tumour induced mice accumulated in the tumours. ^{18}F labelled azides were injected a day later and were localized in the tumour. Amplified signals could be visualized 2 h post the azide injection (Lee et al. 2013).

5.4.6 *Single Photon Emission Computed Tomography (SPECT)*

Single Photon Emission Computed Tomography is a non-invasive imaging technique which works similar to PET in providing functional information. It provides a 3D imaging of various tissues and organs by detecting gamma radiation emitted by the radionuclides localized in the target tissue or organ (Van Audenhaege et al. 2015). The functional information provided might then be required to be coupled with the morphological information typically provided by CT and hence nowadays it is often referred to as SPECT-CT (Maccora et al. 2019). Compared to PET, SPECT is less sensitive but can however image biochemical processes. Comparison of the advantages and drawbacks of both PET and SPECT are tabulated in Table 5.1. Nanoparticles when used in SPECT are functionalized with the radionuclides on the surface or conjugated to the core during the process of synthesis (Arms et al. 2018). Magnetic nanoparticles, carbon-based nanomaterials and noble metal nanoparticles are some of the commonly used nanomaterials in SPECT imaging. For example,

Table 5.1 Comparative account of PET and SPECT imaging

	PET	SPECT
Sensitivity	More sensitive than SPECT	
Availability		More widely available
Cost effectiveness		Is cheaper than PET due to the longer half-lives of the radionuclides
Resolution	Higher resolution than SPECT	

Zhao et al. (2016), have utilized gold nanoparticles doped with ^{199}Au atoms for SPECT imaging of cancer in a mouse tumour model. Limitations of both PET and SPECT imaging may include radioactive exposure (Han et al. 2018).

5.4.7 Photo Acoustic (PA) Imaging

Photo Acoustic imaging is a non-invasive imaging technique which can provide molecular data such as oxygen concentration and saturation in hemoglobin (Hb) as well as information on living tissues (Song et al. 2016). Most of the excitation sources used in PA imaging are in the near IR to IR region since near IR region has a higher penetration depth than visible light due to lower Hb absorption and tissue scattering (Cho et al. 2010). In addition, organic dyes under photobleaching whereas nanoparticles exhibit better photo stability. Nanoparticles can absorb strongly in the near IR region and therefore enable higher contrast and penetration depth when used in PA imaging (Zhou et al. 2017). Nanoparticles hold great promise in imaging tumors, vasculature, brain mapping and identifying plaques in the arteries (Li and Chen 2015). In general nanoparticles in the size range between 20 and 150 nm are commonly used for imaging studies since they emit a strong signal and have substantial residence time in the target tissues. Studies indicate that nanoparticles <10 nm are quickly cleared from the system and hence nanoparticles of larger size range as mentioned are preferred (Lemaster and Jokerst 2017). In view of their studied biocompatibility, inorganic metallic nanoparticles were among the first type of nanoparticles to be studied as potential contrast agent in PA imaging (Thakor et al. 2011). Among the metal nanoparticles, different configurations of gold nanoparticles have been widely studied in the laboratory due to their ease of preparation and easier process control. For example, Song et al. (2016) have synthesized functionalized "smart" gold nanoparticles which can absorb in the near IR region and function as PA imaging agents responsive to tumour microenvironments.

5.5 Significance of Characterization Techniques

Factors such as size and shape of the nanoparticle, functionalization of the surface of nanoparticle and the type of nanoparticle can ultimately determine the interaction of the nanoparticle with tissues, its compatibility and fate in the body (Michalet et al. 2005). For example, biofunctionalization of the surface of the nanoparticle can protect it from protein adsorption by creating a neutral hydrophilic surface. The effective coating on the surface can provide protection against aggregation and agglomeration due to the presence of proteins in the vicinity and ultimately provide stability (Naseri et al. 2018). It is of utmost importance to ensure that the selected coating/functionalizing agent is biocompatible as well as provides stability against aggregation. Some of the commonly used functionalizing agents for coating nanoparticles are chitosan, PEG, alginate, dextran and starch (Hong et al. 2010). The functionalizing agents can either be added during the course of synthesis or after the synthesis of the nanoparticle. Control over the shape and size of the nanoparticle can be obtained by fine tuning the process of synthesis. Size of the nanoparticle plays a role in determining the residence time in the target tissue as well as influences the strength of the signal produced when used for imaging applications. Therefore, characterization techniques play a critical role in determining the end applications of nanoparticles in imaging techniques. They provide information on the shape and size, degree of aggregation, active surface area and surface charge (Mourdikoudis et al. 2018).

5.5.1 Techniques to Confirm the Morphology of Nanoparticles

Shape and size of the nanoplatforms synthesized can be confirmed using some of the techniques mentioned below. However, the list is not exhaustive and an extensive review on various characterization techniques for nanomaterials can be found at Mourdikoudis et al. (2018).

5.5.1.1 Dynamic Light Scattering (DLS)

DLS measures the hydrodynamic diameter of particles and is based on the Brownian movement of particles. It measures light scattering as a function of time. The technique measures the secondary particle size which can be affected by forces such as van der Waals forces of attraction. DLS also provides information on the polydispersity or uniformity of the sample and is used for the measurement of spherical particles.

The stability of the nanoparticle in the system can be determined by measuring the zeta potential in solution. This measurement is usually performed by suspending the nanoparticle in a background electrolyte such as KCl. A zeta potential above +20 mV and below −20 mV is considered to be stable in solution (Prathna et al. 2011).

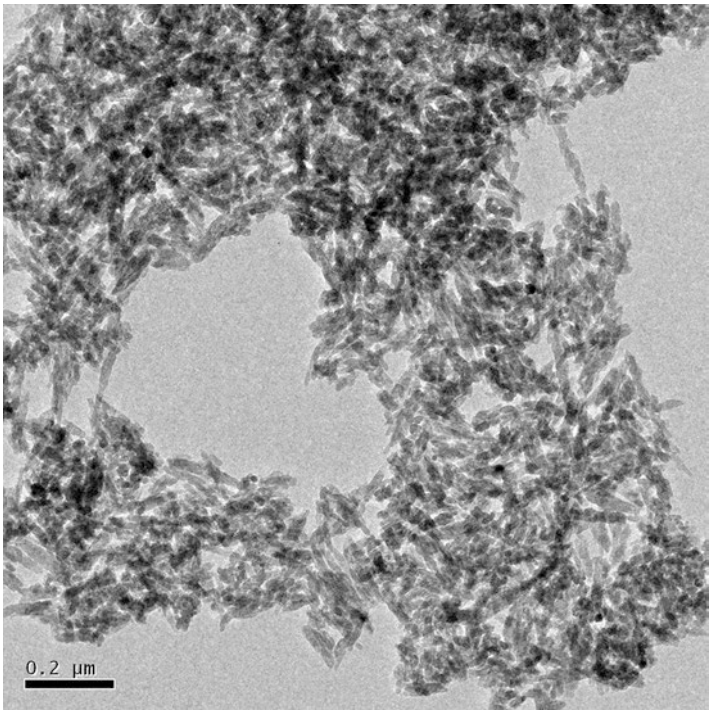

Fig. 5.1 Magnetic iron oxide nanomaterials visualized using HR-TEM

5.5.1.2 Microscopic Techniques

Electron Microscopic techniques such as Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) are used to study the morphology of the synthesized nanoparticles. The shape and size of the nanoparticles can be visualized using an electron beam under different magnifications. Polydispersity of the nanoparticle sample can also be identified using the electron microscopy technique. HR-SEM is known to achieve resolution down to 1 nm (Kempen et al. 2013). The uniformity of the coating on the surface of the nanoparticle can also be visualized using the electron microscopic techniques. Nanoparticles internalized within cells or tissues can be visualized using SEM once the samples have been sputter coated with metal. This can however lead to higher chances of damage to the biological material (Fig. 5.1).

Atomic Force Microscopy is another microscopic technique which is used to analyse the topography of the nanoparticle sample. A three-dimensional image of the sample is then constructed by measuring the force between the probe and the sample surface. This force microscopic technique can be operated in both contact and non-contact mode (Vanhecke et al. 2014). One of the major advantages of AFM is that it can provide information on the depth or height of the nanoparticle which

otherwise cannot be measured using electron microscopic techniques. Studies indicate that the size measurements by AFM are comparable to that obtained using electron microscopic techniques such as SEM and TEM (Oćwieja et al. 2013).

5.5.2 *Techniques to Quantify the Concentration of Nanoparticles*

Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES), Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS) and Atomic Absorption Spectroscopy (AAS) are some of the techniques used to quantify the concentration of nanoparticles present in the sample. ICP-OES for example is very sensitive and can detect minor changes in concentration and can detect multiple elements at the same time (Elzey et al. 2012).

5.6 Environmental Impact and Way Ahead

Even though there are numerous in vitro studies on the efficacy and efficiency of nanoparticles in imaging applications and in vivo pre-clinical and clinical stages in different stages, long term toxicity needs to be ascertained. As of now, only iron oxide nanoparticles have been utilized in clinical studies in view of limitations to achieve monodisperse particles inside the body and concerns on elimination from the body and toxicity (Kiessling et al. 2014). Studies on excretion profiles of the nanoparticles, optimized dosage required to achieve the desired effects, systemic accumulation and biodistribution and long-term toxicity effects on the body, if any, is of immediate need. Toxicity profiles should be correlated with the size, shape, coating agent, stability, charge and surface chemistry of the nanoparticle. More concerted efforts are required to synthesize stable, biocompatible nanoparticles with good excretion profiles as well as significant accumulation in the target tissue such as tumour.

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Chapter 6
Nanosponges: In Perspective to Therapeutic Medicine

J. Shanuja, Kajal Singh, R. Sai Nandhini, and Jeyanthi Palanivelu

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6.1 Introduction

Nanomedicine seeks to enhance the existing drug delivery and treatment processes using nanoparticulate processes. Nanosponges based systems can be used as a vessel for pharmaceutical principles to tackle the issues related to solubility, absorption, penetration, bioavailability, in vivo stability, and can achieve continuous and selective drug delivery for a variety of pharmaceutical entities with maximum therapeutic efficacy (Osmani et al. 2018). Advances in technology have further expanded the research, manufacture, and advancement of new technologies for selective drug delivery. Secure and controlled delivery of drugs may boost the efficiency of certain classic medicines currently on the market and would also have consequences for the production and effectiveness of novel clinical approaches such as the supply of anticancer medications, peptide and protein, and gene therapy (Parveen et al. 2012).

Nanosponges are a type of nanoparticles that are small mesh-like systems that can encapsulate a wide number of substances such as active drug molecules for the application of an innovative drug delivery system (Trotta et al. 2012). Such

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nanoparticles made by integrating polymers and crosslinkers with encapsulated drug molecules circulate through the body until they reach the surface of a tumor cell where they bind to the surface and start releasing the drug in a stable and controllable manner. The nanosponges have a three-dimensional scaffold (backbone) or naturally degradable polyester network (Bhowmik et al. 2018). Nanosponges often show a tremendous benefit in comparison to other nanoparticles due to their properties. Nanostructured nanosponges lock the drug molecules into the porous biodegradable polymer and move across body fluids to directly target biological processes and distribute drugs by extended release. Nanosponges can be used as various dosing types such as parenteral, aerosol, topical, tablets, and capsules because of their small size and spherical shape (Shringirishi et al. 2014). Cyclodextrins (cyclic oligosaccharides) shows an external hydrophilic surface and a hydrophobic inner cavity, an uncommon feature that defines their well-known ability to shape stable inclusion complexes in aqueous media with low-polarity guest molecules (Yang et al. 2010).

The key applications of the nanoformulation delivery method are the dental, topical, and parenteral delivery of various medications, proteins, and peptides. Hence, Nanoscale-based biomaterials comprising of nanosponge systems have structural features designed to offer future drug delivery and nanotherapeutic applications. In this chapter, we present the utilization of nanosponges-based approaches and advances which provide high precision tools to diagnose human diseases and their treatment to improve human health.

6.2 Nanosponges: Types

The nanosponges are a 3D scaffold (backbone) which is capable of degrading naturally. It is mixed in solution with small molecules called crosslinkers which acts as tiny grappling hooks to fasten different parts of the polymer together (David 2011) (Fig. 6.1).

Commonly synthesized types of nanosponges are Titanium-based nanosponges, Carbon coated metallic nanosponges, Beta cyclodextrin based nanosponges, Hyper cross-linked polystyrene nanosponges, Silicon-based nanosponge particles,

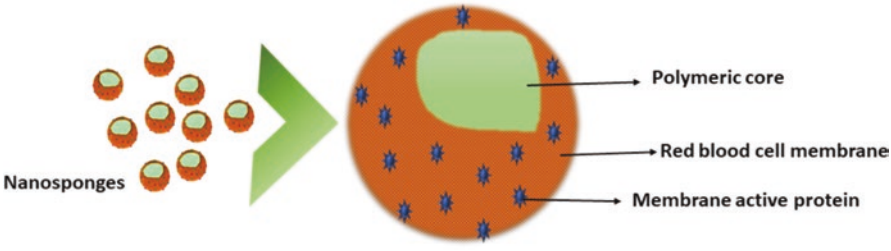

Fig. 6.1 Structure of nanosponge

Polyester based nanosponges, Metal-based nanosponges, Peptide nanosponges, and RNA nanosponges.

The classification of types of β-cyclodextrin-based nanosponges depending on the cross-linking agent used are β-Cyclodextrin-based carbamate nanosponges, β-Cyclodextrin-based carbonate nanosponges, β-Cyclodextrin-based ester nanosponges, polyamidoamine nanosponges, and modified nanosponges (Pawar et al. 2019).

6.3 Methods of Nanosponges Synthesis

The methods of preparation of nanosponges are simple, as they can be regenerated without difficulty by different treatments. Polymers are integrated with cross-linkers by the following methods to prepare the nanosponges containing chemotherapeutic drugs (Fig. 6.2). The different types of constituents used for the synthesis of nanosponges are mentioned in (Table.6.1).

Ultrasound-assisted synthesis: In this method, the polymer and the crosslinker are made to react in a flask without any solvent. The flask is placed in ultrasound and it is heated up to 90 °C and sonicated for 5 h. The mixture is then cooled down and brought to room temperature. The product obtained is broken down roughly. For the removal of unreacted polymer, the product is washed multiple times and refined in the Soxhlet apparatus with ethanol solvent (Khan et al. 2016). Figure 6.3 depicts the preparation of nanosponges using ultrasound assisted synthesis method.

Emulsion solvent diffusion method: This method is based on the emulsification principle. The dispersed phase of the drug and the polymer and is added drop by drop into a definite amount of emulsifier PVA with water. The solution is

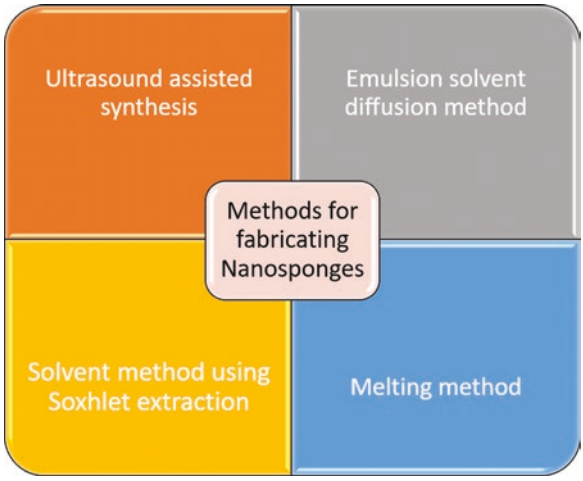

Fig. 6.2 Different methods for fabricating nanosponges

Table 6.1 List of constituents used for the preparation of nanosponges

S. No	Constituents	Types	Reference
1	Polymers	Hyper cross-linked polystyrenes, methyl β-cyclodextrin, alkyloxycarbonyl cyclodextrin, 2-hydroxy propyl β-cyclodextrin, Eudragit RS100, β-cyclodextrin, α-cyclodextrin	Singh et al. (2016)
2	Co-polymers	Poly(valerolactone-allylvalerolactone), Poly(methyl methacrylate) (PMMA), Poly(valerolactone-oxepanedione), poly vinyl alcohol, hydroxypropyl methylcellulose (HPMC), ethyl cellulose (EC)	Sharma et al. (2011), Rahi and Kumar (2017)
3	Aprotic solvents	Methanol, ethanol, dimethylformamide, dimethyl sulphoxide, dimethylacetamide	Patil et al. (2017)
4	Cross-linkers	Diphenyl carbonate, diaryl carbonate, hexamethylene diisocyanate (HMDI), carbonyldiimidazole (CDI), carboxylic acid dianhydride, toulene-2,4-diisocyanates (TDI), epichlorhydrin, pyromellitic anhydride (PMDA), 2,2-bis(acrylamido)acetic acid, dichloromethane, polyamidoamine	Rahi and Kumar (2017)

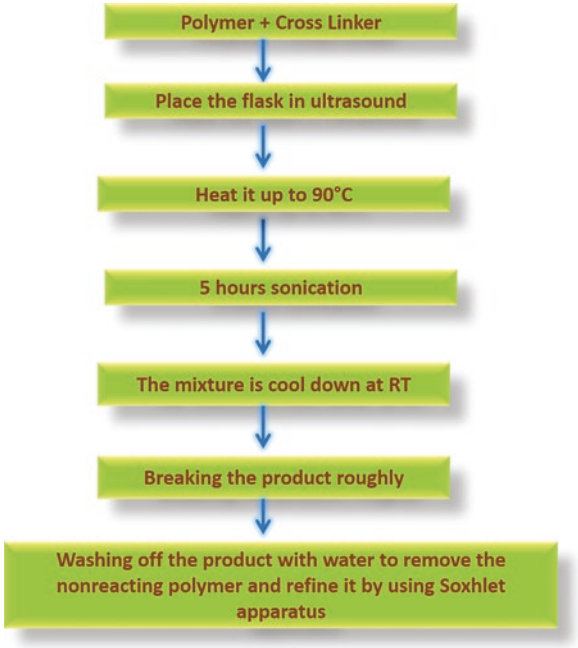

Fig. 6.3 Flow chart for the preparation of nanosponges by using ultrasound assisted synthesis method

constantly stirred by a magnetic stirrer. An emulsion will be formed due to the evaporation of the dispersed phase and the nanosponges formed can be collected by filtration and dried in the hot air oven (Jilsha and Viswanad 2015). Figure 6.4 illustrates the preparation of nanosponges by Emulsion solvent diffusion method.

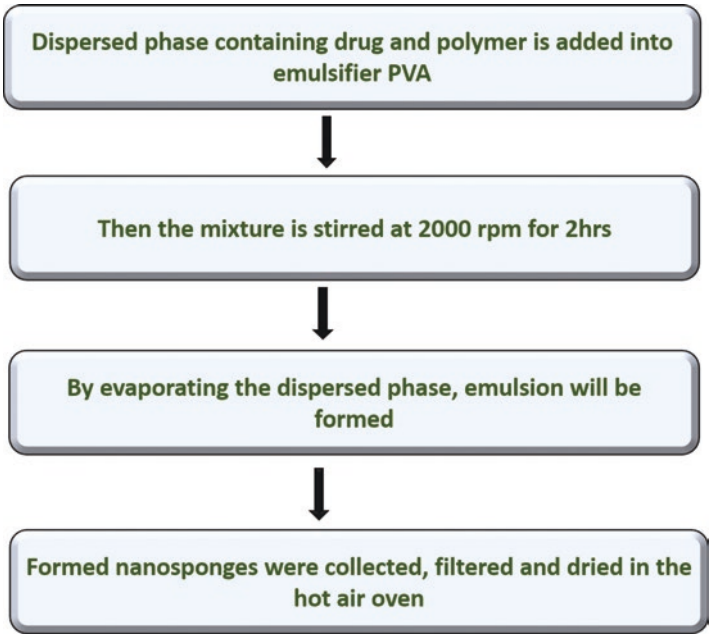

Fig. 6.4 Flow chart for the preparation of nanosponges by using Emulsion solvent diffusion method

Solvent method using Soxhlet extraction: The polymer solution and crosslinkers are added in a molar ratio of 1:4 and heated together up to 80 °C in a round bottom flask until a clear solution is obtained. Catalysts can be added to carry out the reaction. The crude obtained is centrifuged and resuspended with water. The residue is desegregated by vortex and centrifuged again. By using Soxhlet extraction methods the slurry is washed with ethanol for 8 h to remove unreacted crosslinker. The obtained nanosponges samples are maintained at −78 °C for 4 h and then the resultant solid is lyophilized overnight to keep their original intact structure (Singh et al. 2018).

Melting method: In this method, the nanosponges are synthesized by melting the crosslinker and the polymer together by heating in a flask at 100 °C. The mixture is constantly stirred in a magnetic stirrer for 5 h and cooled down. The nanosponges can be collected after subjecting the obtained mixture for multiple washing with an appropriate solvent to get rid of unreacted reagents and polymer (Jain et al. 2020).

6.4 Loading of Drug

Nanosponges are pretreated by suspending in water and sonicated to create more dispersion and avoid agglomeration. The excess drug is added into the aqueous solution of nanosponges and mixed with the assistance of magnetic stirrer continuously for complexation to take place. Removal of the supernatant after centrifugation helps in separating the unbound drug from the immobilized drug. The solid crystals of the nanosponges are collected by using a freeze dryer or by solvent evaporation method. The prepared drug/dye-loaded nanosponges are purified by dialysis against distilled water. Figure 6.5 depicts the steps for drug loading to nanosponges. The loading efficiency is calculated by = (amount of drug content in nanosponges/theoretical drug content) × 100 (Chilajwar et al. 2014). By using UV spectrophotometer and high-performance liquid chromatography, the amount of drug-loaded into the nanosponges and the loading efficiency can be estimated (Bolmal et al. 2013). The loading efficiency is calculated using Eq. (6.1)

$$Loading\ Efficiency\left(LE\right) = \frac{Amount\ of\ drug\ content\ in\ nanosponges}{Theoretical\ drug\ content} \times 100 \quad (6.1)$$

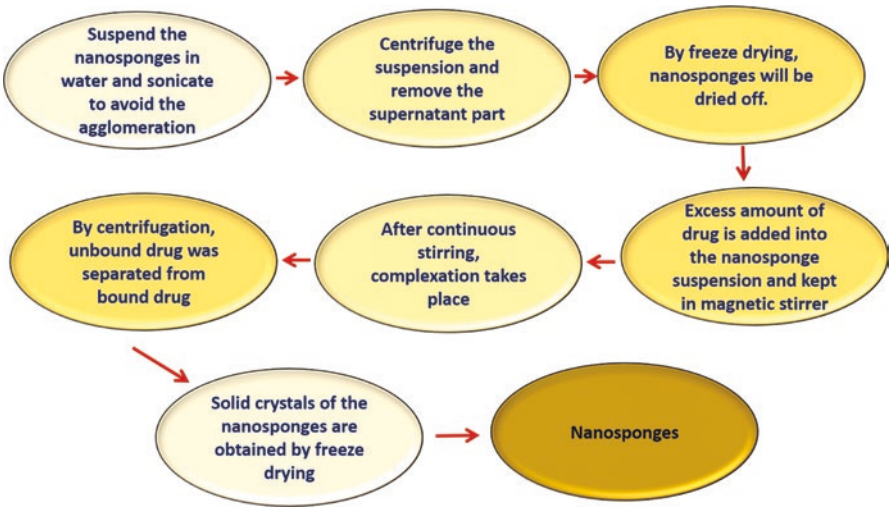

Fig. 6.5 Steps for loading the drug into nanosponges

6.5 Drug Releasing Kinetics

The Nanosponges are made up of many open structures on its surface. Nanosponges can carry the water-insoluble drug because of their tiny porous structure. Drug molecules are added to the mesh-like structure of nanosponges with nanocavities in the vehicles in encapsulated forms where they move freely from the particles into the vehicle until equilibrium is obtained. When applied locally on the skin or taken orally, the vehicles containing the drug molecules cause a disturbance in the equilibrium and cause unsaturation of the vehicle containing the active drug molecules. The drug molecules from the nanosponges flow consequently to the skin epidermis until the vehicle is either absorbed or dried completely. This process continues until all the drug has been utilized by the body. The release of the active drug molecules continues for an extended period even after the nanosponge particles retained on the skin surface (Khan et al. 2016).

The drug release of administered β-CD-NSP-Dox (β-cyclodextrin based nanosponges with doxorubicin) and β-CD-NSP-CHS-Dox (β-cyclodextrin based nanosponges with cholesterol and doxorubicin) were analyzed by dissolution apparatus against buffer at pH 6.8 and pH 1.2. CHS grafted NSP showed relatively fast release at starting in the phosphate buffer at 6.8 pH while the release profile was nearly the same after 2 h. NSP and CHS grafted NSP displayed a high profile of drug release at pH 1.2 and nearly 82% of drug release within 15 min. This pattern obtained agreed with previously reported research. The amount of Dox released form the NSP and CHS grafted NSP was determined by HPLC with a UV detector at 233 nm (Singh et al. 2018).

The Korsmeyer-Peppas release exponent (n) ranged between 0.331 and 0.418, established diffusion as the principle of mechanism of drug release of econazole nitrate (EN) nanosponges loaded hydrogel. The mechanism of drug release was further verified by calculating the ratio of exponents A/B ratio derived from the Kopcha model. This drug delivery system developed as a nanosponge hydrogel provides a solubilizing matrix for poorly soluble drugs and also provides a rate-limiting matrix barrier for drug release modulation (Sharma et al. 2011).

6.6 Advantages of Nanosponges

1. Nanosponges are chemically and physically stable.
2. Nanosponges can increase and alter solubility.
3. Nanosponges can carry hydrophilic and lipophilic substances.
4. Drug delivery using nanosponges formulations is nontoxic nonallergenic, and nonirritant and can provide many advantages for the delivery of anticancer molecules, such as the protection of the incorporated molecules, the controlled and sustained release of drugs, the capacity to overcome biological barriers, the localization in targeted tissues (Trotta et al. 2014).

5. Nanosponges are small and spherical in shape. So, it can be developed as multiple dosage forms like topical, tablets and capsules (Bezawada et al. 2014).
6. Nanosponges can be administered either by topical delivery system, oral and intravenous administration.
7. Nanosponges play a vibrant role in the delivery of highly efficient anti-cancer drugs and better-targeted action to improve bioavailability and reduce side effects (Pawar et al. 2019).
8. The nanosize of nanosponge particles can penetrate the cells, bind with the nucleus membrane, and release the drugs.
9. Nanosponges can be labeled with specific linkers to target diseased cells hence achieving greater efficacy while reducing side-effects, dosing frequencies, and improving patient compliance (Ahmed et al. 2013).
10. HNT-CDs nanosponge hybrids are good nano adsorbent for selective adsorption of cationic dyes compared to the anionic ones in a wide pH range (Massaro et al. 2017).
11. Nanoformulations could increase drug concentration at the tumor site since they are solid particles with spherical morphology and with very high solubilizing effect to form inclusion and noninclusion complexes with various drugs (Mendes et al. 2018).
12. Self-sterilizing activity is owed to the average pore size of 0.25 μm preventing the penetration of bacteria in the nanosponge (Bhowmik et al. 2018).
13. Nanosponges network enables further modification of the surface due to the presence of free hydroxy groups within the structure. The polarity and size of the polymer mesh can be adjusted by changing the form of cross-linker and degree of cross-linkage (Trotta et al. 2012).
14. BNS-DOX (β-cyclodextrin nanosponges containing Doxorubicin) proved to be a promising effective nanoformulation in the treatment of breast cancer (Argenziano et al. 2020).
15. β-cyclodextrin and NN-methylene bisacrylamide Nanosponge hydrogel formulation is ideal for improving the solubility and bioavailability of poorly water-soluble drugs such as celecoxib (Gangadharappa et al. 2017).
16. The use of nanosponge CD polymers and their modified forms in adsorption processes have provided ways of removing water contaminants and pollutants efficiently and successfully (Leudjo Taka et al. 2017).
17. A significant reduction of the induction time of oxidation in the presence of polypropylene-based composites containing β-cyclodextrin nanosponges alone is achieved using nanosponges (Alongi et al. 2011).

6.7 Disadvantages of Nanosponges

1. Incidences of drug dose dumping cannot incorporate large molecules.
2. Different loading capacities of the paracrystalline and crystalline form (Dubey et al. 2017).

3. Applications are unfortunately hampered in many cases by their non-binding nature.
4. Lack of interaction with specific proteins or cell membranes.
5. Loading affects the degree of crystallization.

6.8 Therapeutic Application of Nanosponges

Nanosponges activate insoluble drugs and prevent the physiological degradation of active drug molecules. Due to the diverse applications in anti-cancer, antiviral, antiplatelet, and antihypertensive therapy nanosponges play an important role in new arrays of therapeutic medicine because of its rapid growth in dose reduction, controlled drug release and retained long term stability.

Nanosponges to overcome Multidrug resistance: WP6-based nanosponges are developed as drug carriers to surmount MDR (multidrug resistance). MDR cancer cell line MCF-7/ADR has been used to evaluate the efficacy of drug delivery. In comparison, nanosponges loaded with DOX (doxorubicin hydrochloride) displayed high cytotoxicity even at low concentrations. When both nanosponges and WP6 were tested against the cell line in in-vitro conditions, both groups displayed limited cytotoxicity, which showed that nanosponges could deliver DOX to overcome MDR. The endocytic mechanism of DOX-loaded nanosponges into MCF-7/ADR cells has been investigated. Flow cytometry compatible with fluorescence microscopy was also used to assess the cellular uptake of DOX-loaded nanosponges. The fluorescence observed in the cytoplasm of cells for the short duration of incubation time suggested that DOX loaded nanosponges were internalized by endocytosis. Weak DOX fluorescence could also be observed in cell nuclei, confirming that DOX was delivered to nuclei (Liu et al. 2020). P-gp (P glycoprotein)-mediated efflux pump is often considered to be the key factor for MDR (Markman et al. 2013). MDR is often overcome by the use of nucleic acid or small molecule P-gp inhibitor. The possible reasons for MDR overcome with WP6-based nanosponges: (1) stable encapsulation of the pre initialization of DOX to avoid exocytosis of DOX; (2) efficient use of cells for these negatively charged nanosponges; (3) effective release of drugs after internalization. This reported research on host-guest chemistry is a promising method of loading cargo to resolve MDR and other biomedical purposes (Liu et al. 2020).

Nanosponges for improved antibacterial and effective antifungal activity: To study the development of cyclodextrin-based nanosponge of norfloxacin (NFX) to improve its physicochemical characteristics to assess the antibacterial activity of nanosponges, CLP (cecal ligation and puncture) model was used. The kidney was the organ chosen for the assessment of antibacterial activity. Rats treated with norfloxacin loaded nanosponges presented a smaller number of CFU when compared to animals treated with the drug alone. This is due to the higher

solubility of the drug in the nanosponge system and the mucoadhesion caused by this system. NFX-loaded NS provided a controlled release of NFX that prolonged the antibacterial in vivo activity induced in rats. This paper reports that due to its stronger antibacterial behavior, nanosponges are an effective carrier for norfloxacin. This system could increase the therapeutic benefits of being a potential alternative to the existing formulations of NFX drugs (Mendes et al. 2018).

In this study, lemongrass-loaded ethyl cellulose nanosponges were developed with topical hydrogel with an enhanced antifungal effect and decreased irritation. Male albino rats were used to assess the in vivo antifungal activity of the nanoformulation of nine prepared hydrogels integrating lemongrass-loaded nanosponges called F9. SEM and TEM analysis of the nanoformulation revealed the spongy structure with minute pores and the sustained integrity of the nanosponge structure when incorporated in the hydrogel. The obtained results were concluded to be positive in terms of the practical application of the incorporation of lemongrass oil in pharmaceutical formulations to reduce hazards (Aldawsari et al. 2015).

In this study, lysozyme impregnated surface-active carbonyl diimidazole crosslinked b-cyclodextrin nanosponges were synthesized to preserve its conformational stability and break bacterial cell walls by catalyzing the hydrolysis of 1,4-b-linkages between N-acetyl-D-glucosamine (NAG) and N-acetylmuramic acid (NAM) residues present in peptidoglycan layer around the bacterial cell membrane. To deliver the hydrolytic enzyme it works as a smart polymer-based nanoformulation. The stable nanosponges formulation will be a promising carrier for preventing the calcium depletion in antibiotic-associated hypocalcemic conditions and for antibacterial protein (Deshmukh et al. 2016).

Nanosponges for Targeted drug delivery: For the preparation of therapeutic medicine for oral administration, the nanosponges are dissolved in a suitable excipient like lubricants, diluents, and anti-cracking agent (Bhowmik et al. 2018). To study the targeted drug delivery, a novel theragnostic agent was prepared using a surface modification of magnetite nanoparticles with CDNS-FA (cyclodextrin nanosponge polymer decorated with folic acid) for targeted drug delivery of curcumin. Curcumin was the hydrophobic model drug loaded into the CDNS cyclodextrin cavities and the polymeric matrix. The goal of this study is to apply novel magnetic CDNS as a theranostic agent in cancer. The nanosponge system exhibited acceptable loading capacity of the drug and release profile for cancer therapy. The nanoformulations proliferation inhibitory effect against the cancerous line of the folate receptor-positive M109 was more than that of folate receptor-negative MCF 10A normal cell. The targeted drug delivery system revealed excellent biocompatibility with blood and the ability to selectively target further studies (Gholibegloo et al. 2019).

In this study, it narrates the new polyaminocyclodextrin nanosponges synthesis and characterization methods. The adsorption capabilities and sequestration tests at different pH values were checked against an acceptable collection of model guests, and were mainly controlled by electrostatic interactions and assessed the pH-dependent adsorption capabilities against organic guests with various

structures. Nanosponges appear as promising "pH-smart" materials, for potential applications as drug carrier/delivery systems or in the environment (Russo et al. 2016).

Nanosponges for topical applications: Cyclodextrin nanosponges can be regarded as a multifunctional nanoscale system appropriate for the delivery of active drug molecules in nanomedicines. Cyclodextrin nanosponges have a wide variety of uses in pharmacy, medicine, and other fields such as diagnostics, enzyme-catalyzed reactions, environmental regulation, and agrochemistry (Sherje et al. 2017). In this research, three types of nanosponges using α, β, γ-cyclodextrin cross-linked with carbonyldiimidazole (1:4 molar ratio) were developed as oxygen delivery systems for topical applications. The oxygen-encapsulating nanosponge and Pluronic F127 hydrogel were tested either as aqueous nanosuspension or as a gel. This gel could be a suitable carrier for oxygen as it provided a normal, continuous release of oxygen in the presence and the absence of ultrasound (Cavalli et al. 2010). In another study, the inclusion complex of Gamma-oryzanol b-cyclodextrin-based nanosponges was studied. They were chosen for their ability to encapsulate drugs to reduce their side-effects and to protect them from deterioration. In vitro studies performed on Franz diffusion cells found that the mechanism of complexation does not prevent the aggregation of Gamma-oryzanol in porcine ear skin. The nanoformulation complex indicated that it may have potential as a carrier for topically active substances (Sapino et al. 2013). In another study, there is a first report to produce semisolid formulations for drug delivery to the skin using multifunctional ingredient of β-cyclodextrin nanosponge and pyromellitic dianhydride (β-NS-PYRO) cross-linked. It can balance light-sensitive drugs and modulate the transportation of highly penetrating drugs in the external skin layers. It suggests that β-NS-PYRO will be a promising multifunctional ingredient in topical monophasic and biphasic formulations (Conte et al. 2014). In this work, β-cyclodextrin modified with a reactive group (monochlorotriazinyl group) is used in textile finishing. The permanent fixation of cyclodextrins allows with intriguing properties to strengthen. The cyclodextrins can complex different compounds of human sweat to offer new possibilities in medical diagnostics. The diagnosis and treatment of extensive skin diseases can be improved by the addition of pharmaceutically active substances to the complex by fixed cyclodextrins (Buschmann et al. 2001).

Nanosponges for cancer therapy: In this study, the formulation of three kinds of nanosponges, i.e., β-CD-1/2 nanosponges, β-CD-1/4 nanosponges, and β-CD-1/8 nanosponges for a delivery system for camptothecin was recently shown to be effective nanotechnology for the treatment of both androgen-sensitive and castrate-refractory prostate cancer in cell-line studies. The findings obtained in this research indicate that by displaying their inhibitory function, the nanosponges can integrate and distribute camptothecin in prostate cancer cells, and the nanosponges prolong camptothecin exposure longer (Minelli et al. 2012).The formulations of nanosponges are spherical in shape and colloidal in dimension. The nanoformulations loaded with CAM displayed marginal hemolytic activity and heavy cytotoxicity to HT-29 cells (Swaminathan et al. 2010).

The platelet-neutrophil hybrid membrane (PNM)-camouflaging gold nanocages (AuNCs), defined as nanosponges and nanokillers (NSKs) can deliver chemo-photothermal therapeutic agents to circulating tumor cells. Using high-affinity membrane adhesive ligands, the nanoformulation can trap and eliminate the circulating tumor cells and migrate tumor-related exosomes entirely. In this study, 4T1 xenograft and orthotopic breast tumor-bearing mice were administered with the nanoformulation. The findings obtained revealed that the NSKs do not only completely ablate the primary tumor and effectively prevent the metastasis of tumors. This approach is, therefore, a modern viewpoint on the therapeutic application of NSKs to metastasis inhibition of breast cancer (Ye et al. 2020).

In a study, it was demonstrated that free paclitaxel (PTX) and PTX-loaded in pyromellitic nanosponges (PTX-PNS) were both more effective than PTX in inhibiting the in vivo growth of melanoma cells in a mouse model. It was found that at lower concentrations, PTX-PNS were able to suppress, than free PTX. PTX nanoformulation has demonstrated lowering the anti-tumor effective doses and increasing the efficacy in inhibiting melanoma growth in vivo. The development of melanoma was significantly reduced in mice treated with PTX-PNS whereas no significant inhibition was obtained with the same dose of free PTX (Clemente et al. 2019).

In this study, a protein and lipid bilayer-capped ultrasmall graphene nanosponge supported lipid bilayers were synthesized having incorporated features of porous carbon nanosheets and liposomes to address the various obstacles of drug delivery to tumors. This uses photolytic therapy to release on near-infrared irradiation, a drug blast of docetaxel (DTX), and gasified perfluorohexane (PFH) and intense heat energy. The reported findings showed no recurrence of tumors for more than 60 days following diagnosis. This nanoformulation delivery system is a medium for penetrated, photo responsive, and combination gasification/chemotherapy to enable tumor care and use in other biological applications (Su et al. 2016).

Toxin-absorbing nanosponges to treat microbial infections: In this research, a biologically inspired toxin nanosponge is designed with a polymeric core wrapped in natural red blood cell bilayer membranes. It absorbs membrane damaging toxins and diverts them away from their cell targets. The nanosponges significantly reduced the toxicity of staphylococcal alpha-hemolysin (a-toxin) in a mouse model and thus proved to improve the survival rate of toxin-challenged mice. The variety of injuries and diseases caused by pore-forming toxins were treated by detoxification method of nanosponges (Hu et al. 2013). In a similar study, an advanced hybrid material that incorporates a unique hydrogel which retains toxin-absorbing nanosponges for antivirulence treatment of local methicillin-resistant Staphylococcus aureus infection was developed. The obtained reports from the in vivo treatment of MRSA infection and toxin neutralization by controlling skin lesions in mice models showed collectively that the formulation represents a new and successful detoxification technique for the treatment of localized bacterial infection (Wang et al. 2015).

Nanosponges for removal of dyes and bioremediation: To study the removal of dyes from aqueous solution, CDNS was fabricated in a one-step solvothermal method by β-cyclodextrin (β-CD) and diphenyl carbonate (DPC) using two of the familiar dyes basic red 46 and rhodamine B as the model contaminants. The experimental data of the adsorption capacities of both the dyes were studied. The uptake of the two dyes suggested that CDNS are qualified as environment-friendly bio adsorbent for the removal of dyes from water (Li et al. 2020).

Nanosponges for selective adsorption and antioxidant properties: Another study reported the viability of the material nanosponges based on halloysite nanotubes and cyclodextrins as a wastewater decontaminant by studying its adsorption capacity toward an organic dye Rhodamine B. The inclusion of cyclodextrin in the hybrid improves the halloysite adsorption efficiency. The obtained results showed that HNT-CDs nanosponge hybrids are good nano adsorbents in a wide pH range for selective adsorption of cationic dyes as compared to the anionic ones (Massaro et al. 2017). In another report, preliminary studies of organo-clay hybrid nanomaterials based on halloysite covalently linked with modified cyclodextrin highlighted that the carrier is promising for the delivery of quercetin and curcumin and can serve as a reservoir for the extended-release of the drug over 96 h. Antioxidant measurements have demonstrated that the curcumin in the hybrid preserves its properties, which can be beneficial for the treatment of many pathologies (Massaro and Riela 2018). In another study, novel nanoformulations were developed to increase the potential of resveratrol as a skin targeting antioxidant. Resveratrol (RSV) is a potent lipophilic antioxidant with poor aqueous solubility. These nanoformulations could be used to promote the skin delivery of RSV and to potentially exert an antioxidant effect on the skin. Effective transdermal delivery of RSV has potential benefits because the antioxidant effects may mitigate skin damage following exposure to UV light and slow down signs of skin aging (Nastiti et al. 2020).

Nanosponges for combination drug therapy: This approach involves the co-administration of multiple nano delivery systems containing active agents or the co-delivery of different active drug molecules in the same nanocarrier. Cyclodextrin-based nanosponges are ideal for the construction of codelivery drug-delivery systems due to their properties. Co-delivery of multiple anti-infectious agents in a single nano-based system is beginning to show substantial advantages over monotherapy, such as synergism, enhanced anti-microbial activity, broad anti-microbial range, decreased development of resistance, and improved and cost-effective treatment (Walvekar et al. 2019). In this research, multidrug delivery through combination drug therapy using nanosponges has been explored to promote a successful combination of monotherapy. Sequential HVGGSSV peptide targeted nanosponges of PTX and CPT were synthesized and scrutinized against murine LLC and A549 cell lines. The in vivo results supported the in vitro observations with improved antitumor efficacy showing cell arrest in step G2/M phase, destruction of microtubules, decreased vascular density, cell proliferation, and improved cellular deaths (Rawal and Patel 2019).

Nanosponges for the treatment of hyperphosphatemia: In this study, the novel engineered cyclodextrin-based nanosponge with calcium carbonate formulation has produced an enteric and controlled release of calcium in the management and treatment of hyperphosphatemia. Nanosponges serve as a reservoir for calcium carbonate delivery and adsorbent for moisture uptake and the moisture contents of the nanosponges varied from 0.1% to 0.7% (Shende et al. 2013).

Nanosponges for the treatment of Parkinson's disease: For the treatment of Parkinson's disease, β-cyclodextrin crosslinked with 1,1′-carbonyldiimidazole in DMF for the synthesis of Molecularly imprinted nanosponges (MIP-NSs) with a L-DOPA as a template molecule. Good efficiencies in encapsulation and drug loading capacities were achieved. The NMR results showed the effective performance of molecularly imprinted drug delivery systems. The findings of the NMR demonstrated the successful efficiency of the molecularly influenced drug delivery system. The in vitro experiments showed gradual and controlled release kinetics over time, indicating that the MIPs have a good capacity to store the drug and sustain its release. Polycarbonate βCD-based MIP-NS is a promising new drug delivery device for oral administration safety and sustained release of L-DOPA (Trotta et al. 2016).

Nanosponges producing biofunctional fabrics: In this study, carbonate nanosponges were developed from β-cyclodextrin and 1,1′-carbonyl imidazole. The XRD analysis of the nanoformulation emphasized that melatonin produces a molecular dispersion in the cavities of nanosponge. Melatonin loaded nanosponges were dispersed on cotton fibers, which proved to be a suitable substrate for durable nanosponge long-lasting adsorption. The in-vitro release tests have demonstrated zero-order kinetics creating a biofunctional fabric capable of controlling the melatonin release through the skin (Mihailiasa et al. 2016).

Nanosponges to improve water solubility: In this research, hyper-cross-linked cyclodextrin nanosponges were developed to encapsulate dexamethasone as a model molecule. The presence of cross-linking and cyclodextrin cavities in their structure facilitates interaction with drug molecules. The dexamethasone release profile showed good complexation between the drug and the nanosponge structure. These nanosponges can be used to improve the solubility of poorly water-soluble drugs. Contaminants like heavy metals like cadmium, chromium, zinc, lead, and many persistent organic substances such as chlorobenzenes, chlorotoluenes, and polychlorobiphenyls can easily be removed as nanocarriers for biomedical applications (Trotta and Cavalli 2009).

Nanosponges to improve oral bioavailability of drugs: In this research, griseofulvin (GRI) loaded β-cyclodextrin (β-CD) based nanosponges were successfully developed to mask the bitter taste of GRI, improve dissolution rate, and eventually boost oral bioavailability. This study reported that the GRI complexation with NS is a viable approach for masking the bitter GRI taste and improving oral bioavailability. Human panel gustatory response palatability studies confirmed the potential of F1 for GRI bitter taste masking. The formula F1 in the form of dry reconstitution suspension could be used as a successful GRI dosage form (Omar et al. 2020). In another research, β-cyclodextrin based nanosponge of

erlotinib hydrochloride (ERL-NS) was successfully developed and implemented to increase solubility, the efficiency of dissolution, and oral bioavailability of erlotinib. It was concluded that the inclusion complex of nanosponge formulation is a successful approach to enhance its solubility and oral bioavailability may result in a reduction in dose and dose-related side-effects (Dora et al. 2016).

6.9 Conclusion

Nanosponges due their "adjustability" and "tailoring" property lead to an interest to explore the many ways to achieve controlled drug delivery while guaranteeing drug stability and minimizing toxic effects. Nanosponges are adopted in removing bitter components of food and drug products. Some of the applications to be explored include removal of dangerous chemicals from industrial waste and organic air solvent vapors. Nanosponges are fabricated to solve physical, chemical and biological problems relevant to disease treatments. Interestingly, Nanosponges could be explored as diagnostic agents, for example in cancer imaging. Though they share wide range of applications, to date, only conventional approach and ultrasound-assisted synthesis are the synthetic methods reported for nanosponges. Research should throw light on methods for synthesizing nanosponges. Nanosponges role in downstream management requires thorough research due to their unique structure.

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Chapter 7
Recent Development in Peptide-Nanosystems for Combating Multidrug Resistant Cancer Cells

Mani Arulkumar, Varatharajan Mathivanan, Ramalingam Revathi, and Zhao-Yang Wang

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7.1 Introduction

Independent administration of drugs, including inhibitory RNAs, photosensitizers, and anticancer drugs is found not suitable for curative therapy primarily due to weak solubility, poor clinical outcomes as well as developing resistance during the clinical practice. Among them, rising resistance (MDR) against commonly prescribed therapeutic strategies such as chemo, hormonal, and radiotherapy regardless of the

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type of cancer cells is challenging obstacles for the treatment of cancer (Gottesman et al. 2002; Carlson et al. 2011). In resistance mechanisms, genetic influences (occurrence of mutation in alpha and beta-tubulin (Yang et al. 2005) and mutation in ABCG2 (Robey et al. 2003)), biochemical and molecular parameters (the transition from hormone-dependent to hormone-independent, gene amplification, overexpression of androgen receptor (AR) and AR coactivators in castration-resistant prostate cancer (Fujimoto et al. 2007; Maughan and Antonarakis 2015)), drive out anticancer drug by an elevated level of ABC transporters (P-glycoprotein, P-gp) (Liu et al. 2019), alteration in targeting enzymes (topoisomerase (Hwang and Hwong 1994) Glutathione S-transferases (Tew 1994)), stimulation of autophagy (Levy et al. 2017), inhibit the induction of apoptosis (Sun et al. 2019), and also additional modes have been reported. Apart from these specific factors, some of the resistance phenomena are multi factorials in nature and interrelated.

To fight MDR, exploration of new pharmaceutics from plants (Yu et al. 2016), marine species (Abraham et al. 2012; Dyshlovoy and Honecker 2015), synthetic medicinal chemistry approaches inspired from natural products (Hwang et al. 2016; Lopes-Rodrigues et al. 2017), and nanosystems (Patel et al. 2013) demonstrated impressive outcomes including no cross-resistance, no compromising in its potential, target the MDR cancer cells precisely, and averting recognition from P-gp. Among the above mentioned therapeutic modalities, nano-based strategies are resilient in terms of spatiotemporal response, sensitivity to various stimuli (pH, photothermal therapy, magnetic influence, light, etc.) and provide an area to bestow distinct camouflaging agents (hyaluronic acids, polydopamine, etc.) that helps to target and ensembles in the microenvironment of cancer cells (Patel et al. 2013; Shi et al. 2020) with the diverse mode of action (Zhao et al. 2019). One of the main advantages of utilizing nanoparticles is their enhanced permeability and retention effect (EPR) in the cancer cells. Notwithstanding, passive diffusion of the nanosystems is not a logical approach to all types of cancer cells; for instance, human sarcomas have fewer vascular structures, hindering the accumulation of nano-sized platforms. Thus, a unique molecular entity is essential to overcome those obstacles and should be versatile to allow various drug cargoes.

In addition to their significant role in immune and physiological, various biomedical applications of peptides (Rad-Malekshahi et al. 2016; Sunna et al. 2017; Qi et al. 2019) were developed owing to the structural conformations (Dougherty et al. 2019), sequence-defined targeting (Zhang et al. 2017; Han et al. 2016; Sun et al. 2017a), simple attachment methods including self-assembly (Chen et al. 2017) to form as nanoformulations, and provide sites to tether with various chemotherapeutics (Rong et al. 2018). These features accelerate progress in the drug delivery systems (DDS) towards cancer cells including MDR cancer cells. Peptide perpetually occupies as a crucial component to be appended onto nanoplatforms relating to its high sensitivity, conferring stability, enhanced attachment on the membrane of tumor cells, internalization, and allowing attachment of distinct chemotherapeutics, photosensitizer, and various stimuli-responsive elements. Therefore, peptide aided nanoplatforms realize an efficient DDS for the annihilation of resistance traits in the cancer cells and would open up the new avenue of therapeutic choice.

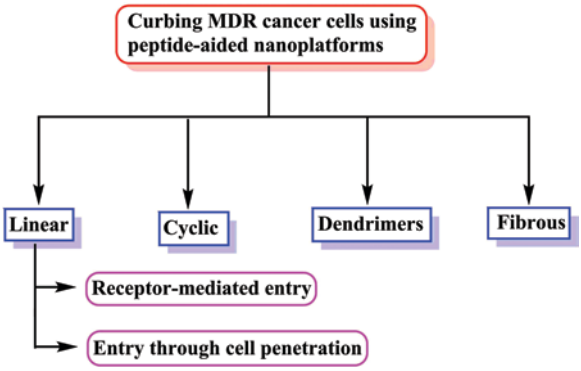

Fig. 7.1 Classification of peptide-aided nanoplatforms for the reversal of MDR

A recent development for the circumvention of resistance by the peptide integrated nanoplatforms reported from 2017 to the present date is categorized into four sections according to the structural characteristics of peptide that are attached to nanoplatforms, as shown in Fig. 7.1.

7.1.1 Linear Peptide Aided Nanoplatforms (Receptor-Mediated Entry)

Ranging from short (3 amino acids) to long (50 amino acids), peptides are utilized as cancer-targeting moiety by tethering on various platforms, including nanoparticles for enhanced and precise uptake of drug cargos into resistant cancer cells.

The application of linear peptides in DDS is of significant merits in terms of targeting ability based on sequence-defined and facilitating sites for attachment of the range of drugs, other camouflaging agents, and polymers. The short linear peptide that has three amino acids is found adequate for transporting even big molecular weight anticancer agents, establishes secure attachment, and internalizes onto the membrane of the resistant cancer cells. As stated in the preceding paragraph, RGD (*Arg-Gly-Asp*) peptide grafted core-shell nanocarrier orderly released the tethered verapamil (Ver), a P-gp inhibitor, and mitoxantrone, a chemotherapeutic onto MDR tumors (Wang et al. 2018). It is known that RGD is precisely recognized $\alpha_v\beta_3$ integrin receptors that are highly expressed in the MDR cancer cells rather than healthy cells (Shan et al. 2015). Identically, RGD peptide (Cys-*Arg-Gly-Asp*-Lys/Arg-Gly-Pro-Asp/Glu-Cys) conjugated lipid polymer hybrid nanosystem comprising of core-shell milieu were successfully delivered the attached drug payloads, P-gp inhibitor (tetrandrine, a bis-benzylisoquinoline alkaloid from *Stephania tetrandra* S. Moore) and chemotherapeutic (paclitaxel, PTX) to the paclitaxel-resistant ovarian carcinoma (A2780/PTX) (Zhang et al. 2017). Conjugation of another RGD containing peptide (Gly-Phe-Leu-Gly-His-His-His-Arg-Arg-Gly-Asp-Ser) and sequence cleavable by lysosomal cathepsin B (Gly-Phe-Leu-Gly) with mesoporous

silica nanoplatforms avert both efflux mechanism of molecular transporters (P-gp) and endolysosomal recognition. This peptide based-approach ensures to deliver the encapsulated curcumin and doxorubicin (adriamycin, ADR) to the resistant MCF-7 (MCF-7/ADR). After accumulation and cleaved inside the lysosome, Histidine abundant amino acid residue remained in drug payloads facilitates to penetrate the lysosomal membrane and ensures the export of drug payloads to their destination without entrapment (Sun et al. 2017b). Furthermore, encapsulation of Dox and curcumin in the nanoparticles incorporated with distinct features including pH-responsiveness and functionalization with U11 peptide (Val-Ser-Asn-Lys-Tyr-Phe-Ser-Asn-Ile-His-Trp) is targeted particularly to the overexpressed Urokinase plasminogen activator receptor (uPAR) in lung tumor cells and tissues (Hong et al. 2019). Without peptide targeting, these nanoplatforms exhibit less potentiality for the circumvention of resistant cancer cells, presenting evidence of the peptide as a targeting component in the nanoplatforms.

7.1.2 Linear Peptide Aided Nanoplatforms (Entry Through Cell Penetration)

Cell penetrating peptide (CPP) is the protein transduction domain, possessing inherent targeting ability towards the membrane and internalizing a range of attached cargoes, including nanomaterials, into cancer cells. CPP exhibits distinct mode of attachment and delivery; firstly, cationic amino acids (mostly arginine and lysine) sequence exerts positive charge to the attached nanosystems in order to bind with the negatively charged cancer cell membrane, and aids to endocytosize into the target cancer cells; secondly, a small array of amino acids facilitate to cross the cell membrane due to its intrinsic transduction abilities (Zahid et al. 2010). The functionalization of nanoplatforms with the arginine-enriched sequence in a peptide exerting positive charge shows a high affinity towards the negatively charged cell membrane of resistant cancer cells. For instance, Octa arginine (Arg_7) and folic acid tethered nanosystems with pH-sensitive moiety (poly sulfadimethoxine) display enhanced targeting and delivery of docetaxel (DTX) into resistant cancer cells due to pH variation between healthy and cancer cells (Wang et al. 2017a). Belonging to the CPP family, TAT peptide exhibits efficient transportation of various types of cargoes, including nanosystems along with the cytotoxic agents. Furthermore, exploiting ultrasound with micro bubbling technology enhanced the intracellular delivery of TAT peptide-nanoconjugate by causing pore formation in the resistant cancer cells, thus, introducing alternative endeavor in transforming disadvantage into practically feasible strategies for clinical practice in future (Wang et al. 2017b). Due to conformational change in pH low insertion peptide (pHLIP), another class of CPP at weak acidic microenvironment of cancer cells, construction of the nanosystem with pHLIP successfully released its drug payloads into cancer cells, including MDR cancer cells. Remarkably, the circumvention potential of camouflaged

gold nanocages by the thermoresponsive element highlighted that the decorated pHLIP is an essential component to be conjugated for the internalization and execution of anti-MDR activity even though reasonable photothermal conversion rate and hollow caged structure of gold nanoparticles (Huang et al. 2019). Further, irradiation using near-infrared (NIR) causes shrinking of the thermoresponsive element, thus, instantly expose the hole in the gold nanocages, which in turn ideally release the appended Dox into the resistant cancer cells on-demand manner. Thus, cumulative efforts such as efficient internalization, photothermal conversion rate, nanogold cage structure, and peptide aided internalization offer multi-staged nanoplatforms to surmount resistant cancer cells.

LAH4 is another Histidine enriched cationic CPP, having antimicrobial activity and interacts with the negatively charged membrane of cancer cells. Its derivative, LAH4-L1 peptide integrated with RNA interference (RNAi) technology offers practically feasible approach to reverse drug resistance in cancer cells by suppressing MDR in gene level. Loaded siRNA (siMDR1) onto self-assembled polypeptide nanoplatforms (LAH4-L1/siRNA) enhanced the cellular internalization via endocytosis and macropinocytosis and unloaded siRNA in order to silence overexpressed gene responsible for the multidrug resistance. A combination of the LAH4-L1/siRNA with PTX deliberately increased the inhibition of cell growth through apoptosis as compared to PTX alone treatment (Liu et al. 2019).

7.1.3 Cyclic Peptide Aided Nanoplatforms

In addition to linear peptide, closed circular form of the peptide, the cyclic peptide is integrated into various biomedical applications due to their excellent binding affinity, leave no charged terminal at their structure (Claro et al. 2018), less susceptible to the degradation, opsonization and low antigenicity (Mäkelä et al. 2008) and known cell penetration mechanism (Dougherty et al. 2019). To target p53 receptor and peritumoral lymphatic vessels, LyP-1, a 9-amino acid-containing cyclic peptide (Cys-Gly-Asn-Lys-Arg-Thr-Arg-Gly-Cys) to which coupling of a conjugate containing low molecular weight heparin (LMWH)-quercetin tenders inner hydrophobic quercetin core and hydrophilic LMWH outer shell (PLQ) (Tian et al. 2018). The integration of LyP-1 peptide facilitates tumor targeting, internalizes PLQ nanoparticles into the lymph node, and involves in co-delivery of gambogic acid to intercept the growth of the resistant cancer cells, and prevent metastatic spread of cancer through lymphatic vessels as well as the formation of new blood vessels. Overall, the PLQ nanosystem overwhelmed the resistant and relapsed cancer cells via a combinatorial approach. Recently, the cyclic form of RGD peptide (cRGD) decorated on nano-micelles along with chemotherapeutic DTX modifies DTX resistance in U87/DTX cells (Qilong et al. 2019).

7.1.4 Dendritic Peptide Aided Nanoplatforms

Dendritic peptide is a type of branched architectural arrangement that offers indispensable pharmacological properties, including robust contact with the membrane of the resistant cancer cells owing to branched hydrophobic moieties, preventing leakage of the loaded drug molecules, and flexibility in drug release by modifying the length of branches. One such dendritic peptide is a synthetic amphiphilic dendritic peptide, Cys-Arg-Arg-Lys(Arg-Arg-Cys-Gly(Fmoc))$_2$, which is enriched with basic amino acids, typically arginine. Encapsulation of Dox into self-assembled nano-micelles is stabilized through the hydrophobic Fmoc group of the dendritic peptide. The self-assembled nano-micellar systems are intended to escape from molecular pumps (P-gp) and endolysosomal identification to prevent the expulsion of Dox. Thus, the arginine sequence directs the internalization of nano-micellar systems and aids its localization into the nucleus of MDR cancer cells (Chen et al. 2017).

Apart from the amphiphilic dendrimers, Gu group utilized self-assembled Lysine dendronized amphipathic peptide to form nano-aggregates. It disturbs the membrane of resistant cancer cells by leaking its biomolecules and perturbs the membrane of organelles, including mitochondria, to release apoptosis factor, thus innovatively killing resistant cancer cells (Zhang et al. 2018). Gu group further investigated the scope of peptide dendrimeric prodrug (PDP) nanoplatforms targeting distinct enzyme known as telomerase (the vital enzyme involved in conserving telomeric end) and found that its inhibition sensitizes resistant cancer cells (MCF-7/R) (Wu et al. 2020). Thus, construction of multi responsive dendritic nanoplatforms integrated with different tumor-specific responsive factors such as acid-activated Histidine, a redox-sensitive poly(ethylene glycol) (PEG), BIBR1532, and acid-sensitive hydrazone bond in order to foster the cellular uptake, escape from endosomal recognition, the release of a telomerase inhibitor, and release of the chemotherapeutic drug, respectively is involved for effective combating of resistant MCF-7/R.

Furthermore, they developed another dendrimeric structure supported by MMP sensitive peptide (Gly-Pro-Leu-Gly-Leu-Ala-Gly). The formed nano scaffold shows the advantage of being multi responsive elements whose attachment facilitated to cross various physiological obstacles posed by resistance phenomenon (Li et al. 2017). The passive diffusion of the dendrimeric nanostructure, effortless internalization, intense penetration, cytoplasmic stimuli-induced disintegration of drug cargoes, depletion of ATP, induction of apoptosis, and delivery of chemotherapeutics to the nucleus are some of the listed advantages of dendrimeric nano assemblies against resistant cancer cells. Gu group again successfully established peptide-based dendritic nanosystem loaded with Dox along with cathepsin B recognizable peptide, Gly-Phe-Leu-Gly (GFLG) to deliver the attached drug cargoes to cancer cells. Once the delivery of mPEGylated Dendron-GFLG-Dox conjugates into the lysosome, the level of cathepsin B was increased, which in turn increases the level of caspase-3 and ROS as well as decreases level of Bcl-2 (critical inhibitor of apop-

tosis). In vivo result also supported the therapeutic efficacy of peptide-dendrimeric combined nanosystem with Dox for circumvention of resistant cancer cells as compared to Dox alone (Wang et al. 2020).

7.1.5 Nanofibers: Cancer Cells Instructed Self-Assembly

Xu group introduced novel concept designated as enzyme-instructed self-assembly (EISA) for the annihilation of cancer cells, particularly drug-resistant cancer cells (MES-SA/Dx5, T98G, and A2780-cis) whose ectopic alkaline phosphatase (ALP) expressed on the surface is involved to convert D-tripeptides (DTPs) into the nanofibrous net. Nanofibers interact with both CD95 and DR5 receptors and present TNF-α to kill MES-SA/Dx5 and A2780-cis resistant cancer cells or assist for the interaction of ligands such as TNF-α, TRAIL, and CD95L to their respective receptors intending to stimulate apoptosis in T98G resistant cells (Du et al. 2017). Similarly, the formation of nanofibrous architecture from D-phosphopeptide (derivative of DTP) was formed due to the instruction from prostatic acid phosphatase (PAP) in drug-resistant prostate cancer cells (castration-resistant prostate cancer) (Feng et al. 2019). Phosphatase (ALP and PAP) mediated-dephosphorylation of the terminal phosphate group of DTPs produced the nanofibrous net-like structure, which activated the death receptor and presented proapoptotic ligands to their respective receptors for the destruction of resistant cancer cells, thus, exploiting phosphatase as druggable targets to curb MDR cancer cells.

His group further discovered another EISA-mediated formation of nanofibers that are mainly targeting the mitochondria of cancer cells, including Dox-resistant cancer lines (MESA/Dx5) (He et al. 2018). The action of enterokinase enzyme (ENTK) cleaved the substrate precursor tethered with protein tag (FLAG-tag) to generate micelles, which further undergo intracellular cleavage to afford negatively charged nanofibers. Once enter into cells, the branched peptide along with cleaved products accumulated on mitochondria due to affinity between negative nanofibers with positive charged H^+ in the intermembrane space of mitochondria. The attached Dox with D-2TFLAG enhanced the synergistic anticancer activity of Dox in the Dox-resistant cancer cells (MESA/Dx5) and notably, branched architecture is a unique structural prerequisite for targeting mitochondria.

7.1.6 Polypeptide Aided Nanoplatforms

The nanocomposite (PNOC-PDA) were constructed using poly (L-Cysteine)$_{20}$, PC as core component with a copolymer of poly(ethylene oxide)$_{45}$ with a coating of polydopamine (PDA) around heat-sensitive nitric oxide (NO) releasing element in order to combat the resistance in synergistic fashion (Ding et al. 2019). PNOC-PDA nanocomposites upon NIR irradiation release NO; a gas molecule that can able to

kill cancer cells at its high concentration and diminish the level of expression of P-gp as well. The attached anticancer drug Dox (PNOC-PDA/Dox) and PDA provide a module to perform chemotherapy and phototherapy without harming healthy cells, thus, paving new track in killing MDR cancer cells.

7.1.7 Peptide Aided Nanogel Systems

Self-assembling properties of short peptides afford another type of nanostructure named nanogels, which is reported as a potential platform with various biomedical applications (Eskandari et al. 2017). Nanogel formed from short peptide (Fmoc-Gly-Phe-Leu-Gly-Gly) has been conjugated with Dox through pH-sensitive hydrazone bond (Peptide-Dox, PD). Upon further co assembled with P-gp inhibitor Ver, PD/Ver nanosystems displayed potent ability to traverse along with the attached drug cargoes (Dox and Ver) into Dox resistant ovarian A2780/ADR cancer cells and reached lysosome where the cleaving of acid-sensitive hydrazone bond helps to unload drug cargoes. The liberated Dox and Ver emerged from lysosome are directed to the cytosol and nucleus to perform its intended tasks, inhibition of topoisomerase activity and P-gp, respectively. Therefore, PD/Ver nanogels are considered as a smart approach due to delivering anticancer drug and P-gp inhibitor simultaneously, and masking its recognition by the MDR cancer cells (Lyu et al. 2017). The overall strategies exploiting peptide-nanoplatforms for the reversal of MDR are illustrated in Fig. 7.2.

Important structural characteristics, target, and appended chemotherapeutics in the peptide-nanoplatforms for the reversal of MDR are summarized in Table 7.1.

7.2 Peptide Aided Nanoplatforms to Eradicate Cancer Stem Cells (CSC)

Cancer stem cells (CSC) are a small subpopulation of cancer cells whose sustaining role in cancer resistance, relapse, and metastasis are inevitable (Eduard and Hans 2017). Remarkably, targeting CSC in resistant cancer cells via its specific characters, including receptors, markers, etc. was also achieved by the nanosystems with the appropriate appended components (Zhao et al. 2013; Tsai et al. 2019). Thus, targeting specific receptors that are expressed uniquely on CSC is one of the paradigm strategies. There are two types of approaches in peptide-aided eradication of CSC; one approach is to integrate a peptide with high affinity to receptors unique for CSC (ligand-receptor approach), and another approach is to integrate a peptide possessing different function other than acting as a ligand for the specific receptor (Fig. 7.3).

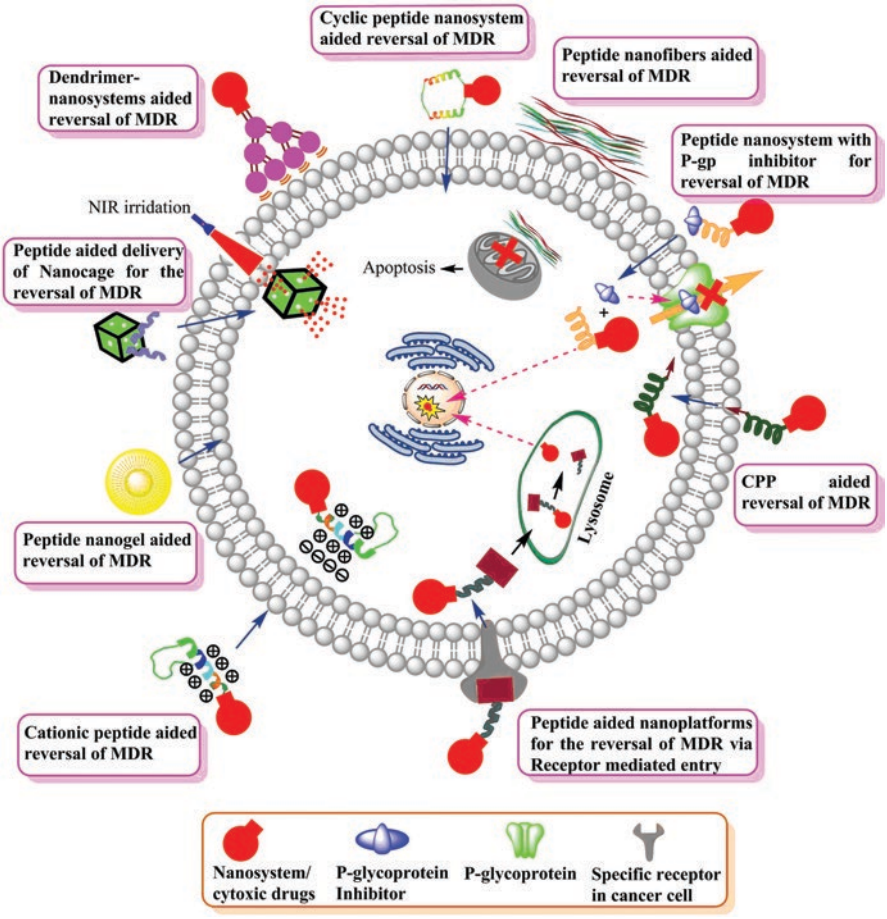

Fig. 7.2 The distinctive approaches towards curbing MDR using peptide-nanosystems

With rising resistance phenomenon to chemotherapy and radiotherapy, glioblastoma multiforme (GBM) is considered as a severe brain tumor owing to the existence of CSC. Gonçalves et al. developed innovative strategy by exploiting GBM stem cell marker (Nestin, Nes)-destined peptide along with PEG ether thiol camouflaged-gold nanorods (PEG-AuNR) system, which exhibited selective accumulation and eradication of single Nes positive GBM CSC and its multicellular tumor spheroids system (MCTS) (Gonçalves et al. 2017, 2018). The appended modular peptide is particularly found as an essential component whose terminal sequence drives to Nestin receptors and establishes stable and selective binding only with Nes positive CSC of GMB. Once internalized, on-demand cytotoxic action initiates heat from gold nanorods upon irradiated with NIR. This approach retards the survival of MCTS derived from GBM CSC and may exploit to eradicate the CSC population in other resistant cancer cells, recurrence, and relapse.

Table 7.1 Structural characteristic, target, appended chemotherapeutics, shape, and size of peptide aided nanoplatforms against MDR

S. No	Name of the peptide	Characteristic features	Tethered drug/s	Resistant cell line/ name of the drug to which resistance has arisen	Target	The shape of nanocarrier (size)	Reference
1	RGD	Linear	Mitoxantrone[a] Verapamil[b]	BEL-7402/MDR	$\alpha_v\beta_3$ integrin receptors	Sphere (20 ± 6 nm)	Wang et al. (2018)
2			PTX[a] and Tetrandrine[b]	A2780/PTX		Sphere (<150 nm)	Zhang et al. (2017)
3			Dox (Adr)[a] and Curcumin	MCF-7/Adr		Sphere (~70 nm)	
4	U11 peptide		Dox[a] and Curcumin	A549/Adr	Urokinase plasminogen activator receptor	Sphere (121.3 nm)	Hong et al. (2019)
5	Octaarginine		DTX[a], GDC0941[a] and Folic acid	MCF-7/Adr	Negatively charged membrane of cancer cell (cell penetration)	Sphere 151.8 ± 9.5	Wang et al. (2017a, b)
6	TAT peptide		Dox[a] and microRNA (miR-129-5p)	MCF-7/Adr	P-gp	Sphere (90 ± 4.2 nm)	Wang et al. (2017a, b)
7	pHLIP		Dox[a]	MCF-7/Adr	Acidic pH environment of the cancer cells	Cube (>160 nm)	Huang et al. (2019)
8	LAH4		PTX[a] and siRNA (siMDR1)	MCF-7/Adr	MDR1 gene	Irregular shape (110 ± 5 nm)	Liu et al. (2019)
9	LyP-1	Cyclic	Gambogic acid[a] and low molecular weight heparin (LMWH)-quercetin	MCF-7 cells (over-expression of p32 receptors)	p53 receptor and peritumoral lymphatic vessels	Quasi-Sphere (172.2 ± 2.8 nm)	Tian et al. (2018)
10	RGD		Docetaxel[a]	U87/DTX	$\alpha\alpha_v\beta_3$ integrin receptors	Sphere (159.2 ± 0.2 nm)	Qilong et al. (2019)

S. No	Name of the peptide	Characteristic features	Tethered drug/s	Resistant cell line/ name of the drug to which resistance has arisen	Target	The shape of nanocarrier (size)	Reference
11	Synthetic amphiphilic dendritic peptide	Dendrimers	Dox[a]	MCF-7/Adr	The membrane of cancer cell (cell penetration)	Sphere (119.4 nm)	Chen et al. (2017)
12	Lysine dendronized amphipathic peptide		Dox[a]	Drug-resistant human ovarian, SKOV3/R	Plasma/organelle membrane of tumor cells	Sphere (150 nm)	Zhang et al. (2018)
13	Peptide dendrimeric prodrug (PDP)		Dox[a] and BIBR1532 (telomerase inhibitor)	MCF-7/R	The membrane of cancer cell (Cell penetration)	Uniform Sphere (92.4 ± 2.8 nm)	Wu et al. (2020)
14	Dendrimeric prodrugs		Lonidamine (hexokinase 2 inhibitor) and MMP sensitive peptide	MCF-7/R	Tumor metabolism	Sphere (~130 nm)	Li et al. (2017)
15	Peptide dendritic nanoparticles		Dox[a]	MCF-7/Adr	Lysosomes of resistant cancer cells	Uniform Sphere (92 nm)	Wang et al. (2020)
16	Enzyme-instructed self-assembled nanofibers	Fibrous	–	MES-SA/Dx5 and A2780/Cisplatin	CD95 and DR5 receptor and presents TNF-α	Net-like structure (7 ± 2 nm)	Du et al. (2017)
17			Dox[a]	MESA/Dx5	Mitochondria	Net-like structure (18 ± 3 nm)	He et al. (2018)
18	PD nanogels	Gel	Dox[a] and Verapamil[b]	A2780/Adr	Lysosomes of resistant cancer cells	Gel-like state (198.9 ± 7.0 nm)	Lyu et al. (2017)

[a]Anticancer drug
[b]P-gp inhibitor

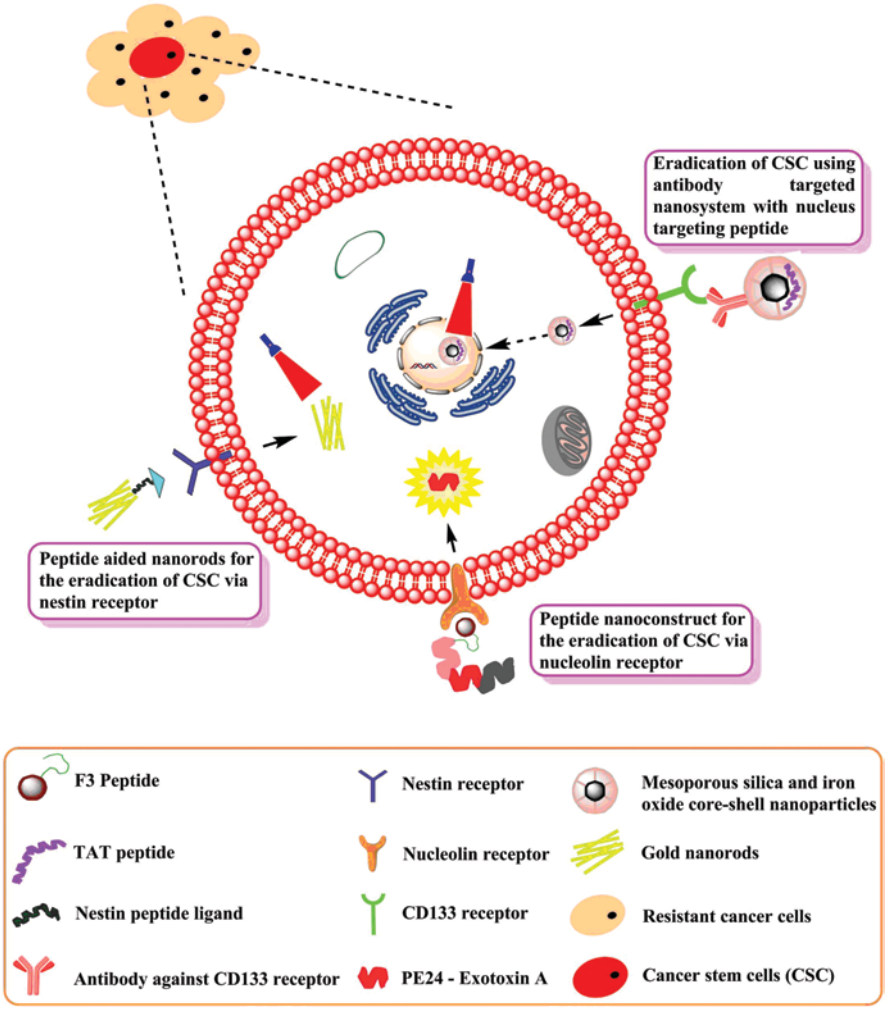

Fig. 7.3 Eradication of CSC using peptide-nanoplatforms

Furthermore, in an investigation to find out the five specific ligands of surface receptors of CSC, including CXCR1, nucleolin, CMKLR1, and CD44v6 in triple negative breast cancer (TNBC), researchers found F3 peptide ligand (a synthetic peptide from phage display technology), which showed maximum affinity towards nucleolin receptors (Pesarrodona et al. 2020). Existing proof of efficient internalization into MDA-MB-231 cancer stem cells further leaves room to exploit F3 peptide for transporting a range of drug cargoes. Initially, F3 functionalized nanoplatforms F3-RK-GFP-H6 (where RK stands for cationic peptide, GFP stands for a green fluorescent protein, and H6 stands for Histidine amino acids) formed in self-assembled fashion have the capability of nucleolin mediated-internalization. To further improve cytotoxic action of the nanoconstruct, the substitution of GFP with *Pseudomonas*

aeruginosa exotoxin A (PE24) whose preclinical antitumor activity and cell death encouraging its incorporation into the nanosystem (F3-RK-PE24-H6), thereby affording the highest internalization efficacy of nanosystems. This nanoconstruct with PE24 easily penetrates into CSC enriched-populations and aids its death.

In contrast to the peptide ligand targeted approach mentioned above, the peptide is fabricated as an integral part of the nanosystem with a specific role. Antibody against CD133 (specific for membrane receptor) as CSC targeting element is integrated along with nucleus targeting TAT peptide into the core-shell framework of nanosystem. Incorporated CSC-specific antibody in the nanosystem recognizes CD133 receptor and internalizes into CSC. Further exposure to an external magnetic field released TAT peptide, which guided nanosystem to reach the nucleus, followed by the release of loaded anticancer drug tirapazamine and simultaneous external stimuli-induced heat effectively killed CSC (Li et al. 2019).

Thus, the multi responsive strategy combined with peptide-functionalized nanosystems, chemotherapeutic, and thermotherapy targets CSC as well as completely eradicates the relapsing resistance phenomenon in cancer cells.

7.3 Conclusion and Future Perspectives

Nevertheless, nanostructure offers a range of benefits in the DDS, combination with the peptide-defined transport of cargoes to the cancer cells offers tremendous advantages such as selectively killing of cancer cells, resistant variants (MDR), and a subset of cancer stem cells (CSC) because of versatility in the synthesis of the peptide using solid-state chemistry. Linear to the cyclic structural conformation of peptide contributes to the stabilization and provides attachment sites to various nanoplatforms to curb resistance properties in cancer cells. Practical utilization of the self-assembly properties of peptide or another camouflaging agent in nanosystems is desirable strategies for the reversal of MDR cancer cells. Peptide aided nanoplatforms avoid its entrapment in the vesicular compartments offering a sequence-directed escaping mechanism for the eradication of MDR cancer cells.

Moreover, the unique sequence of peptide acts as a ligand for a specific receptor in CSC. Attached peptide (ligand) as a single entity along with chemotherapeutic improves the ability of conjugated nanoplatforms to target and eradicate CSC; on the other hand, CSC aimed-nanoplatforms whose conjugation with specific peptide (directs to the nucleus) performs intended role once internalized into CSC. This peptide-based approach entirely curbs the resistance by eradicating relapse and recurrence phenomena. Different designs such as EISA and combinatorial approach for the circumvention of MDR emerged out while using peptide-aided nanosystems, giving further opportunity in treating complex molecular diseases like MDR cancer. Successfulness in in-vivo of the peptide-aided nanoplatforms endorses the potential applicability in human clinical trials for the effective eradication of resistant cancer cells.

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Chapter 8
Emerging Role of Nanomedicine for Targeted Drug Delivery in Brain Tumor

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8.1 Introduction

Brain is a complex organ protected by the skull and serves as the center of the nervous system, co-coordinating all the activities of the body and any abnormal growth of cells inside this restricted area can cause various problems (Frackowiak 2004). Brain tumors are categorized into cancerous (malignant) or noncancerous (benign) tumors based on their invasive nature. Benign tumors are generally referred as harmless and do not invade the nearby tissues and can be removed by surgical resection, whereas malignant tumors have the potency to evade the neighboring tissues and cause metastasis (Hutterer and Stockhammer 2009). These primary tumors can be further classified into glial and non-glial tumors which when tends to grow, cause increased pressure inside the skull, resulting in brain damage and pose a serious life threat to the affected individuals (Bondy et al. 2008). The majority of the benign tumors which arise from the brain and brain associated tissues comprise of meningioma, pituitary adenoma, acoustic neuroma, craniopharyngioma, epidermoid tumor, colloid cysts and hemangioblastomas, whereas malignant tumors comprise of astrocytes, oligodendroglioma, medulloblastoma and glioblastoma multiforme (Black 1991).

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Benign Tumors Benign tumors are described as non-aggressive and harmless tumors which do not metastasize and can be easily treated. These tumors can be classified based on their origin and location of the tumor into the following types: Meningioma are the non-cancerous tumors which are believed to originate from the meningothelial cells and accounts for about 13–26% of all intracranial tumors which show an increasing incidence with age. They occur more commonly in females when compared to males (3:2 female:male incidence ratio) and occur throughout the cranial cavity (Marosi et al. 2008). Meningioma is characterized by homogeneous additional axial mass of cells located in cerebral convexity and arise from the sphenoid wing. They may sometimes even originate from locations such as paranasal sinus (Mirimanoff et al. 1985). Surgeries in case of meningioma require skilled clinicians since it is more difficult to excise total tumor mass. Meningiomas are curable and the rate of recurrence is based on the site of the tumor and its biological aggressiveness (Black 1991; Marosi et al. 2008). On the other hand, pituitary adenomas belong to a diverse array of tumors which is believed to arise from the adenohypophyseal cells of the pituitary gland and has been classified into two main categories namely, microadenoma and macroadenoma based on the size (Ezzat et al. 2004). They can also be subdivided into chromophobic, acidophilic and basophilic adenoma based on the tinctorial characteristics of the cytoplasm (Kovacs et al. 1977). When the hormone secretion from the pituitary gland is inactive, the tumors cannot be clinically detected. However, when the secretion of hormone is in excess, they can give rise to several syndrome such as acromegaly or Cushing's syndrome which can be much more lethal even though the tumor growth is not evident enough. Pituitary adenomas are uncommon during the childhood and only about 3.5–8.5% of the tumors are diagnosed before 20 years of age. Resection of the tissues in case of pituitary adenoma results in the loss of hormone secretion which may result in several disorders in children such as growth retardation (Asa and Ezzat 1998). Acoustic neuroma belong to a class of non-cancerous tumors which are said to grow from the nerve sheath of the vestibular nerve, usually within the internal auditory canal and sometimes can even be inherited by the dominant autosomal disorder on chromosome 22 (Black 1991). Approximately, acoustic neuromas account for 6% of intracranial tumors, where approximately ten tumor patients are diagnosed per million people each year (Nikolopoulos et al. 2010). The tumors are said to grow in the internal auditory canal which tends to extend till the cerebellopontine angle and is reported as the main cause of hearing loss and the initial symptoms of acoustic neuroma. The patients initially suffer gradual hearing reduction followed by dizziness, vertigo and sensation of fullness in the ears. As the tumor progression begins, the pressure developed causes the compression of the trigeminal nerve, thereby resulting in the numbness and altered sensation on the face and tongue. When left untreated, this condition could lead to severe headache, hoarseness and difficulty in swallowing. The patients usually opt for treatment procedures such as surgery and radiation therapy based on the tumor size and proportion (Wiegand et al. 1996). Craniopharyngioma occurs occasionally in the craniopharyngeal canal, but most of them can also arise in the sellar or parasellar region. It is quite rare and only a few cases of about 0.5–2 per million person per

year is reported, out of which 30–50% of the cases are present during childhood and adolescence. However, craniopharyngioma can be detected at any age but the bimodal distribution with maximum peak incidence rate are shown in children between the age groups 5 and 14 years and in adults it ranges from 50 to 74 years (Müller 2014). Patients often complain neurological symptoms such as headache, visual disturbances and sometimes endocrine dysfunctions such as growth retardation and delayed puberty. However, the two main strategies involved in the treatment is the gross resection of the tumor and the second one is surgery followed by radiotherapy which aims at debulking the tumor to reduce the tumor mass, which could otherwise affect the optic pathways leading to ocular related disorders (Garnett et al. 2007). Epidermoid tumors belong to the class of congenital tumors that arise from the ectodermal rests from the incomplete cleavage of the neural cells from the cutaneous ectoderm which is said to occur during the third and fifth week of embryogenesis and comprise for about 1% of the central nervous system neoplasms (Chambers et al. 1977). The symptomatic onsets of epidermoid tumors are usually low and last for a period of about 2 or more years. Patients suffer symptoms such as hemorrhage, bacterial meningitis, acute brain swelling and trauma (Netsky 1988). Hemangioblastomas are the benign tumors that originate from the remnants of the mesoderm in the cerebellum, which would eventually grow into the central nervous system during the third fetal month (Rivera and Chason 1966). Hemangioblastomas of the brainstems are rare, with an incidence rate of 5.8% in a year. Patients suffering from these tumors usually complain about symptoms such as muscle weakness, numbness sensation, nausea and frequent headaches. The tumors can be removed by surgery or sometimes the patients even prefer radiotherapy (Wolbers et al. 1985).

Malignant Tumors Malignant tumors are described as the aggressive tumors which have the potency to metastasize and pose serious concern to the life of affected individuals. These tumors can be classified into various categories based on the site of occurrence and median survival rate of the patients (Maher et al. 2001). Astrocytoma is the most common type of malignant brain tumor arising from the star shaped cells namely astrocytes, that form the supportive tissue of the brain. The most frequently diagnosed astrocytoma are the pilocytic astrocytoma, anaplastic astrocytoma and glioblastoma multiforme, which can be diagnosed by means of computed tomography and magnetic resonance imaging and treated using radiation therapy, chemotherapy or surgery. Patients often complain of symptoms such as altered mental status, headache, nausea, visual disturbances and sensory anomalies (Kabel et al. 2018). Oligodendroglioma or mixed glioma is defined as the infiltrating tumors characterized by the presence of both oligodendroglial and astrocytic components in the tumor and accounts for about 5–19% of intracranial glioma occurring in individuals between the age group of 33 and 55 years. One of the hallmarks of oligodendroglioma as described by the WHO is the combined allelic loss on the short arm of chromosome 1 (1p) and the other on the long arm of 19 (19q), and these alterations were found in about 90% of the patients suffering from oligodendroma. The median postoperative survival time of the patients suffering from

oligodendroglioma range from 3.5 to 16.7 years, whereas in case of anaplastic oligodendroglioma, it accounts for only about 0.9–7.3 years (Hartmann and Von Deimling 2005). Medulloblastoma is a class of malignant tumor which generally occurs during childhood and is incurable in about one third of the patients. Current treatment strategies have shown a detrimental long term effect on the survivors. Majority of patients suffering from medulloblastoma undergo radiation therapy. Although it is highly effective in terms of tumor shrinkage, it tends to cause severe damage to the developing brain, hence the patients are advised to opt for surgery or chemotherapy. However, patients still complain of discomforts such as depression, headache, seizures and mental imbalance (Gilbertson 2004). Glioblastoma multiforme is the aggressive of all glioma tumors and is generally defined as grade IV glioma arising from the glial cells or their precursors within the central nervous system. They are described as the malignant forms since the patients have a median survival time of 12–15 months despite the therapeutic interventions. One of the main reasons for the resistance of glioblastoma multiforme to various therapies is its complex tumor structure showing characteristic features such as necrosis, hemorrhage and microvascular proliferation which allows the quick metastases to the neighboring tissues and worsens the condition of the patients (Holland 2000).

8.2 Drug Transport Mechanism Across the Blood Brain Barrier

Neurons communicate to the adjacent neurons by means of chemical and electrical signals, which is the key mechanism for maintaining the homeostasis in the neural environment that is brought about by the barrier layers between the blood and the neural tissues (Abbott et al. 2010). The blood brain barrier is created by the endothelial cells that form the walls of the blood capillaries and the blood-cerebrospinal fluid barrier is formed by the interface between the epithelial cells of the choroid plexus facing the cerebrospinal fluid. The blood brain barrier possesses certain vital functions such as protecting the central nervous system from neurotoxic substances and thus ban their entry into the brain by pumping them out by means of ATP-binding cascade transporters which could cause potential damage to the brain. However, the blood brain barrier shows passive permeability to many water soluble nutrients and metabolites required by the neurons to carry out their metabolic activities (Pardridge 2007). Blood brain barrier is also composed of tight junction proteins such as occludins and claudins which maintain the membrane integrity and prevents the uptake of many pharmaceutical drugs; however certain small molecule lipophilic drugs having a molecular weight less than 400 Da can cross the BBB effectively (Pardridge 2007; Kreuter 2013). These lipophilic drugs have the potency to diffuse through the endothelial cell membrane and reach the targeted site; their activity can also be enhanced by using carrier mediated molecules (Abbott 2005).

8.3 Current Treatment Modalities and Limitations

In the recent years, a number of remarkable progress has been made in understanding the hallmarks of brain tumor development. However, with its increasing incidence and recurrence rate, its clinical management poses to be a challenge to deal within the twenty-first century (Xu et al. 2018). The current treatment modalities comprise of radiation therapy, surgery, chemotherapy, hormone therapy and immunotherapy. Out of the current available therapies, 50% of the cancer patients opt for radiotherapy during the course of their illness. The main strategy involved in the treatment procedure is to deprive the cancer cells from multiplying and hence control their growth. When cancer tissues are bombarded with radiation, they transfer a high amount of energy to the cells and tissues which cause genetic damage to the cells and cause their death. However, the affected normal cells surrounding the cancer cells can repair the genetic damage caused to them by base pair mechanisms (Baskar et al. 2012). Though radiation therapy is commonly used by patients, the recent research carried out by Kim et al. proved that radiation could trigger the angiogenic pathways which could eventually lead to the increase in angiogenic signaling molecules such as VEGF, FGF, PDGF and angiogenin and contributing to metastasis, thereby worsening the condition (Madani et al. 2008; Kim et al. 2004). Surgery is another common treatment approach; however, its use in brain tumors is limited in certain instances due to the location of the tumor and its invasive nature and secondly due to the complications in the surgical procedure which could lead to several physiological problems in patients, since the brain is the active organ which co-ordinates all the functions of the body (Vives and Piepmeier 1999). In many of the cases, patients also opt for chemotherapy treatment. Bevacizumab, temozolamide and carmusitine are the well-known drugs approved by FDA to treat brain tumors. These drugs can easily permeate the blood brain barrier and reach the targeted site. However, the bioavailability of the drugs is low and hence, administration of increased dosage can lead to several side effects among patients, thus the dosage should be administered with care. Patients undergoing chemotherapy with these drugs usually complain symptoms of headache, mental stress, hair loss, weakness, and nausea (Omar and Mason 2009). An emerging effort in the treatment process is the immunotherapeutic approach, which includes the administration of targeted antibodies and vaccination strategies. This modality is suitable in the treatment of malignant glioma, since the tumors are well separated from the central nervous system and can be effectively targeted. The main principle involved in immunotherapy is the identification of specific antigens on the tumor cells by the antibodies. Although this is a targeted approach, the main obstacle lies in the poor recognition of specific antigens on human malignant glioma cells, that may result in therapy failure (Roth and Weller 1999).

8.4 Nanomedicine as an Emerging Tool in Drug Delivery

Due to the drawbacks of the current treatment modalities, there is an urge to find an alternative therapeutic approach which ensures targeted delivery, ease the treatment procedure and is also cost effective. In the present scenario, nanotechnology seems to be a powerful tool and as a means of targeted drug delivery in brain tumor management. Biodegradable nanocarriers of various forms and sizes are gaining importance to deliver the drugs to the target tumor tissue. Some of the commonly used nanocarriers include liposomes, micelles, nanoparticles, dendrimers, carbon nanotubes, nanopeptides, exosomes and lipid carriers.

Liposomes Liposomes are the extensively studied nanocarriers which are microscopic in nature and made up of one or more concentric lipid bilayers. Due to their lipophilic nature, they can easily penetrate the blood brain barrier, and act as carriers either for water soluble or lipid soluble drugs. There are three major classes of liposomes which are extensively used as carriers in drug delivery system. Multilamellar vesicles, commonly represented as MLV, are formed when lipids are hydrated in an aqueous solution, and is said to possess a size of about 800 nm. Small unilamellar vesicles (SUV) are formed from MLV by ultra-sonication process and pose an average uniform size of about 200 nm in diameter, which are more stable and remain in the blood circulation for a long time ensuring slow release of the drug. Large unilamellar vesicle (LUV) is formed by reverse phase evaporation technique which is uneven in size but can be made into uniformly sized vesicles by passing them through a polycarbonate filter (Weinstein and Leserman 1984). In order to accomplish brain specific delivery and enhanced circulation, certain macromolecules including peptides, antibodies, polysaccharides and polymers can be conjugated with liposomes (Vieira and Gamarra 2016). A study conducted by Ying et al. explains the importance of liposome carrier in the targeted drug delivery, wherein dual-targeting daunorubicin liposomes were formulated by conjugation with p-aminophenyl-α-D-mannopyranoside and transferrin for the transport of the drug across the blood brain barrier in C6 glioma cells. The results confirmed that the transport of the drug was enhanced by 24.9% compared to the bare drug, which suggests the efficiency of the carrier in drug delivery system (Ying et al. 2010). Liposomal formulations containing daunorubicin encapsulated in DSPC and cholesterol is in clinical trial for the treatment of pediatric brain tumors (Lippens 1999). A PEGylated doxorubicin formulation 2B3-101 is proven to be effective in targeting brain metastases (Gaillard et al. 2014). Liposomal cytarabine was evaluated for its efficacy in targeting malignant brain tumors among children and adolescents. This study confirmed the sustained drug delivery over a period of 1 week when administered intrathecally, with good tolerability and minimal adverse effects (Peyrl et al. 2014). Solid brain tumors have been treated by vincristine sulfate and tetrandrine liposomes (Song et al. 2017) and nanoliposomal irinotecan has been successfully implemented in the treatment of recurrent high-grade gliomas (Clarke et al. 2017). Liposomal formulation of platinum compounds, administered to F98 orthotopic glioma model in Fischer rats, significantly improved the intratumoral

accumulation of the drug with an increase in the survival time by serving as an adjunct to concomitant radiotherapy. In this study, 24 h after the intracarotid infusion of the liposomal formulations, the animals were subjected to gamma knife irradiation at a dose of 15 Gy (2.8 Gy/min). Liposomal oxaliplatin showed 2.4-fold increase in the accumulation potency compared to bare oxaliplatin, while liposomal carboplatin could increase the mean survival time of the tumor bearing rats up to 46.8 days vs. 22 days in sham control, indicating the effectiveness of combination therapy in glioma (Charest et al. 2012).

Micelles Polymeric micelles range in the size of 10–100 nm and serve as a promising colloidal carrier which are more stable, efficient and have a prolonged circulation time *in vivo* when compared to the surfactant micelles and ensures targeted delivery to the tumor location. The characteristic feature of micelles is the presence of a hydrophobic core shell which is made up of a biodegradable polymer such as poly (b-benzyl-l-aspartate) or poly (d-lactic acid) which serves as a reservoir for the storage of drug. The drugs can be incorporated into the micelles by means of chemical conjugation or physical entrapment using emulsification or dialysis technique. Polymeric micelles pose many advantages due to their desired size, controlled drug release and also is an ideal carrier for poorly water soluble drugs. However, the physical stability of the carrier pose a difficulty in making them an ideal carrier (Jones and Leroux 1999). Guo et al. carried out a study wherein they developed Pep-1 and borneol bifunctionalized carmusitine loaded micelles which had the potency to target the overexpressed interleukin-13 receptor that could cross the blood brain barrier and reach the target site. The synthesized micelles which were loaded with the drug had an average size of about 32.6 ± 1.1 nm and the release of the drug was quick in slightly acidic environment. The micelle loaded drug was tested against human glioma BT325 and the cellular studies carried out reveals the increased blood brain barrier penetration, cytotoxic effect and cellular apoptosis when compared to the bare drug. The study was also carried out using *in vivo* models and the results revealed the strong fluorescence as well as longer retention of the drug in the brain tissues when compared to the control groups (Guo et al. 2019). Overall the study concludes the importance of micelle loaded drugs as an efficient tool in drug delivery system and could have potential benefits in the therapeutic strategies. In a pre-clinical study on U87MG glioma bearing mice, transferrin modified micelles containing paclitaxel significantly enhanced the survival time compared to that with bare taxol (39.5 days vs. 33.6 days) along with greater intratumoral accumulation (Zhang et al. 2012). C6 glioma bearing rats administered intranasally with coumarin-6 loaded micelles modified with a cell penetration peptide Tat resulted in the brain targeted delivery of the drug (Kanazawa et al. 2011). Cyclic RGD-tagged polymeric micelles conjugated with docetaxel markedly increased the cytotoxic efficacy of docetaxel with enhanced intratumoral accumulation without systemic toxicity symptoms, indicating its efficacy as a brain tumor chemotherapeutic (Li et al. 2015).

Nanoparticles Nanoparticles are the widely used carriers in drug delivery system from a decade of years and exists either in amorphous or crystalline form. They can be synthesized by various techniques such as nanoprecipitation, emulsion, mechanical attrition, chemical precipitation and chemical vapor condensation. Nanoparticles range from the size of about 10 to 100 nm and can be synthesized easily in the laboratory using a basic biodegradable copolymer such as Polylactic glycolic acid (PLGA), Polylactic acid (PLA) and Polycaprolactone (PCL). These nanoparticles can be then loaded with the choice of drug and tagged with the suitable markers, which will ensure the targeted delivery exactly to the site of the tumor (Caruso et al. 2011; Vauthier and Bouchemal 2009). A study carried out by Cui et al. wherein they aimed to synthesize transferrin conjugated magnetic silica PLGA nanoparticles loaded with doxorubicin and paclitaxel and checked its efficacy against U87MG glioma cell lines to cross the blood brain barrier. Results revealed that the cellular uptake of the drug which was encapsulated by PLGA was enhanced due to the combination of transferrin targeting ligand and magnetic field. The cells treated with this combination showed the highest toxicity when compared to the free drug and the nanoparticles which was not combined with transferrin and magnetic field (Cui et al. 2013). A similar study was carried out by Mendoza et al. wherein they used a lipid nanoparticle loaded with synthetic alkyllysophospholipid edelfosine as an anticancer agent against C6 glioma cells. The study revealed that the lipid nanoparticle loaded with the drug showed potential antiproliferative effect and the study was further proceeded in *in vivo* conditions, taking xenograft mouse model of glioma. The *in vivo* study concluded that the oral administration of the lipid nanoparticles loaded with the drug showed high accumulation of the drug in the brain tissue at the tumor site, which led to the significant reduction in the tumor growth at the end of the 14th day after the treatment. This study suggests that these nanocarriers could be potential tools in the drug delivery system (de Mendoza et al. 2011). Functionalized iron oxide nanoparticles encapsulating chlorotoxin enhanced the total drug uptake by brain tumor cells in transgenic ND2:SmoA1 mice models along with sustained drug release and no toxicity on healthy cells (Veiseh et al. 2009). Cellular transduction of the anticancer drugs can often be enhanced by integrating them into metallic nanoparticles using microwave-induced hyperthermia technique (Stockwell et al. 2014). A study by Kreuter reported the enhanced penetration capability of gadolinium nanoparticles and their subsequent uptake by brain tumor parenchyma (Kreuter 2014).

Dendrimers Dendrimers are highly branched structures having their size in the nano meter scale dimensions, which are made up of three regions consisting of the core, interior branch and the peripheral groups. The core and the interior branches are designed using natural compounds such as polyamidoamine which can interact with the cell surface receptor (Liu et al. 2012). Therapeutics based on RNA interference mechanisms play a very important role in the management of several multidrug resistant cancers and the delivery of these siRNA and oligonucleotides can be made possible by the incorporation of polyamidoamine dendrimers (Kesharwani et al. 2015). Circulation half-life of the drug was found to be prolonged due to the

ability of dendrimers to bind to plasma proteins and certain other circulatory biomolecules (Patel et al. 2012). A study was carried out by Bhadra et al. where they synthesized PAMAM dendrimers using ethylenediamine as core and methyl methacrylate as propagating agent, which was then PEGylated using N-hydroxysuccinimide-activated carboxymethyl MPEG-5000 to deliver 5-fluorouracil. The study revealed that the carriers had a prolonged and sustained delivery which gradually decreased the leakage of the loaded drug, showing targeted delivery to the tumor site (Bhadra et al. 2003). Upon systemic delivery of hydroxyl terminated PAMAM to a 9L gliosarcoma model with intracranial tumors, it was observed that the dendrimers retained in the tumor tissue at least for 48 h along with speedy accumulation and succeeding deposition in the tumor associated macrophages (Zhang et al. 2015). Paclitaxel thiamine conjugated PPI dendrimer showed enhanced cytotoxicity *in vitro* on IMR32 neuroblastoma cells in comparison with paclitaxel alone (Patel et al. 2012). Theranostic dendrimers prepared by conjugating G5 PAMAM dendrimer with polyethylene glycol and chlorotoxin were used by aiding in SPECT and radiotherapy for targeting glioma xenografts that overexpress matrix metalloproteinase-2. Biodistribution investigations of these dendrimer formulations revealed the specific accumulation of the drug in the tumor tissues. This nanoplatform, being relatively stable and of higher purity, could be impressively labelled with radioactive 131I and utilized for targeted drug delivery (Zhao et al. 2015).

Carbon Nanotubes Carbon nanotube carriers are gaining a lot of importance in drug delivery system due to their advantageous features such as enhanced cellular uptake, high surface area and biodegradability. Carbon nanotubes are made of either single walled or multi walled nanotubes which are obtained from graphene sheets and have the capacity of adsorbing or conjugating themselves with many therapeutic molecules and drugs and hence can be an excellent choice in drug delivery (Elhissi et al. 2012). In a study carried out by Ren et al. multi-walled carbon nanotubes were synthesized and modified using angiopep-2 loaded with doxorubicin to target against C6 rat glioma. The *in vivo* studies carried on the Balb/c mice suggests that doxorubicin loaded using carbon nanotubes enhanced the anti-glioma effect when compared to the bare drug alone. The biological safety of the synthesized carbon nanotubes was also evaluated using bovine cerebral endothelial cells which suggested that the carriers were biocompatible and showed low toxicity (Ren et al. 2012). A similar study was carried out by Santos et al. wherein they used the combination of carbon nanotubes coupled with fluorescein-5—thiosemicarbazide and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide along with near-infrared radiation and induced hyperthermia for the treatment of primary brain tumors against U251, U87MG, LN299 and T98G cell line. The cells lines were treated with the carbon nanotubes and incubated for 24 h followed by exposure to the near infrared radiation for 10 min. Upon internalization with the carbon nanotubes and exposure to the radiation, the glioma cells underwent necrosis due to hyperthermia leading to cell death. The study was also carried out in *in vivo* conditions in the rodent tumor model, wherein the nanotubes caused the shrinkage of the tumor and no recurrence

of the tumor was observed (Santos et al. 2014). Multiwalled nanotubes have been used as immunotherapeutics due to their preferred affinity towards macrophages, thereby stimulating them to destroy glioma cells in GL261 murine intracranial glioma model (VanHandel et al. 2009). Pre-clinical trials performed using carbon nanotube X-ray array in a LINAC set up revealed the feasibility of generating microbeam radiation which was effective in treating U87MG orthotopic glioma. This approach significantly decreased the rate of tumor proliferation. Results of this study suggest the future utilization of carbon nanotubes as glioma therapeutics after appropriate clinical validations (Yuan et al. 2015).

Nanopeptides Peptides are also used as carriers in drug delivery system due to their simple structure and biocompatibility properties. The peptides can be synthesized through the molecular self-assembly of the peptide sheets and then conjugated with their respective carrier ligand to target the specific cell surface receptors. The most common forms of peptides used are either dipeptides or tripeptides ranging in the nanoscale dimensions. A broad range of peptides and proteins have proven to produce highly stable nanofiber structures called amyloid fibers which are trending and created potential interest among the researchers in the field of targeted drug delivery (Faintuch et al. 2011). These peptides have also developed a modern technique of molecular switches in which these carriers can gradually change their molecular structure when they are subjected to temperature fluctuations or change in pH. Cell penetrating peptides are another class of peptides bearing the length of 5–30 amino acids which possess the ability to penetrate through the plasma membrane. Among them, peptide amphiphiles (PAs) are the most efficient peptides which are said to transport the drug more precisely to the targeted site (Mohammadi et al. 2015). Angiopep, a peptide sequence, in conjugation with PEG-PCL has been successfully used for the treatment of U87MG intracranial glioma and acted as dual-targeting drug delivery system efficiently crossing the blood brain barrier and selectively targeting the low density lipoprotein receptor-related protein (Xin et al. 2011). RGDyk peptides were found to affect brain tumor vasculature thereby acting as anti-angiogenic therapeutics (Yan et al. 2011). Polyacrylamide nanoparticles conjugated to coomassie brilliant blue and PEG/F3 peptides effectively delineated the tumor margins during resection surgery and assisted in brain tumor targeting (Nie et al. 2012). Synthetic d-reverse peptides derived from the type 1 repeats of thrombospondin (TSP1) were evaluated for antitumor efficacy on rat C6 glioma and 9L gliosarcoma. Intravenous injection of the peptide 10 days after the tumor transplantation resulted in tumor shrinkage via reduced angiogenesis and antiproliferative effects on tumor cells (Bogdanov et al. 1999). cMBP peptides conjugated with G4 dendrimer significantly inhibited the tumor cell division and invasion of U87MG glioma cells by targeting mesenchymal to epithelial transition factor (MET) and altering the levels of pAKT and pERK1/2. The same peptide showed an increase in the mean survival time of tumor bearing mice by 59% upon intravenous injection, thereby proving its *in vivo* efficacy (Wu et al. 2018). Zhai et al. evaluated the anti-glioma activity of vincristine sulfate encapsulated into GKRK peptide ligand and apoferritin. *In vitro* and *in vivo* results of this study revealed the tumor-specific

targeting by the peptide with enhanced antiproliferative effects (Zhai et al. 2018). Cyclic peptide R, a CXCR4 antagonist, reduced the tumor size and microvasculature in intracranial glioma model (Mercurio et al. 2016). Union of the K237 and CVNHPAFAC-NH2 peptides, termed as CK peptide encapsulating paclitaxel, upon intravenous administration to the brain tumor bearing mice, resulted in specific intratumoral accumulation of paclitaxel and enhanced the mean survival time (Feng et al. 2015).

Exosomes Exosomes are the small intracellular membrane bound vesicles that are produced by the endosomes within the bilayer of the lipids inside the cells and serve a major role in many of the biological process in the human body. They are composed of various types of lipids such as sphingomyelin, phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine. These exosomes bear the size of about 10–100 nm and can also be used as excellent carriers in the delivery of the drugs. These carriers do not cause any non-specific immunological reactions in the host body since they have the similar composition as that of the human body (Ha et al. 2016). Graner et al. derived exosomes from the spent media of cultured mouse glioma and human glioma cells and these exosomes were the rich source of heat shock proteins such as HSP 27, 60, 70 and 90 (Graner et al. 2007). A study was carried out by Yang et al. wherein they derived exosomes from brain endothelial cells to test its efficiency to deliver the drugs across the blood brain barrier in zebra fish model. Brain neuronal glioblastoma-astrocytoma U87MG, endothelial bEND3, neuroectodermal tumor PFSK-1, and glioblastoma A-172 cell line was used for the isolation of the exosomes. The isolated exosomes were characterized to determine the particle size, morphology and protein markers. The particle size of the carrier ranged between 30 and 100 nm in diameter. The brain distribution of the synthesized exosomes loaded with rhodamine, paclitaxel and doxorubicin combination was evaluated using xenotransplanted zebra fish model. The images of the zebra fish model showed that the synthesized exosomes loaded with the drug combination could easily penetrate the blood brain barrier and target the tumor site and efficiently deliver the drug which lead to the significant decrease in the tumor growth. The results from the study paved way for exosomes as an excellent carrier in drug delivery system (Yang et al. 2015). Exosomal proteins conjugated with iron chelators reduced the numbers and size of tumor spheroids as well as inhibited the tumor stem cell population in neuroblastoma cells (Bisaro et al. 2015). Embryonic stem cell derived exosomes reduced the cell viability and promoted cellular apoptosis in U251 and U87MG glioma cell lines. In continuation, upon intravenous injection of the ESC-Exos into glioma xenograft bearing mice, tumor size and weight was found to be significantly reduced against sham control group. Further, these ESC-Exos combined with cRGD peptides loaded with paclitaxel were injected into subcutaneous glioma bearing xenograft mouse model and assessed for their accumulation at the tumor site, which was found to be greater, indicating the efficient blood brain barrier permeability potential of these exosomes (Zhu et al. 2019). Genetically engineered exosomes prepared by fusing uracil phosphoribosyltransferase to suicide gene mRNA and cytosine deaminase were tested for their antitumor efficacy against

orthotopic murine models of schwannoma. Results of this study revealed potential anticancer efficacy of these exosomes when coupled with 5-fluorocysteine prodrug treatment (Mizrak et al. 2012).

Lipid Carriers Nanostructured lipid carriers have gained importance in the last few years in targeted drug delivery system. Lipid carriers are formed by bilayered vesicles which are surrounded by phospholipid membrane that are designed to adhere to the cellular membrane and deliver the drugs by means of endocytosis (Chen et al. 2016). A study was carried out by Song et al. aimed at the synthesis of arginine-glycine-aspartic acid peptide in combination with nanostructured lipid carriers to deliver temozolamide for targeting glioma. *In vitro* studies were carried out using U87MG cell line to test the efficiency of the combined lipid nanostructures loaded with temozolamide, whereas the *in vivo* antitumor efficacy was evaluated using mice bearing the glioblastoma multiforme tumor. The results revealed that the lipid carriers loaded with the drugs showed the best results when compared to the bare drug both in case of *in vitro* and *in vivo* studies (Song et al. 2016). A similar study was carried out by Madan et al. where they synthesized solid lipid nanoparticles loaded with noscapine and targeted it against U87MG glioma to test its efficiency in crossing the blood brain barrier and evaluation of plasma half-life. Noscapine is a well-known anticancer drug having a short plasma life and rapid elimination from the body which results giving multiple injections to the patients for successive results in chemotherapeutic procedures. But when it was administered into the body using these solid lipid nanoparticle form, the plasma half-life was increased up to 11-fold significantly and the nanocarriers could easily cross the blood brain barrier and deliver the drug to the targeted site (Madan et al. 2013). A transferrin conjugated nanostructured lipid carrier containing artemisinin was found to possess greater cytotoxicity on U87MG glioma cells compared to that of free drug (Emami et al. 2018). Curcumin loaded lipid nanocarriers showed significant cytotoxic activity on U251MG glioma cells *in vitro*. These lipid carriers also decreased the tumor size in C6 glioma bearing rats and prolonged their survival time (Zanotto-Filho et al. 2013). Solid-lipid nanocarriers composed of Compritol and Precirol loaded with the anticancer drug edelfosine possessed antiproliferative effect and tumor regression in *in vitro* and *in vivo* rat C6 glioma models (Estella-Hermoso De Mendoza et al. 2011).

8.5 Conclusion

This book chapter emphasizes on the different types of brain tumors and the current strategies employed in its treatment. It also deals with the limitation of the current treatment modalities and an urge to find a new means to tackle the existing problem in order to improve the quality of therapeutic strategies. Nanomedicine offers several advantages in targeting brain tumors in terms of its efficacy in permeating the blood brain barrier, ease of application in drug delivery as well as its utilization in

tumor imaging in a parallel manner. Nanotechnology seems to be a promising approach by increasing the efficiency of certain chemotherapeutic drugs via enhancing intratumoral accumulation and sustained drug release that will significantly contribute to the clinical management of brain tumors.

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Chapter 9
Fate of Biomaterials Post Payload Delivery: Current Understanding and Future Perspectives

Sanjeeb Kalita, Ashish Dhayani, Vikas Kumar, E. Sujanthi, and Praveen Kumar Vemula

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9.1 Introduction

Biomaterial based drug delivery vehicles/NCs have shown a tremendous impact in the field of biomedical engineering with a significant increase in the therapeutic index of drugs for numerous diseases. They are used as imaging tools and markers for diagnosis, in gene therapy for correction of damaged gene copies, improving patient compliance through long term or stimuli responsive drug delivery etc. Despite great potential in pre-clinical settings, most NCs don't make it past clinical studies. The failure to overcome translational barriers is due to the lack of thorough understanding of absorption, distribution, metabolism, excretion, and toxicity (ADMET) profiles of the NCs.

9.1.1 Role of NCs in Regenerative Medicine

Biomaterials have played different roles in regenerative medicine, from having started with being passive components such as carriers for drug delivery, structural scaffolds for cell and cellular components; to currently being hailed as active components aiding tissue regeneration and repair by responding to physiological cues. The earliest known and designed biomaterials were inert materials such as polymethylmethacrylate, with no known degradability profile; but as our knowledge in the field increased, more materials with biodegradable and biocompatible profiles were designed and used. Currently, there is a need for biomaterials which can be targeted to desired locations, fulfill the intended function, slowly degrade and get absorbed by the body without leaving any by-products. Such materials can be injectable or implantable *in vivo*, eliminating the need for secondary surgery for device removal and even long-term health hazards associated with permanent devices (Sadtler et al. 2016).

9.1.2 Essential Features for Efficient Design of NCs for Biomedical Applications

Herein we outline, some of the essential features for the design of biomaterials for regenerative medicine (see Fig. 9.1):

(a) *Biocompatibility*—Decellularized tissues consisting of ECM (dECM) proteins and other tissue factors necessary to provide inductive cues for cellular growth have been used as biocompatible materials. Moreover, native proteins, polysaccharides and lipids are used as highly biocompatible materials.
(b) *Biodegradation profile*—Native polymers such as hyaluronic acid, chondroitin sulphate etc., are already found in our body and are remodeled by ECM proteins. Thus, such materials can be easily degraded in the body over a period of time without generating an immune response.

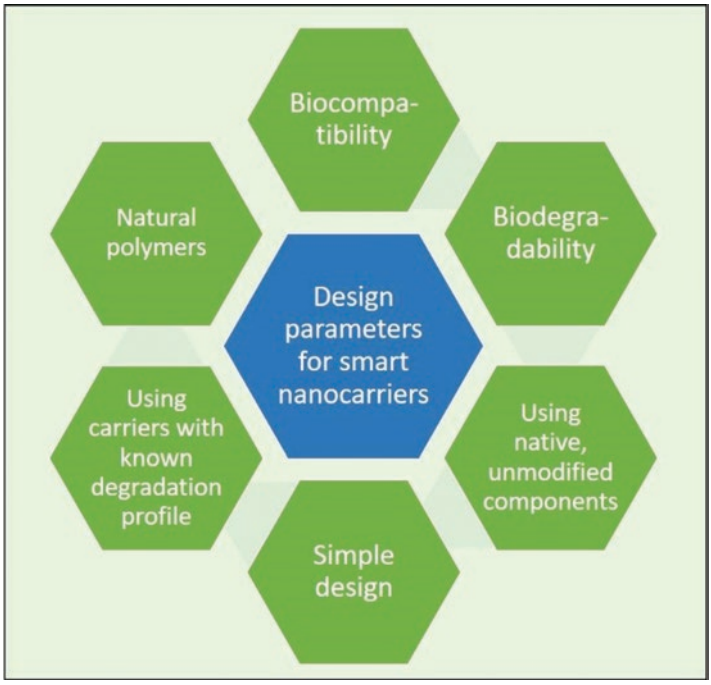

Fig. 9.1 Important design considerations for the choice of NCs and their biomedical applications

(c) *Minimal modification for simplistic design*—Native polymers used with minimal modification such as covalent attachment of a drug or click chemistry to make certain kinds of scaffolds can be used to support cell proliferation and tissue repair. Moreover, minimal modification ensures that once the payload is delivered, the remaining polymer is easily cleared off either by the immune system or biochemical processes in the tissue microenvironment.

(d) *Use of native, unmodified and natural biomaterials such as silk, collagen, fibrin and polysaccharides such as starch, amylose, alginate, chitin and chitosan as opposed to synthetic (polymethyl methacrylate, polystyrene based polymers)*—Natural polymers are similar to biological macromolecules. Thus, they can be biodegradable and susceptible to enzyme mediated degradation and remodeling. However, some of their limitations are weak mechanical properties, high immunogenicity, structural complexity and batch variability.

Synthetic polymers such as polylactide (PLA), polyglycolide (PGA) and their co-polymer poly(lactide-co-glycolide) (PLGA) are extensively used in biomedical applications but typically lead to accumulation of the acidic degradation products in the tissue causing a substantial increase in the activation of complement system and opsonization, leading to inflammation and significant increase in foreign body reaction (FBR) (Li et al. 2020).

(e) *Use of NCs with known breakdown profile for efficient clearance in vivo*—Triglycerolmonostearate (TGMS) and Ascorbylpalmitate (AP) based small amphiphilic molecules which are broken down into native components *in vivo* for efficient clearance (Gajanayake et al. 2014; Zhang et al. 2015). Starch and amylose are examples of natural polymers that can be easily cleared. Silk is another biodegradable and biocompatible polymer which does not generate a strong immune response and degrades into non-toxic products (Li et al. 2020). These are some of the FDA approved GRAS (*generally recognized as safe*) compounds and have been used for drug delivery in vascularized composite allotransplantation (VCA), arthritis, inflammatory bowel disease (IBD), diabetes, etc.

9.1.3 Physiological Response to a Nanomaterial

Injection/implantation of a biomaterial *in vivo* is very similar to an injury and leads to a host response from tissue injury, inflammation, to remodeling and repair. This is, in turn governed by the degradation kinetics—the degradation profile of the biomaterial and immune response at material-host interface. Even though the immune response is governed by innate as well as the adaptive arms of the immune system; macrophages are undeniably the most important immune cells and play a huge role in this response. There are two major phenotypes of macrophages M1 (pro-inflammatory type) and M2 (anti-inflammatory type). A favorable clearance of the nanocarrier requires that they interact with M1 macrophages which leads to inflammation and slow degradation and ultimately M2 macrophages which are involved in remodeling and complete clearance. An imbalance between the ratios of M1 and M2 macrophages or an untimely switch would lead to a strong foreign body reaction (FBR) covering and isolating the nanocarrier and impeding the function altogether. Understanding the foreign body response to a biomaterial has helped design immune-engineered materials that can be efficiently cleared from the system. A better understanding of the structure-function relationship of the designed scaffold and the immune response will help us design biocompatible materials with better functionality at the site of action (Li et al. 2020).

In this chapter, we have broadly divided the organic NCs into biological, lipid-based carriers, carbohydrate based, nucleic acid, protein-based, and polymeric carriers. Understanding the biodistribution, metabolism, excretion and toxicity profiles of these NCs will help in designing next generation therapeutics and vehicles with enhanced therapeutic benefits and minimal toxicity. We first describe general ADME properties of NCs and their clearance mechanisms (Fig. 9.2).

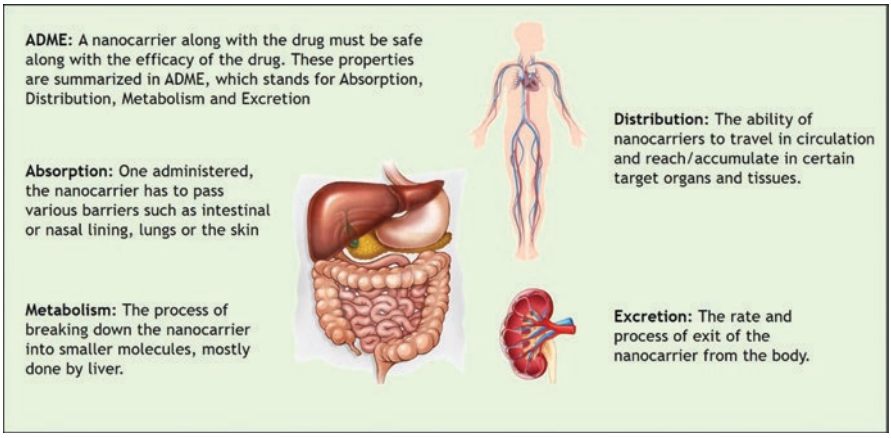

Fig. 9.2 Physiological fate of NCs: Once in the body, the NCs as well as the drug undergoes a series of changes which are defined processes and clear roles to play till the nanocarrier finish their jobs and are excreted

9.2 Factors Controlling the *In Vivo* Fate of Drug Delivery Agents

9.2.1 Absorption in the Body and Entry into the Systemic Circulation

Absorption of drug NCs refers to the process of transport from the site of administration via systemic circulation across biological membranes (Li et al. 2015). Post administration, NCs may undergo three potential fates: (1) Absorption of intact NCs with payload into the systemic circulation; (2) NCs don't absorb, but facilitate absorption of payload by fusing or directly interacting with the epithelial membrane (3) premature release of the cargo at the entry site microenvironments leading to degradation. Thus, the site of administration plays a crucial role in determining the fate of NCs. We describe the most prevalent administration sites and how the fate of NCs might be affected at each of these sites (Fig. 9.3).

Oral administration of NCs requires crossing of multiple barriers such as intestinal mucus, epithelial and endothelial layer. NCs translocate these barriers via a process of transcytosis and paracellular transport. NCs taken up by M cells and transcytosis are eventually delivered to the GALT and lymphoid cells. Absorption of NCs through enterocytes, directly takes them to systemic circulation. Uptake of NCs by enterocytes, M cells or epithelial/endothelial cells are also influenced by its surface characteristics, architecture, size, and composition.

Inhalation delivery of NCs requires their capture and crossing of pulmonary barriers and ultimate clearance by the pulmonary microenvironment (Labiris and Dolovich 2003). Inhalation drug delivery using NCs is an ideal non-invasive way due to the permeable pulmonary epithelium, absence of harsh pH environments and

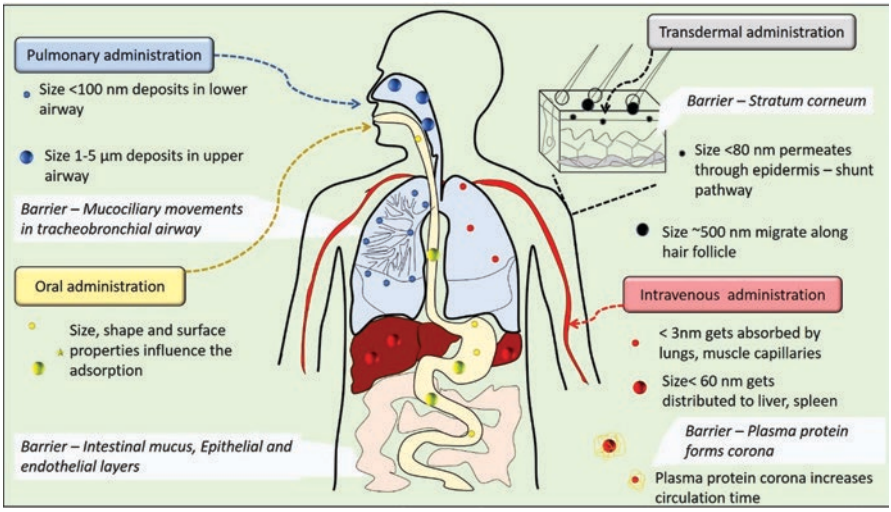

Fig. 9.3 Routes of administration of NCs and their possible *in vivo* fate

a large alveolar surface area. This route offers an increase in the therapeutic index, a reduction in side effects and avoiding off-target tissue drug distribution. Once inhaled, the NCs need to overcome the muco-ciliary movement in the tracheobronchial airway to reach the alveolar airway, where they get cleared by phagocytosis (Chenthamara et al. 2019). The size of the NCs play an important role here with particles <100 nm depositing in the lower airway while 1–5 µm particles are trapped in the upper airway—this is the most crucial role in the delivery and deposition of these particles in the distinct tissues. Larger particles are deposited in oropharyngeal airways (Thomas 2013).

The skin is yet another important site for the administration of drugs. Stratum corneum (SC), the superficial layer of epidermis, poses the biggest hurdle in the absorption of NCs through the skin. Small NCs (<80 nm) can permeate the viable epidermis through shunt pathways facilitated by hair follicles and sweat glands, whereas NCs with bigger size, such as 500 nm, can only migrate along the hair follicles (Schneider et al. 2009). Two pathways have been characterized for the effective translocation of NCs through viable epidermis: (1) the transcellular route and the (2) the intercellular lipid route.

In the transcellular route of translocation; NCs cross through the lipid structures of the interlamellar region and corneocytes with the keratin-enriched intracellular macromolecular matrix. NCs' penetration through intercellular pathway involves translocation through the alternating continuous lipid matrix and small intracellular aqueous space domains. The diffusion rate of NCs through this intracellular space depends on their lipophilicity, solubility, molecular weight (MW) and hydrogen bonding ability. In contrast, large molecules are physically restricted within the lipids. Though considered a shorter path of NCs translocation; the transcellular route involves higher resistance to the transcellular passage of substances because of both lipophilic and hydrophilic barriers (Trommer and Neubert 2006).

During the transcytosis process; trafficking of NCs to the endolysosomal compartment is the biggest hurdle and it reduces the efficiency of translocation. Endocytosis of NCs is substantially easier than the exocytosis; a tendency that can be described as EEDD: easy entrance, difficult discharge. Size, charge, and surface properties of the NCs play crucial roles in endocytosis mechanisms. The pathways for internalization of NCs into epithelial and endothelial cells are: caveolin mediated, clathrin mediated, lipid-raft-mediated endocytosis as well as micropinocytosis, phagocytosis, and receptor mediated endocytosis. A majority of endocytosed NCs get trafficked along the endolysosomal pathway and with translocation from early to late endosomes and their maturation or fusion with lysosome. Once NCs accumulates in lysosomes; it can't escape and gets degraded via lysosomal acidic enzymes or is, depending on the material, indefinitely accumulated. Further, depending on physico-chemical characteristics; the NCs may afford lysosomal escape and may release its cargo inside the cytosol and may be trafficked to a specific cellular organelle or might follow an exocytosis pathway. Exocytosis plays a crucial role in successful transversion of NCs through the membrane barriers (Duncan and Richardson 2012).

Administration of NCs through intravenous route facilitates immediate systemic distribution throughout the body. Stability of NCs in blood along with its physico-chemical characteristics such as shape, size and surface chemistry, determines its fate, circulation half-life, absorption and biodistribution. Study suggested that; after 10 min and 4 h post-injection; the smaller particle had an increase in circulation half-life ($t_{1/2}$) with higher retention. High ionic strength mediated protein corona formation in NCs results in increase in hydrodynamic diameter and aggregation of NCs which can lead to obstruction of blood capillaries and formation of embolism (Dobrovolskaia et al. 2009). Serum protein adsorbed in the NCs surface encourages its uptake by mononuclear phagocytic system (MPS), resulting in decreased availability, reduced utility and rapid clearance of NCs in blood. Avoiding MPS requires the development of material design with stealth tactics and active targeting (Gustafson et al. 2015; Anselmo et al. 2013).

9.2.2 Biodistribution of NCs

Studies on quantitative biodistribution of NCs in organs and tissues of interest concluded that the size, shape and surface properties along with the route of administration and physiological environment, influence the fate of the biodistribution profile. Once in systemic circulation; NCs encounter a rich environment of proteins, tissues and cells. *In vivo* mouse models suggest that particles on the micrometer scale remain in the body much longer than particles on the nanometer scale (Faraji and Wipf 2009). In contrast, another study reported elimination of NCs with size >200 nm by the spleen which compromises its distribution to tissues (Kulkarni and Feng 2013). Studies reported the preferential accumulation of 10–200 nm particles in tumor tissues by virtue of EPR effect (Fang et al. 2019). Further, non-spherical particles

appear to increase circulation time and promote more accumulation in the target organ compared to spherical NCs. NCs accumulate mostly in the liver, spleen and blood. Rapid accumulation of bigger particles in the liver and spleen may be due to increased binding opportunities between the NCs and MPS (Song et al. 2014). Size and morphology of the blood vessel endothelium affects the circulation time and transport properties of NCs into surrounding tissues and organs. Spleen and liver with discontinuous endothelial morphology with larger pore spaces allow NCs less than 60 nm whereas lung and muscle capillaries with continuous endothelial morphology only allow NCs with size range below 3 nm (Sutapa and Samir 2014). Once in the bloodstream, the NCs adsorb a range of proteins on its surface depending on the hydrophobicity, size, curvature and surface charge. Adsorption of proteins in NCs surface results in (1) changes in surface charge, (2) increase in the hydrodynamic diameter, (3) aggregation. Negatively charged or neutral surfaces of NCs facilitate reduction in plasma protein adsorption and minimal nonspecific cellular uptake. In contrast, positively charged NCs undergo high nonspecific internalization resulting in short circulation half-life (Karmali and Simberg 2011). Surface chirality and surface ligands are other two factors which influence blood circulation patterns and cellular uptake (De Paoli Lacerda et al. 2010). Adsorption of specific protein types e.g. opsonin (laminin, complement proteins, IgG) initiates a complement cascade which helps in the recognition and phagocytosis of these NCs by macrophages and other immune cells. Upon NCs uptake, macrophages secrete cytokines stimulating a systemic immune response (Dobrovolskaia et al. 2009). Phagocytic macrophages and monocytes comprising the reticuloendothelial system (RES) reside largely in the spleen, liver, bone marrow and lymph nodes which results in sequestration of NCs in these organs. This further results in reduction of systemic circulation time of NCs, undesired localization in non-target organs and hinders therapeutic outcomes. Coating of these NCs with hydrophobic polymers such as poly (ethylene glycol) (PEG) reduces protein adsorption and thereby extends the circulation time with minimal uptake by RES cells. Protein corona formation does not always accelerate the clearance of NCs from systemic circulation. Adsorbed proteins which do not present a relevant receptor and are not recognized by any cellular receptors, provide stealth to particles. With reference to physiological and anatomical context, modelling a general trend for biodistribution of NCs is not possible as these relationships appear to be nanoparticle type specific (Ge et al. 2011; Moghimi et al. 2001).

9.2.3 Metabolism

Metabolism of NCs is influenced by physicochemical properties of NCs and route of administration. Once delivered, NCs interact with biological fluids, cell components, membranes, DNA, proteins etc., forming a nano-bio interface that comprises dynamic, physicochemical, kinetic, and thermodynamic exchanges between the nano-surfaces and the biological components, thereby imparting new features (Nel et al. 2009; Stark 2011). Current understanding suggests that the shape, size, surface charge, surface functional groups, aspect ratio of NCs and tissue microstructures have strong influ-

ence on organ-site specific extravasation and clearance profiles. With the help of structural design modification; *in vivo* metabolism of NCs can be modulated with improving biocompatibility and reducing toxicity. RES mediated overaccumulation of NCs in tissues and organs limits natural biodegradation and excretion, resulting in undesired toxicity. Large surface to volume ratios with relatively more atoms or molecules on the NCs limits their mobility in various *in vivo* compartments. NCs with diverse physicochemical characteristics with abundance of chemically active sites, initiate complex biochemical reactions; resulting in alteration of adsorption, aggregation, dissolution behaviors and eventually influence their *in vivo* behavior. Further, biophysical interaction occurring between NCs can potentially influence phase transitions, degradation, dissolution, protein adsorption, agglomeration and surface reactivity. The dynamic alteration in the physicochemical properties of NCs makes the metabolism complex and difficult to predict (Deng et al. 2007).

NCs may undergo structural degradation or can be excreted from the body in a non-degradable form. NCs with different structural components produce a wide range of metabolites. Organic NCs dissociate due to enzymatic activity and then metabolize to smaller components. The smaller size structural components of NCs travel through transcellular pores, access the Disse space and then accumulate in hepatocytes whereas bigger components of NCs fail to do so. Hepatic enzymes such as epoxide hydrolase, transferases, monooxygenases, and esterases degrade the structural components of NCs and the metabolites are either excreted through urine or transported to the bile and excreted in the feces (Sun et al. 2012).

9.2.4 Elimination

To reduce the potential toxicity profile of NCs, effective excretion is an essential process. In contrast to bulk materials and molecules, NCs show a unique and gradual clearance process. Upon entry into systemic circulation; it accumulates in different tissues and organs of the body. As per FDA regulations; NCs should be excreted from the physiological system within a certain time window. There are two major excretion routes for NCs: renal (urine) and hepatic (bile to feces). Biological clearance is classified into three categories: (1) opsonization-mediated NC removal by immune cells, (2) disintegration of NCs by protein adsorption, and (3) filtration of NCs by organs with fenestrated vasculature. There are two major elimination pathways: (1) Mononuclear Phagocytic System (MPS) Clearance, (2) Renal Clearance

1. **Mononuclear Phagocytic System (MPS) clearance**
   MPS is the dominant clearance pathway for NCs, resulting in accumulation of NCs with particle hydrodynamic diameter >10 nm in liver and spleen. Adsorption of opsonin onto the surface of NCs initiates receptor mediated rapid recognition and uptake by macrophages (e.g. Kupffer cells in the liver) in the MPS. It is reported that velocity of NCs reduces 100 folds upon entry into the liver leading to 7.5 times more interaction with hepatocytes compared to peripheral cells. PEGylation decreases the speed of protein adsorption and thereby delays the

MPS mediated elimination. Non-biodegradable NCs with hydrodynamic diameter >6nm, are excreted exclusively in the bile. Bile is composed of bile acids, phospholipids, cholesterol, proteins, and water. Low MW compounds are excreted through various active transporters whereas higher MW compounds undergo transcellular and distinctive paracellular transport (Soo Choi et al. 2007). The passage of NCs from the perisinusoidal space to bile canaliculi is regulated by hepatocytes. Vesicular-dependent transcytosis occurs either through receptor-mediated or fluid-phase endocytosis. Asialoglycoprotein receptor (AGPR), Apo-E and other receptors play a crucial role in elimination of NCs through bile (Furumoto et al. 2001). NCs which can't be recognized by hepatocytes must undergo fluid-phase endocytosis (Gruber et al. 1980). Surface charge of NCs also influences the process of biliary excretion as cationic charges promote the biliary excretion whereas anionic surface charge hinders the process. Investigation on isolated perfused rat liver has shown that the biliary excretion of macromolecules (proteins, dextran, PEG, inulin) seems to be independent of MW, from 2 to 500 kDa (Gruber et al. 1980). Biliary excretion is a slow process and the excreted quantities are usually small (≤5–10% of the injected dose over 8–48 h). Detection of small quantities of NCs in bile raises questions on the effectiveness of this system in removal of NCs from the body.

2. **Renal Clearance**
   MPS mediated extended residence time and the negative consequences can be overcome by excretion through renal route. Physicochemical characteristics such as shape, size and surface charge play the most crucial part in renal clearance. NCs of smaller size <5.5 nm, with positive surface charge easily transverse through a negatively charged glomerular capillary membrane, enter the urinary bladder and can be effectively excreted through urine (Yu and Zheng 2015). In Bowman's space, some NCs undergo tubular reabsorption and return to systemic circulation. For polymeric NCs, a MW below 5000 is associated with renal clearance, although the density of the polymer also influences this cut-off. A recent study showed that the renal mesangium could be targeted by NCs with a defined size ~75 nm which easily permeate through endothelial fenestrae and trapped in glomerular basal membrane (GBM) for at least 24 h and eventually cleared by residing macrophages (Soo Choi et al. 2007). Nonetheless, compared to NCs concentration found in MPS related organs; the total amount found in the kidneys remained low (≤5%) which warrants additional studies to confirm the efficiency of renal filtration in preventing accumulation and toxicities in clinical context.

9.2.5 Toxicity Considerations

The following aspects can be considered with respect to the toxicity of NCs in the body. First, when the NCs are introduced to the systemic circulation; it interacts with the blood cells and components, and results in hem-toxicity. Second, RES mediated extensive accumulation of NCs in the lung, liver, spleen, and kidney

resulting in severe toxicities in these organs (Aillon et al. 2009). Furthermore, generation of multiscale immune responses due to the recognition of NCs as foreign material causes substantial immunotoxicity. Molecular mechanisms of toxicity of NCs involve the formation of free radicals and induction of oxidative stress. The free radicals interact with cellular components like nucleic acid, proteins, and lipids and alter or disrupt their function and therefore are believed to be the major contributor to the toxicity of NCs (Elsaesser and Howard 2012).

Material properties of NCs, depending on their biodegradability, biocompatibility, administration route and dose, type of material can lead to dramatically different *in vivo* toxicities. There are not many reports investigating the effect of physicochemical properties on the *in vivo* toxicity of NCs. However, the available limited studies suggest that surface chemistry has a huge role to play in determining the *in vivo* toxicity (Nel et al. 2006; Albanese et al. 2012).

We now discuss a few synthetic polymers that have evolved over time as versatile biomaterials becoming the backbone for various NCs developed and being FDA approved for clinical use since the second half of twentieth century. We further discuss some biological macromolecules such as proteins, polysaccharides, lipids and viral vectors that have evolved overtime as excellent NCs for drug/gene delivery along with their ADME properties.

9.3 Synthetic Polymers as Drug Carriers

9.3.1 Dendrimers

Dendrimers are a supramolecular assembly of macromolecular size, widely studied for diagnostic and therapeutic applications (refer Fig. 9.4). These are characterized by their compact cascade branching and high group functionalities and commonly used in MRI (magnetic resonance imaging). Dendritic polymers are generally classified as dendrimers, dendrons, dendronized (hyperbranched) polymers. Dendrimers and dendrons have a molecular size of 1–10 nm (hydrodynamic radius), while dendronized have a larger size ranging up to microns (Khandare et al. 2012; Stiriba et al. 2002). ADMET properties of dendrimers are governed by the size, structure and surface functionality. Kaminskas L.M. studied the effect of physicochemical parameters on the pharmacokinetic behavior of dendrimers in detail and recently developed an *in silico* ADMET prediction model "dendPoint". This is a free tool which can be accessed using http://biosig.unimelb.edu.au/dendpoint (Kaminskas et al. 2019).

Biodistribution Small-sized dendrimers with lower generations (G1 and G2) generally have prolonged circulation time as compared to higher generation dendrimers (G3 or more). In a biodistribution study comprising G3, G5 and G7 PAMAM Starburst™ dendrimers, the G3 dendrimers with MW 5147 Da showed maximum

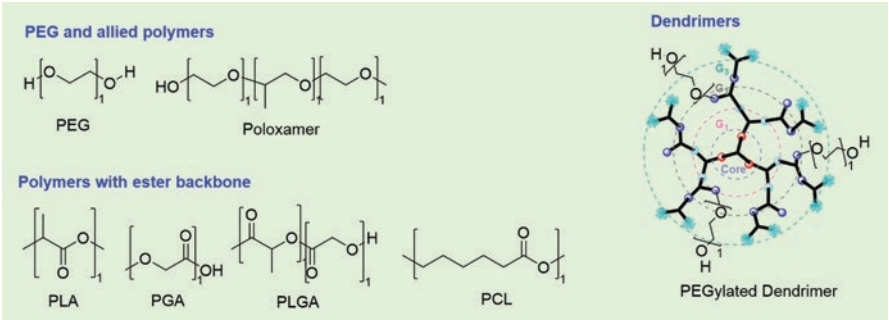

Fig. 9.4 Synthetic polymers and their use as NCs: PEG and its allied polymers, different generation dendrimers and various lab synthesized polymers with ester backbone are the first biomaterials used in various biomedical applications

accumulation in the kidney after 48 h; G5 and G7 with MW 21,563 and 87,227 Da respectively showed localization in pancreatic tissue after 24 h (Roberts et al. 1996).

Charges on dendrimers play a crucial role in interaction with the membrane, cell surfaces and plasma proteins. Cationic dendrimers showed rapid systemic clearance and are known to have higher hemolytic and cytotoxic properties in comparison to uncharged and anionic dendrimers (Malik et al. 2000). Boyd et al., found that *iv* administration of poly-L-lysine (cationic) dendrimers led to a sudden decrease in plasma concentration due to their binding with endothelial surfaces followed by its hydrolysis to lysine. This finding directs the possible biodistribution and reabsorption in the form of free amino acid of cationic poly-L-lysine dendrimers (Boyd et al. 2006). PAMAM dendrimers are among the most widely studied dendritic structures. Cationic PAMAM dendrimers are found to have higher cell internalization and tissue distribution in comparison to neutral PAMAM dendrimer as a consequence of opposite charge of the cell membrane (Nigavekar et al. 2004). A lower generation PAMAM dendrimers (G4) were also found to be taken up by neuronal cells *in vivo*, with enhanced internalization on pre-activation of the microglia. Neuronal intake was also dependent on surface modification of the dendrimer (Albertazzi et al. 2013).

Metabolism Metabolism of dendrimers is also dependent on their size, end-capping nature and degree of surface charge. Keminskas and coworkers had observed that end-capping of poly-L-lysine dendrimer with anionic group resulted in metabolism resistance which was even higher with anionic benzene disulfonate capping (Kaminskas et al. 2007). Neutral dendrimers with size less than 3.5 nm are readily eliminated unchanged in the urine. PEGylation increases the circulation time of dendrimer, avoids its interaction with proteins and cell surfaces, makes it less accessible to the metabolic enzymes, prevents electrostatic interaction and phagocytosis-based clearance (Mignani et al. 2019). Elimination of such assemblies is mainly size-dependent, i.e. small sized assembly will be cleared through renal secretion while large-sized (>20 kDa) will primarily be removed by RES (Lee et al. 2005).

Elimination Elimination too, can be size dependent. For instance, large aryl sulfonate capped dendrimers are systemically cleared by opsonization followed by elimination through the reticuloendothelial system (RES). In contrast, small aryl sulfonate and succinate capped dendrimers are more readily metabolized and eliminated by the kidney (Kaminskas et al. 2007). Cationic PAMAM dendrimers (third and fourth gen) showed rapid clearance (<2% recovery) from systemic circulation in comparison to anionic dendrimers (20–40% recovery) of the same generation after 1 h of *iv* administration at 10 μg/ml in Wistar rats (Malik et al. 2000). Generally, dendrimer having MW >30 kDa have less volume of distribution and take a long time for elimination from the body with an elimination half-life of 1–3 days, whereas dendrimers with MW <20 kDa eliminated quickly from the body with an elimination half-life of only 1–10 h (Kaminskas et al. 2008). Dendrimers up to G5 have hydrodynamic radii of 5 nm, preferentially eliminated by renal secretion while dendrimers above G7, due to their large size, are not able to pass through glomerular filtration and cleared through RES (Kaminskas et al. 2011).

Toxicity Dendrimers are used for both therapeutic and diagnostic applications and usually meant for *iv* administration, so the safety of dendrimers needs to be carefully evaluated. Dendrimers are evaluated so far via *in vitro* cell viability assays, *in vivo* toxicity, haemolytic potential, biocompatibility and their tendency for immunogenicity. Due to highly charged surfaces, dendrimers are very much prone to have interaction with the cell membrane, proteins, DNA and many other charged species present in the body.

G4-PAMAM dendrimers were found to be safe in Swiss albino mice in repetitive low to high dosing (Chauhan et al. 2010). PAMAM Starburst™ dendrimers were found non-immunogenic. However, G7 dendrimers resulted in pharmacological complications (Roberts et al. 1996). Melamine based G3 dendrimers were found to be cytotoxic, *in vitro* at 100 μg/ml concentration. In acute toxicity study in mice, the melamine dendrimers were found lethal at 160 mg/kg intraperitoneal dose with 100% mortality in 6–12 h. Subchronic toxicity studies showed lethality at 40 mg/kg. Liver damage was also observed in both studies (Neerman et al. 2004). In further toxicity evaluation, cationic end-capped melamine dendrimers were found to be more toxic in comparison to the anionic and neutral dendrimer. PEGylation of melamine dendrimer resulted in the loss of cytotoxicity and acute toxicity associated with melamine dendrimers (Chen et al. 2004).

Similarly, G4-PAMAM dendrimers also resulted in a loss in cell cytotoxicity after anchoring with PEG (2000 Da) and folic acid (Diaz et al. 2018). Malik et al., had reported that dendrimer with -NH2 termini showed concentration-dependent, while PAMAM dendrimers have generation dependent hemolysis and cell morphology change effect even at 10 μg/ml. They found that cationic dendrimers were more cytotoxic with IC_{50} value ranging 50–300 μg/ml in a panel of cell lines (Malik et al. 2000). Amino terminated cationic PAMAM dendrimers were found lethal due to platelet activation and morphology change, which resulted in enhanced platelet aggregation and adherence, ultimately giving rise to blood coagulation. Further,

these dendrimers were also found in interacting with various negatively charged blood proteins, including fibrinogen (results in blood coagulation) and albumin (Jones et al. 2012a, b).

In a recent study, cationic G5-PAMAM dendrimers were found to be inhibiting renin-angiotensin system (RAS) by their action on angiotensin-II mediated transactivation of epidermal growth factor receptor (EGFR) and ErbB2. RAS inhibitory effect was observed even at a low non-cytotoxic concentration of dendrimer and was dependent upon generation size, surface group functionality and incubation/exposure time (Akhtar et al. 2016).

From the above case studies, we can conclude that cationic dendrimers have serious side effects due to opposite charge interactions with various charged surfaces and proteins, this adverse effect could be minimized by surface modification and reducing the surface charge of a dendrimer.

9.3.2 PEGs and PEG-Allied Polymers

Poly-ethylene glycol polymers, also known as macrogols, are among the most widely used carriers because of their hydrophilicity, neutral charge and biocompatibility. PEG is synthesized from ethylene oxide in the form of both linear and branched-chain polymers and is also used in making various block copolymers (Fig. 9.4). Safe, inert and non-immunogenicity are the main advantages of PEG and has been approved by the FDA for diverse clinical applications. Water solubility enhancement and increase in the systemic circulation time due to the stealth property of PEG are additional advantages (D'Souza and Shegokar 2016; Kolate et al. 2014). Among PEG-allied polymers, poloxamer, a copolymer of ethylene oxide and propylene oxide, is also widely used in nanocarrier preparation (Su et al. 2019).

Biodistribution Absorption and distribution of PEG polymers in the body after payload delivery will depend on their MW and size. After oral administration in rats PEGs with MW less than 1 kDa showed only partial absorption (~2%) while PEGs with MW more than 4–6 kDa remained unabsorbed until 5 h. These results indicated that high MW PEGs will be excreted unabsorbed from the GIT post oral administration (Knop et al. 2010). Similar, MW dependent absorption after oral administration was observed in humans as well (Shaffer et al. 1950). Polymers with MW ≥50 kDa are eliminated by the Kupffer cells in the liver (Yamaoka et al. 1994). Being water-loving PEGs showed uniform biodistribution after payload delivery with fractioning in highly perfused organs such as kidney, heart and liver. In a biodistribution study, a 40 kDa PEG polymer showed a maximum level of polymer in the kidney followed by lungs, heart and liver (Longley et al. 2013). Further investigation using 2 kDa PEG polymeric nanocarrier after payload delivery following *iv*-administration in tumor-bearing mice resulted in maximum fractionation of PEG in the kidney followed by liver and spleen (Su et al. 2019).

Metabolism PEG polymers, because of their hydrophilic and stealth properties avoid their interactions with proteins and enzymes in the blood; this makes them relatively inert to metabolism. Generally, PEG polymers are non-biodegradable. Only low MW polymers might undergo partial metabolism. Higher MW PEGs are excreted out in unchanged form, only a fraction of metabolite in the form of lower oligomers, glycolic acid or its derivatives and traces of exhaled CO_2 are known (Fruijtier-Pölloth 2005). PEG400 was eliminated by the kidney in unchanged form, and there was no sign of its metabolism to ethylene oxide after both oral and intravenous administration (Shaffer et al. 1950). PEG with lower MW undergoes oxidation of hydroxyl group into the carboxylic group, thought to be catalyzed by alcohol dehydrogenase. *In vitro* studies in isolated liver fraction indicate the presence of sulphated PEG but only to a minor extent (Webster et al. 2007).

Excretion Excretion of PEGs is also MW dependent where low MW PEGs are removed passively by glomerular filtration from the blood, whereas high MW PEGs are excreted in bile and ultimately in feces. Due to the hydrophilic nature, higher MW PEG polymers result in large hydrodynamic size by surface water absorption and result in reduced permeation and renal clearance. Molecular weight threshold for glomerular filtration of PEG is 30 kDa, but this also depends upon the shape of the polymer. Linear PEG-based polymers, even with high MW, can pass easily through glomerular filtration due to the flexibility in PEG structure (Veronese and Pasut 2005). Pharmacokinetics study of PEG polymer of 40 kDa in rats resulted in uniform biodistribution with a terminal elimination half-life of 20 h. The elimination half-life of PEG was also found to be MW dependent, and it was 18 min, 3 h, 16 h and 24 h with a MW of 6, 20, 50 and 190 kDa respectively (Yamaoka et al. 1994; Longley et al. 2013). Further, pharmacokinetic studies in tumor-bearing mice after payload delivery revealed that 2 kDa PEG resulted in 42.7% and 44.0% renal excretion, while excretion in feces was only 0.298% and 2.02% respectively (Su et al. 2019).

Toxicity PEG-based polymers are considered safe and are in clinical use. PEG polymers showed toxicity at a potentially high dose with the kidney being a major affected organ. However, presently used PEGs are very safe with a safety window of ~600 folds (Webster et al. 2009). Ester and ether derivatives of PEG are found safe even for topical application. PEGs with MW less than 4 kDa are rarely absorbed from the skin while higher MW PEGs do not absorb at all from the skin surfaces (Fruijtier-Pölloth 2005).

In vivo pharmacokinetic studies revealed that poloxamer-188 resulted in blood perfusion-based distribution in the body, metabolism was less than 5% with major elimination through the kidney in unchanged form, and was well-tolerated up to 14.5 g/kg in rats, dogs and humans. *In vitro* studies also revealed the safety of poloxamer-188, it was found to not affect metabolizing P_{450} enzymes (Grindel et al. 2002). Metabolites of PEGs which formed only in very few quantities and also known to be non-toxic (Webster et al. 2007). However, PEGs are found safe in a large number

of preclinical and clinical studies and considered as GRAS material by FDA. PEGs are known to attribute some nonclinical toxicity from several marketed products, by its vacuolization in phagocytic cells. But this is due to the normal functioning of phagocytic cells in the body for removal of large molecules, and these vacuoles get cleared with time without any known toxicity (Turecek et al. 2016).

9.3.3 NCs with Polyester Backbone

PLGA, PGA, PLA and PCL are the major ester-linked polymers which are widely explored as NCs either alone or in combination with other polymers (Fig. 9.4). PLGA (poly(lactide-co-glycolic acid)) a biodegradable copolymer of polylactic acid (PLA) and polyglycolic acid (PGA) has been widely explored for controlled drug delivery (Makadia and Siegel 2011). PLA is an aliphatic polyester based biodegradable polymer having wide applications in the biomedical field as well as NCs for the delivery of hydrophobic drugs (Casalini et al. 2019). Poly-ε-caprolactone (PCL) is another aliphatic polyester with similar physicochemical properties and applications, but having semi-crystalline nature with relatively slow biodegradation (Espinoza et al. 2020). All the four polymers, i.e. PLGA, PGA, PLA and PCL have been found safe to human use and approved by the FDA for various applications (Su et al. 2019).

Biodistribution PLGA biodistribution in rats comprising oral and *iv* administration of samarium oxide (radiotracer) loaded PLGA nanoparticles of size 281 ± 6.3 nm was studied. After oral dosing, nanoparticles distributed in various organs indicated uniform absorption. At the same time, after *iv* administration, rapid systemic clearance followed by accumulation in spleen and liver occurred due to RES based capture and uptake of nanoparticles by these organs (Mandiwana et al. 2015). Similarly, in another study 300 nm sized PLGA nanoparticles showed maximum distribution in the liver followed by spleen, lungs, heart and kidney (Saxena et al. 2006). Further, the biodistribution of PLGA was also found to be dose-dependent. In a dose-escalation study, concentration of PLGA in liver and blood was dose-dependent, although there was no change in PLGA concentration in other organs (Panagi et al. 2001).

Metabolism PLGA is biodegraded by hydrolysis and enzymatic degradation to lactic acid and glycolic acid, which are biologically inactive and removed by general metabolic pathways. The liver is the leading metabolic site for these NCs due to presence of a large number of esterases in the liver. Polymeric ester bonds also have pH-dependent stability, and PGA ester linkage was found to be most stable at neutral pH. Oligomeric chains formed after hydrolysis are also known to form salt at free acidic groups sites and the resulting form will have different circulation period and metabolism as compared to protonated form (Göpferich 1996). PLA has the *in situ* self-degradation property by hydrolytic cleavage of the ester bond in its poly-

meric backbone (Casalini et al. 2019). Both PLGA and PLA are metabolized by hydrolytic and enzymatic cleavage with PLA metabolizing faster compared to PLGA (Espinoza et al. 2020).

PCL degrades very slowly in comparison to other biodegradable polymers, and this property is useful for prolonged release applications such as drug releasing implants. PCL capsules having MW 65 kDa were found stable after implanting in rats up-to 2 years followed by its degradation to lower MW species of 8 kDa after 30 months (Sun et al. 2006). Overall, for polyester polymers, metabolism is dependent on the type of nanocarrier, average MW and MW distribution. With wide MW distribution, presence of a large number of carboxylic acid groups results in lowering of microenvironmental pH which may lead to pH dependent auto degradation of the NCs and which eventually affects the drug release (Anderson and Shive 2012).

Excretion Like other polymeric NCs, polyester polymers also have MW dependent elimination from the body. Low MW NCs are supposed to eliminate fast due to their higher hydrophilic nature and carboxylic acid content. Post iv administration of PLGA and its PEGylated (PLGA-mPEG) nanoparticles, PLGA showed dose-dependent nonlinear pharmacokinetics, whereas PLGA-mPEG revealed dose-independent linear pharmacokinetics (Panagi et al. 2001). Among these major polymers, PCL is very slowly degrading polymer. When tritium-labelled 3 kDa MW PCL implant was implanted subcutaneously in rats it showed the first sign of radioactivity in urine and feces after 15 days. Total cumulative excretion of 92% in the form of recovered radioactivity was obtained after 135 days of implantation (Sun et al. 2006).

Toxicity These polymeric materials are biocompatible as well as biodegradable and not known for any severe toxicity, so they are considered as safe and approved by FDA for their medical use. However, some findings also pointed towards the proposed safety of these polymers and nanostructures formed from these polymers. PEG-PCL-PEG (PECE) block copolymer was evaluated for acute toxicity study in BALB/c mice via intrapleural, intraperitoneal and subcutaneous routes at a dose of 10 mg/kg b.w., 25 mg/kg b.w., and 25% b.w. respectively. Post 14 days animals were evaluated for any possible toxicity via histopathology of various body organs, and no sign of toxicity or mortality was observed indicating the safety of administered dose (Gong et al. 2009). However, in another study, PEG-b-PCL copolymer-based nano micelle was found to inhibit vascular angiogenesis during embryo development of zebrafish in a dose-dependent manner (Zhou et al. 2016). PLGA nanoparticles were found to have an inflammatory effect during *in vitro* study, which was higher in case of negatively charged PLGA in comparison to positively charged and uncharged (Grabowski et al. 2013). Inflammatory response of PLGA is thought to be due to induction of dendritic cells maturation which may result in host immune response (Zhu et al. 2015). PLGA induced inflammation is mainly associated with implanted PLGA scaffolds for tissue engineering, but this could be associated with PLGA NCs also.

9.4 Biological Macromolecules as NCs

9.4.1 Protein Based NCs

NCs from natural resources prove to be highly functional material that is tunable for drug delivery. Among the naturally occurring biopolymers, protein based NCs form a major class of potential drug delivery agents because of their abundant availability, non-toxicity, biocompatibility, biodegradability, high binding affinity towards drugs, targeted delivery (Elzoghby et al. 2012; Chen et al. 2006). In this section, we reviewed the pharmacokinetics of protein based NCs such as gelatin, silk, albumin as well as viral proteins which are used as vectors in gene delivery (Ma et al. 2012).

9.4.1.1 Gelatin

Gelatin is a partially hydrolyzed product from collagen, most commonly extracted from skin, white connective tissues and bone as the major sources. Acid based hydrolysis of collagen forms gelatin-A and alkali hydrolysis forms gelatin-B (Salerno et al. 2018). The gelatin thus produced is highly biocompatible and has been extensively explored in generating scaffold and in the pharmaceutical industry for drug and gene delivery. It is commonly used as plasma expander with the property of solubility, biodegradability, biocompatibility and surface charge (Young et al. 2005). Functional groups such as amino and carboxylate, make it easy for drug attachment and PEG coating makes it evade the RES system (Kushibiki et al. 2004). Fibronectin present in cell surface and ECM binds to gelatin (Ruoslahti and Engvall 1977).

Gelatin is capable of forming thermo-reversible gels in water with low melting point, used as a food ingredient and is one of the GRAS agents. Gelation property and ionization of gelatin is based on the pH, thus making it excellent for intracellular delivery. Gelatin B—(pI is 4.8–5.2) makes it negatively charged at physiological pH but in endosome with pH 5, it becomes positively charged resulting in disruption of endosome and release of DNA, RNA, or drug and is thus efficient as a gene delivery vehicle (Morán et al. 2015). Gelatin has the property of non-antigenicity because their tertiary structure is completely denatured by the extensive processing of collagen. Various functional groups can be attached to prevent its opsonization by RES through an aqueous steric barrier and can be used for specific targeting. Gelatin A acid treatment results in more glutamine and asparagine groups, but alkaline treatment of gelatin B hydrolyses these groups into aspartate and glutamate. Gelatin contains Arg-Gly-Asp (RGD sequence) in its chain which is used for tumor targeting. It is a polyampholyte macromolecule. Size of the nanoparticles designed determines its absorption and its bioavailability. Nanoparticles of size 200 nm and submicron particles were easily taken up than microparticles by cells and tissues. Elimination of the particles is also dependent on their size (Sahoo et al. 2015; Elzoghby 2013; Gref et al. 2000, 2003). Smaller particles have larger circulation time which is needed for sustained release and targeted drug delivery whereas larger particles are removed from the system more rapidly.

Gelatin nanoparticles without surface modification are eliminated from the system by the circulating mononuclear phagocytic cells as they are identified as foreign bodies. However, with surface modifications and depending on their size they circulate in the vasculature for a longer time which aids in targeted delivery (Won and Kim 2008; Sushma Kommareddy 2007).

Toxicity Thiolated, PEGylated gelatin (higher EPR effect) have shown longer circulation property enhanced tumor extravasation and increased transfection efficiency. Ionically charged gelatin, antibody attached, carbohydrate, protein coated gelatin also increased its targeting ability by interacting with specific receptors thus reducing its toxicity. Gelatin used as a vehicle for delivering protein drugs has been taken up by dendritic cells, APC and degraded by collagenase (Sahoo et al. 2015).

Even though non-toxic and highly potent intracellular nucleic acid delivery system, their *in vitro* and *in vivo* efficacy in transfection is variable. Thus, surface functionalization of gelatin is utilized to conjugate it with targeting ligands and fusion peptides for gene delivery shows efficacy based on their surface and chemical modification. Gelatin/silica nanovectors show cell viability of more than 80%, further chemical modification results in a decrease in cell viability based on the functional group attached. When modified with PEGylation, overall amounts of NPs in tissues were higher in the liver compared to tumor, kidney and spleen (Zhao et al. 2016; Saraogi et al. 2010; Tran et al. 2014).

Gelatin nanoparticles are used for delivery for various drugs including chloroquine, amphotericin B, and paclitaxel, have been explored for application in gene and peptide delivery. Crosslinking agent like glutaraldehyde is used in gelatin NPs preparation. Significant cell viability of more than 90% was observed with only gelatin NPs and drug encapsulating gelatin NPs. When orally administered maximum accumulation of gelatin NPs are observed in macrophage rich sites like lungs, liver and spleen.

Gelatin-oleic nanoparticles functionalized with folic acid as ligand for targeting cancer tissues showed active targeting towards tumor and also were passively targeted to tissues with leaky vascular endothelial functions (Tran et al. 2014). Gelatin derivatives are typically injected subcutaneously and in very specific cases injected intravenously. When used for siRNA delivery, thiolated gelatin is used with thiol modified siRNA Thiol modified showed a long circulation time and reduced uptake RES unlike simple gelatin NP system (Lee et al. 2013). Thus gelatin nanoparticles system with safe biodegradability profile provides potential for functionalization, efficient targeting and minimal toxicity.

9.4.1.2 Albumin

Most nanoparticles after delivery are coated by a protein corona made up of plasma proteins that are collected from the circulation. Albumin is one of the abundant proteins present in plasma. As most nanoparticles are coated by albumin possess longer circulation time, its potential is extensively tested in drug delivery. It also

forms one of the easily obtainable, biocompatible, bio-degradable and non-toxic drug delivery agents. Albumin coated particles are taken up by tumor tissues by binding with overexpressed secreted protein, acidic and rich in cysteine (SPARC) (Schnitzer 1992). It also binds to glycoprotein receptors such as Gp60, Gp30 and Gp18 that results in formation of transcytotic vesicles (Desai et al. 2006). When NPs are coated with albumin, the adsorption of opsonin was significantly less compared to simple NPs, further leading to reduced complement activation (Peng et al. 2013; Hawkins et al. 2008).

9.4.1.3 Silk

Silk is another biodegradable, biocompatible self-assembling protein obtained from domesticated silk worm, spiders and other insects. Non-thrombogenic, anti-inflammatory, cell adhesive, cell responsive and regenerative property makes it a highly desirable material. Sericin coated silk showed inflammation *in vivo* by upregulation of inflammatory cytokines. Removal of sericin reduced thrombogenic and inflammatory responses in implanted silk fibroin (Wenk et al. 2011). Degradation of most protein-based biopolymers occurs through proteolytic enzymes with mostly non-toxic metabolites *in vivo* (Gobin et al. 2006). As most biopolymers, silk undergo degradation by proteolytic enzymes elastase and trypsin (Lammel et al. 2011).

9.4.2 Polysaccharides

Polysaccharides are a group of naturally occurring biodegradable polymers which are obtained from various animal and plant sources including algae and microbes and characterized by *O*-glycosidic linkage between monomeric units. Biocompatible, degradable, hydrophilic, non-toxic, targetable, amenable functionalities in chemical structures, low cost, and abundance are the major advantages of polysaccharides as NCs. Different types of polysaccharides have been explored so far for various drug delivery applications. These include chitosan, hyaluronic acid, dextran, starch, pullulan, pectin, cellulose and hemicellulose, dextrin (hydrolyzed polysaccharide), heparin, chondroitin sulfate, alginic acid, and a number of natural gums (Swierczewska et al. 2016; Barclay et al. 2019) (Fig. 9.5). These materials have been used for making nanoparticles, self-assembled nanostructures, liposomes, nanogels, mucoadhesive, targeting, vaccines, and non-viral gene delivery.

Biodistribution Biodistribution of polysaccharides depends significantly upon their application apart from the size, surface charge and surface modification. Low MW polysaccharides showed faster absorption, biodistribution and penetration in peripheral body tissues as compared to higher MW polysaccharides which tend towards the systemic circulation (Chae et al. 2005; Wang et al. 2016). Water-soluble chitosan (50% deacetylated chitin) post intraperitoneal administration in mice

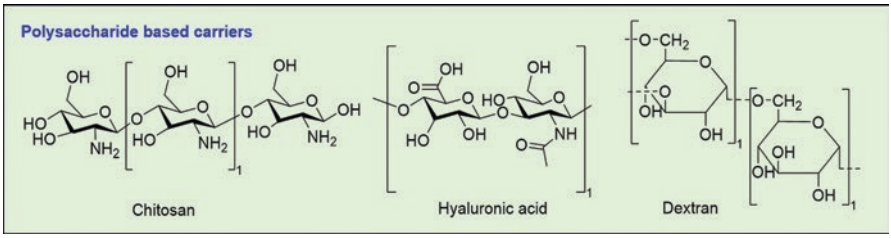

Fig. 9.5 Polysaccharide based carriers: Multiple polymers such as chitosan, hyaluronic acid, dextran, pullulan, various natural gums such as xanthan gum, guar gum, chondroitin sulphate etc. have been utilized as vehicles for drug delivery since they can be easily degraded *in vivo* and the pathways of metabolism are well known

resulted in complete elimination via kidney with negligible distribution in liver, spleen, abdominal dropsy, and plasma after 14 h of dosing. Further, in the same study urine recovered chitosan was found to be of low molecular weight in comparison to *in vitro* incubated chitosan indicating possible biodegradation (Onishi and Machida 1999). Succinylated chitosan resulted in enhanced accumulation in tumor sites in sarcoma180-tumor bearing mice with reduced fractional elimination through the kidney (Kato et al. 2000). ^{99m}Tc labelled hyaluronan after *iv* administration showed maximum radioactivity in liver, kidney and spleen irrespective of the MW; whereas no systemic radioactivity was observed after oral administration and it was only restricted to GIT indicating the absorption limitation of hyaluronan (Laznicek et al. 2012). Few studies with different radioactive labels confirmed the intestinal absorption of hyaluronic acid, so the absorption of hyaluronic acid after oral absorption is still controversial and needs further investigation.

Metabolism Various polysaccharides based NCs easily get metabolized by enzymes present in our body. High MW polysaccharide NCs get metabolized before elimination from the body, while low MW polysaccharides are removed unchanged, also by the kidney. Metabolic sites for polysaccharides in our body mainly include liver, kidney and intestine. Both chitosan and hyaluronic acid are found to be metabolized in their lower oligomeric forms. Chitosan is thought to be degraded/metabolized by lysozymes and enzymes present in intestinal bacteria, further degradation of chitosan is also dependent on its surface modification and will reduce with the degree of deacetylation (Kean and Thanou 2011).

Bacteria derived hyaluronic acid showed fast clearance from the blood with accumulation only in the liver. Its maximum excretion in the form of exhaled air warrants its rapid and excessive metabolism (Nimrod et al. 1992). Oral administration of 300 kDa hyaluronic acid resulted in metabolism by bacterial enzymes of intestine in oligosaccharides followed by intestinal absorption and distribution in different sites in rats including skin and absence of hyaluronic acid excretion in feces confirms its intestinal degradation (Kimura et al. 2016).

Excretion Excretion of polysaccharide depends both on its physicochemical properties as well as metabolism. Generally, polysaccharide metabolites are excreted out by the kidney, but reports also suggest their excretion even as exhaled CO_2. Hyaluronic acid after *iv* administration in rats and rabbits resulted in quick elimination with a half-life of 3.7 and 5.3 min respectively. However, elimination in rabbits was significantly affected by the route of administration, and it was 10.5 h after intraocular administration (Nimrod et al. 1992). PEGylation is well-adopted for enhancing systemic circulation of polysaccharide-based NCs too. PEGylation of dextrin-based nano-magnetogel of size 110 nm resulted in increased circulation time resulting in elimination half-life of 4 h in mice with lesser distribution in liver, kidney and spleen (Gonçalves et al. 2015).

Excretion is highly correlated with biodistribution of polysaccharide and higher MW polysaccharides possibly eliminated earlier due to their lesser peripheral distribution. Dong et al., studied the ADME of FITC labelled water-soluble carboxymethyl chitosan with a different MW in rats. Post 15 days of intraperitoneal administration of low MW FITC labelled carboxymethyl chitosan, 71% excretion was recorded which was lower than 88% of high MW in the same time (Dong et al. 2012). Further, as ADME of polymeric carriers is highly MW dependent and polysaccharides are of biological origin and control over their average MW and distribution is very difficult. So, these issues need to be considered thoroughly for successful translation of polysaccharide based NCs.

Toxicity Polysaccharides are well known for being biocompatible, biodegradable and safe to human use. Chitosan is one of the widely investigated polysaccharide found to be highly biocompatible, well-tolerated and is approved as a food ingredient in Japan. The only concern about chitosan is its cationic nature which may lead to interactions with DNA and other negatively charged proteins. Variation in surface charge of chitosan was also found to be affecting its cellular intake and in turn, the biodistribution of chitosan (Kean and Thanou 2010). Further, positive charged NH_3^+ of chitosan also reacts with an acidic group of bile acids and fatty acid in the intestine and will result in reduced emulsification and absorption of fat from the intestine (Ylitalo et al. 2002). Hyaluronic acid containing intranasal formulations are found highly safe in a human clinical trial for the treatment of dry nose conditions (Thieme et al. 2020). Apart from the safe history of hyaluronic acid, it possesses inherent anti-inflammatory, analgesic and chondroprotective properties, which are advantageous in their therapeutic application, so not a concern for FDA for its safety (Vasvani et al. 2019).

9.4.3 Lipid Based Carriers

9.4.3.1 Use of Liposomes as Carriers for Drug and Gene Delivery

Absorption Administration (s.c/i.p.) of PEGylated liposomes of 100 nm diameter composed of HSPC:CHOL:PEG2000-DSPE showed circulation half-life of 11 h with a peak concentration of approximately 12–24 h. Following s.c. administration;

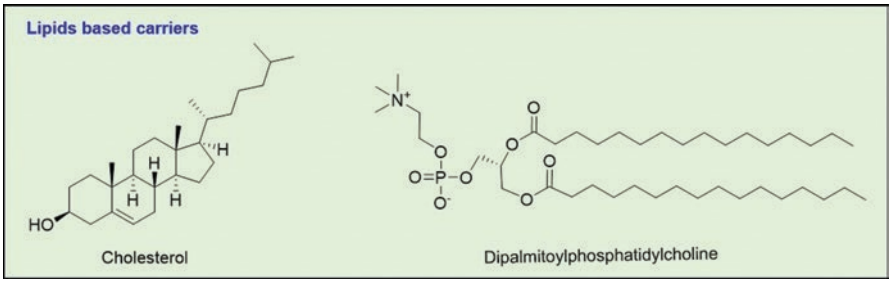

Fig. 9.6 Lipid based drug and gene delivery vehicles. Cholesterol is the most commonly used lipid to impart stability and strength to the liposomes and other vehicles. Dipalmitoylphospatidylcholine (DPPC) is another commonly used lipid for the formation of liposomes and other kinds of vesicles and particles

the liposomes accumulated in the lymph nodes, eventually reach blood through the lymphatic drainage pathways. In contrast; a limited amount of PEGylated DSPC-CHOL liposome showed a movement from blood to peritoneal cavity after i.v. injection (Bally et al. 1994). Whereas non-PEGylated liposomes of 100 nm gets ingested by macrophages to retain in the lymph nodes and fail to reach systemic circulation. PEGylated liposomes administered through i.v. were cleared from the central compartment by first order kinetics (Allen and Hansen 1991) (Fig. 9.6).

PEGylated liposomes exhibit a size dependent absorption to systemic circulation. As an example; particle of >120 nm size doesn't absorb through the pores of interstitial spaces and end up accumulating at the injection sites and failed to reach the systemic circulation (Gregoriadis 2016). DSPC:CHOL liposomes of 100 nm size, move from blood to peritoneal cavity whereas liposomes of 1000 nm size failed to do so. MPS mediated uptake of liposomes remove larger size liposomes from circulation before they could reach the peritoneal cavity. Role of liposome compositions on the fate of absorption was not conclusively studied.

Biodistribution The biodistribution profile of liposomes depends upon a number of factors, namely, surface charge, size, composition, bilayer fluidity, liposome dose and dosing frequency. Once administered through i.v. route; the liposomes are distributed in the MPS cells and the vascular compartments. After a single dose of either PEGylated or non-PEGylated liposomes; volume distribution was not much higher than the total blood volume. Liposome size and presence or absence of capillary discontinuities play the most critical role in biodistribution. Kinetic model investigations revealed that non-PEGylated liposomes are removed from circulation by fixed macrophages in spleen and Kupffer cells in liver. Furthermore, large non-PEGylated liposomes are uptaken by low capacity, high affinity saturable system involving Kupffer cells. However, small non-PEGylated liposomes are taken up by intra and extravascular spaces mostly involving parenchymal cells. In contrast, uptake of PEGylated liposomes by MPS are independent of liposome size and >250 nm size liposomes show the increased distribution in the liver (Hunt 1982).

Hepatic uptake of liposomes with a diameter more than 80 nm are independent of vesicle size and shows high splenic uptake when liver uptake becomes saturated (Liu et al. 1991).

Liposome composition like cholesterol, charged components, rigid lipids and the inclusion of components such as glycolipids, carbohydrates, polymers etc. affects the biodistribution and clearance of the liposomes. Increasing rigidity, by incorporating high phase transition temperature lipids and modifying bilayer composition with cholesterol decreases the distribution to the MPS (Woodle et al. 1992). Small size (~100 nm) rigid non-PEGylated liposomes composed of DSPC:CHOL have several hours of circulation half-life compared to few minutes half-life of large fluid liposomes. Negatively charged carbohydrate containing lipids such as sulphatides, phosphatidylinositol helps in increasing the circulation time (Park et al. 1992). Furthermore, liposomes with other negatively charged phospholipids like phosphatidylglycerol, cardiolipin, and phosphatidylserine are rapidly distributed to liver and spleen compared to their positively charged or neutral counterparts (Senior et al. 1991). Decreasing the MW of PEG to less than 750 Da in HSPC:CHOL:PEG-DSPE liposomes (100 nm in diameter), increases clearance from circulation. Though increasing the MW of PEG above 1000 Da did not further increase the circulation time of the liposomes, PEGylation further increased "passive targeting" mediated distribution of liposomes into ascitic and solid tumours. PEGylated liposomes with ligand-mediated targeting strategies favour desired biodistribution and pharmacokinetics with increased local delivery (Allen et al. 1994).

Metabolism and Excretion Distribution of liposomes in the MPS play the most crucial role in determining the circulation half-life. MPS (liver and spleen) mediated elimination is the major route which does not allow re-entry of liposomes to the systemic circulation.

Low dose (1 pmol PL/mouse) administration of PEGylated liposomes (100 nm size) through i.v. follows a first-order (log-linear) kinetics while non-PEGylated liposomes undergo a Michaelis-Menten-like kinetics (Allen and Hansen 1991). PEGylation increases half-life of liposomes to about 18-fold with a mono-exponential clearance while non-PEGylated one demonstrates biphasic elimination. Elimination of non-PEGylated liposomes gets delayed with increasing doses; typical of a saturation type non-linear pharmacokinetics (Blume and Cevc 1990).
Little information is available for ADMET of lipid-based micelles and solid lipid nanoparticle (SLNs) though it is assumed to follow similar profiles like the liposomes.

Extracellular Vesicles

Extracellular vesicles including exosomes undergo absorption through receptor-mediated endocytosis, micropinocytosis, kinase-dependent phagocytosis, olfactory pathway, and lymphatic active transport (McKelvey et al. 2015). Once being absorbed; exosomes traverse through blood tissue barriers and accumulate mostly

in the liver, kidney, lung, and spleen through MPS and are metabolized (Cataldi et al. 2017). Depending on the nature of the extracellular vesicles, it may or may not be able to escape lysosomal degradation. The metabolite of exosome contains lipids (sphingolipids, fatty acids, sterol, prenol lipids glycerolipids, and glycerophospholipids), nucleosides, amino acids, nucleotides, carboxylic acids, carnitines, sugars, cyclic alcohols, and vitamins. Among other organs, the kidneys, liver, and spleen, where fixed macrophages stay, have been reported to be most closely associated with clearance of exosomes (Zebrowska et al. 2019).

9.4.4 Viral Vectors for Gene Delivery Application

Viruses are biological machines that can efficiently hijack the cellular receptors and pathways for their entry and trafficking of cargo. Recombinant viral vectors have emerged as carriers in gene therapy over a long controversy and clinical trials. Though recombinant viral vectors are efficient in gene delivery, the backlashes faced by viral vectors include the immune response and the non-specific integration of the cargo in host genome. Major classes of virus that have been tested clinically are, lentiviruses, onco-retrovirus, herpes simplex virus-1 viruses (HSV), adenoviruses, and adeno-associated viruses (AAV) (Kay et al. 2001). While lentivirus and onco-retrovirus that are capable of integrating with the genetic material into host chromatin can result in permanent genetic alterations, the other class of non-integrating vectors like adenovirus, AAV, and HSV delivers them as episomes and results in constitutive transgene expression. Targeting and distribution of viral vectors are achieved by their innate ability to infect the host system. However, major roadblocks are found in the form of immune response in terms of pre-existing antibodies and reaction with the complement system (Breun et al. 1999; Kiang et al. 2006; Zhi et al. 2006; Moskalenko et al. 2000). In systemic delivery, virus can get redirected in the blood stream towards the liver by uptake of heparan-sulphate proteoglycans and LDL-receptor related protein or blood cells (Waehler et al. 2007). As other protein carriers, viral vectors get eliminated by protease activity post transgene delivery.

9.5 Future Perspective and Conclusion

Currently, the complexity of the various experimental scenarios used to date, complicates and perhaps even prevents the comparison of data between the studies. Consequently, to collect information useful for developing general rules about the ADME profile of nanoparticles, methodologies must be developed that facilitate the overarching interpretation of resulting experimental data. The data collected from such studies would contribute toward an improved understanding of the potential risk of nanoparticles in human health.

While the physicochemical properties and bio-coating of NPs can be measured in different biological fluids, there are only few appropriate techniques to (kinetically) determine NPs *in vivo* and evaluate their evolution over time through their route in the body. The most drastic change in NP properties may involve *in vivo* degradation, and in this way the fate of all NP components—the core, the engineered surface coating, and the adsorbed biological molecules—need to be analyzed. Assessing biodistribution and clearance would involve multiple labeling strategies, in which all different components can be tracked and analyzed separately. While detailed extracorporeal NP characterization provides information on the products we put "in" and take "out" of the body, the intracorporeal processes are still shrouded in mystery. *In vitro* approaches and models for analyzing the biological effects of NMs could better interpret the overarching information of experimental data, and extract general rules that can be applied to studies of nanotoxicity, design, modification, and applications.

Physiologically based pharmacokinetic (PBPK) modeling along with Quantitative structure-activity relationship (QSAR) models need be used for predicting the absorption, distribution, metabolism and excretion (ADME) of synthetic or natural chemical substances in humans and experimental animals. Furthermore, generation of libraries like "dendpoint, for dendrimer"; with physicochemically diverse NCs, and analysis by hierarchical cluster analysis (HCA) will allow researchers to identify which nanoparticles have the most similar physicochemical properties and *in vivo* fate without having to specify individual physicochemical parameters. In addition to this, Quality Target Product Profile (QTPP) can be developed to optimize the physicochemical properties for a given nanocarrier and evaluating the desired therapeutic effect/targeting and the reduction in the associated toxicity. Studies on the effect of nanoparticle size focus on the energy considerations, especially the interactions between nanoparticles and their environment (including cells) with thermodynamic models can be immensely helpful to predict *in vivo* fate of NCs and will streamline future *in vivo* studies and optimize the design and clinical translation of NCs.

Due to their sizes, a majority of nanoparticles will not be cleared through the renal system. Liver and spleen uptake are the dominant clearance modes for most nanoparticles. Long-term toxicity must be evaluated in these instances. Large-scale synthesis of uniform nanoparticles with controlled surface properties is in high demand. The size and surface coating of nanoparticles profoundly affect biodistribution, clearance, and toxicity. Streamlining nanoparticle synthesis is crucial for standardizing results across studies. Moreover, some MDNS (materials of the drug nanocarrier system) and their metabolites have bioactivity which may lead to undesirable effects. For example, acid metabolites of PEGs may cause acidosis and hypercalcemia; chitosan can reduce emulsification of lipids and promote platelet adhesion; and a metabolite of PLA can cause inflammatory tissue reactions. MDNS and their metabolites may also affect the function of drug transporters and metabolic enzymes leading to drug-MDNS interactions and potential toxicity. Therefore, inertness is a highly desirable feature of MDNS.

To date there are no standardized protocols for evaluating the ADME of NCs and MDNS resulting in problems when trying to compare the results of different studies. Therefore, the development of evaluation standards has high priority. Because of big differences in the PK of parenteral drugs and NC systems, a deep understanding of the ADME of MDNS is important to ensure the safe clinical use of NCs. Although much is known about the PK of drugs, more work is needed to reach the same level of insight into NC DDS. We hope this review will serve to promote this greater understanding.

More quantitative studies that explore and assess essential mechanisms of nano-bio interactions in great detail are needed to provide solutions for overcoming biological and physical barriers that currently limit the clinical translation of nanomedicines. With the development of new analytical techniques in recent years, the transport of *in vivo* administered nanoparticles can be assessed with spatiotemporal information that can ultimately guide the engineering of more effective nanomedicine. There are lots of studies available for targeting and drug release from NCs in different animal models, however the study specific to *in vivo* fate of NCs post cargo delivery is somewhat limited. Pharmacokinetic studies exploring the ADME of various polymeric systems are there, but the ADMET of a nanocarrier will be significantly different from the polymer based upon their size, MW and surface modification. So, for successful translation of a nanocarrier a thorough investigation of polymeric nanocarrier ADMET is well desired.

As loading efficiency of most NCs is limited to ~10–12%, so in order to get a desired therapeutic drug concentration, we need to give a large volume of NCs. There is a lack of pharmacokinetic models exploring the nanocarrier dose transformation from animals to humans. A thorough understanding about the route and extent of metabolism, excretion, and possible toxicity in humans based on animal studies will certainly have a positive impact on the successful translation of NCs to clinics with superior drug delivery.

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Chapter 10
Polymers in Nanomedicine

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10.1 Introduction

Nanomedicine is the field of study that involves both biotechnology and nanotechnology, aiming at structures, molecules, the study of device and mechanism on the nanoscale level, comprising 1–10 nm (Fig. 10.1). Due to their size, structure, and high surface area, researchers have gotten an insight into nanoscale materials in several biomedical applications such as tissue engineering, cancer therapy, drug delivery, bioimaging, etc. Nanoparticles are extensively used to increases the effi-

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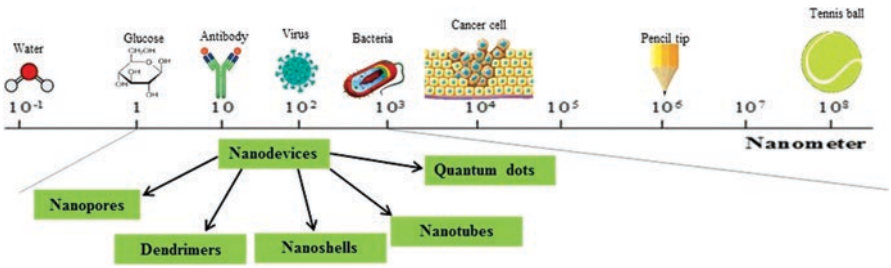

Fig. 10.1 Nanoscale size representation and type of nanodevices

cacy of a drug and reduce side effects through direct targeting and site-specific controlled delivery (Daniel-da-Silva and Trindade 2011). However, due to the toxicity of inorganic nanoparticles, polymers are generally used as a nanoparticle carrier to reduce the toxicity and increase the cellular uptake (Mohanraj and Chen 2006). For decades, polymers have been extensively studied based on their chemical and physical properties and their uses in bioscience applications. Designing polymer that responds to pH, ultrasound, and magnetic field has given rise to smart polymers that have used in surgeries for insertion of self-inflating bulky medical devices (Kumar et al. 2007). Cellular regeneration plays a vital role in the healing process from pathological conditions. However, some of the main issues that might affect tissue regeneration include inflammatory responses, deregulation of proteases, infection, and inadequate localized angiogenesis (Eming et al. 2014). Chronic impairment in tissue regeneration would also lead to loss of cellular senescence or facilitating the formation of cancers. Hence, novel biomaterials are crucial to improve the wound dressing properties that include antioxidative, anti-infective, adhesive, migrating, and proliferating cells (Griffith 2000). Polymeric nanoconstructs are broadly known as cellular support systems for therapeutic application. Table 10.1 illustrates a list of polymers used in nanomedicine applications. The present work focuses on some of natural and synthetic polymers that have been used in the nanomedical field. Most of these polymers are either used as native or chemically modified structures.

10.1.1 Chitosan

Chitosan is a linear polymer of β-(1–4)-linked D-glucosamine and *N*-acetyl-D-glucosamine and is rarely found in its native form in nature. Chitosan is produced through the deacetylation of the amino polysaccharide chitin, the building block of fungi cell walls as well as insect cuticles and crustacean exoskeletons. These sources are abundant and inexpensive by-product of the food industries. However, the extraction of chitin from these by-products is a relatively lengthy process from many crustaceans, unlike barnacles. Barnacles present the least crystalline chitin post demineralization and deproteinization from the starting material (Elieh-Ali-

Table 10.1 Representative list of polymers used in nanomedicine

Classifications	Polymer	Nanomedical applications	References
Natural polymer			
Protein based polymers	Collagen	Drug delivery	Yoshikawa et al. (2008)
		Wound healing materials	Rho et al. (2006), Zhou et al. (2016), Sun et al. (2018)
	Casein	Diagnostic bioimaging	Huang et al. (2015)
		Wound healing materials	Selvaraj et al. (2018)
		Tissue engineering	Fan et al. (2013)
	Albumin	Diagnostics and therapeutic monitoring	Chen et al. (2019b), Parodi et al. (2019)
		Drug delivery	Cui et al. (2013), Bilthariya et al. (2015)
	Gelatin	Diagnostic bioimaging	Li et al. (2013)
		Drug delivery	Gaowa et al. (2014), Nguyen (2017), Yasmin et al. (2017)
	Fibrin	Drug delivery	Lanza et al. (2006)
		Diagnostic imaging	Morawski et al. (2004), Pan et al. (2011)
		Tissue engineering	Sahni and Francis (2000), Kalbermatten et al. (2008)
	Fibrinogen	Drug delivery	Rejinold et al. (2011)
		Tissue engineering	Rajangam and An (2013)
Polysaccharides	Agarose	Tissue engineering	Lewitus et al. (2011)
		Diagnostic tracking	Kong et al. (2019)
		Drug delivery	Kolanthai et al. (2017)
	Alginate	Drug delivery	Zahoor et al. (2005), Li et al. (2008), Sharma et al. (2013)
		Wound healing materials	Coşkun et al. (2014), Kataria et al. (2014)
	Carrageenan	Drug delivery	Bulmer et al. (2012)
		Tissue engineering and regeneration	Gan and Feng (2006), Thakur et al. (2016)
	Hyaluronic acid	Drug delivery	Rho et al. (2018), Rao et al. (2016)
		Tissue engineering	Hemshekhar et al. (2016), Ji et al. (2006)
	Dextran	Drug delivery	Bisht and Maitra (2009), Heo et al. (2017)
		Tissue engineering	Wang et al. (2017), Jia et al. (2011)
	Chitosan	Tissue engineering	Zhang et al. (2006), Shalumon et al. (2009)
		Drug delivery	Fonte et al. (2011), Anitha et al. (2012)
	Cyclodextrins	Drug delivery	Kanwar et al. (2011), He et al. (2013), Lakkakula and Maçedo Krause (2014), Ruiz-Esparza et al. (2014)

(continued)

Table 10.1 (continued)

Classifications	Polymer	Nanomedical applications	References
Synthetic polymer			
Biodegradable			
Polyesters	Poly(lactic acid)	Tissue engineering	Hsu et al. (2011)
		Diagnostic imaging	Nottelet et al. (2015)
		Drug delivery	Fernandez-Fernandez et al. (2011)
	Poly(glycolic acid)	Drug delivery	Chen et al. (2019a), Yoshikawa et al. (2008)
		Tissue engineering	Patrascu et al. (2013)
	Poly(hydroxyl butyrate)	Tissue engineering	Asran et al. (2010)
		Drug delivery	Chaturvedi et al. (2015)
	Poly(ε-caprolactone)	Drug delivery	Wang et al. (2010)
		Tissue engineering	Duan et al. (2013)
	Poly(β-malic acid)	Drug delivery	Lee et al. (2006), Ljubimova et al. (2008)
Polyanhydrides	Poly(sebacic acid)	Drug delivery and nanovaccines	McGill et al. (2018), Yan et al. (2020)
	Poly(adipic acid)		
	Poly(terphthalic acid)		
Phosphorous-based polymers	Polyphosphates	Drug delivery	Alexandrino et al. (2014); Aljuffali et al. (2015)
	Polyphosphonates	Drug delivery	Monteil et al. (2018)
	Polyphosphazenes	Drug delivery and nanovaccine	Schulze et al. (2017)
		Diagnositc imaging	Hu et al. (2013)
Others	Polydihydropyrans	Drug delivery	Gupta et al. (2013)
	Polyacetals		
	Poly(vinyl) alcohol	Drug delivery	Kayal and Ramanujan (2010), Nadeem et al. (2016)
		Diagnostic imaging	Strehl et al. (2015)
		Tissue engineering	Bakhshandeh et al. (2011), Dattola et al. (2019)
Synthetic polymer			
Non-biodegradable			
Silicones	Polydimethylsiloxane	Drug delivery	Mishra et al. (2017)
	Colloidal silica	Diagnostic imaging	Tang and Cheng (2013), Lee et al. (2015)
		Drug delivery	Tang and Cheng (2013)
Acrylic polymers	Polymethacrylates	Drug delivery	Pool et al. (2017)
	Poly(methyl methacrylate)	Drug delivery	Cui et al. (2009), Lazzari et al. (2012)
Others	Poloxamer and poloxamine	Drug delivery	Csaba et al. (2005), Chiappetta et al. (2011)

Komi and Hamblin 2016). The water-solubility of chitosan is assured only via a homogenous deacetylation process, whereas a heterogeneous reaction would result in an insoluble water product (Younes and Rinaudo 2015). The variation in deacetylation and resulting solubility that gives chitosan a range of physical states with tunable porosity, biodegradability, and applicability. Chitosan is a modifiable polymorphic polymer due to its crystalline order in the presence of reactive amino functional groups with a positive charge and liberal hydroxyl groups. Chitosan is a basic natural polymer. Being able to customize chitosan derivates via carboxymethylation, acylation, esterification, alkylation, or the addition of further hydroxyl groups makes it very lucrative to utilize them as an antibacterial, non-cytotoxic, biocompatible and biodegradable materials for nanobiomedical applications (Pacheco et al. 2020).

Chitosan nanoparticles have the potential to be applied in multiple disciplines including diagnostic bioimaging and as drug delivery vehicles. Diagnostic bioimaging using chitosan nanoparticles has been used in the detection of cancer. These chitosan-based nanoparticles carry ligand targeting conjugates on their outer surface to permit binding to a specified target site that is primarily found on cancer cells. Folate receptors, which are overexpressed in cancer cells, are preferred target site for folic acid bound chitosan nanoparticles (Mathew et al. 2010). The diagnostic bioimage is a result of the correspondence of the nanoscale magnetic core that contains a quantum dot within the chitosan nanoparticle and fluorescent microscopy. Ligand targeting chitosan nanoparticles demonstrate target specificity and imaging accuracy required for the diagnosis of cancer (Mathew et al. 2010).

In drug delivery, chitosan nanocapsules can deliver a drug cargo entrapped in the center or located on the surface of the nanoparticles. This increases the bioavailability of the drug by increasing its circulation time and resulting interfacial interaction with the cancerous cells. However, without a ligand-targeted delivery system, lack of specificity occurs in regards to chemotherapy. The *N*,*O*-carboxymethyl chitosan nanoparticles have been used to systemically deliver an anti-cancer drug; 5-fluorouracil with a 65% drug entrapment efficiency (Anitha et al. 2012). Furthermore, chitosan-based solid lipid nanoparticles have been used as successful insulin drug carriers. Their physicochemical characteristics allow them to be very stable and mucoadhesive in the gastrointestinal tract. This improves the drug adsorption properties suitable for diabetes treatment (Fonte et al. 2011). Chitosan has also proven itself ideal for brain drug delivery bypassing the blood-brain barrier to deliver siRNA and resulting a halt of mRNA synthesis, which has the potential to inhibit HIV replication and thus be a preventive measure to HIV infections (Gu et al. 2017). These examples show the diversity of chitosan nanoparticle applications in the aspects of drug delivery for treating some diseases. Its modifiable physiochemical properties and high in protein and loading efficacy aid in maintaining the required stability for the application while providing an improved alternative to the conventional route of administration and dosage-related issues.

Chitosan has found its application in tissue engineering and regenerative materials as electrospun scaffolds that can provide a similar environment alike to the extracellular matrix, which is suitable for the cell seeding and support of multiple cell

types. Furthermore, the ability to optimize tensile strength, porosity, water, and oxygen permeability and surface area of electrospun chitosan nanofiber-based scaffolds enables the desired mechanical and structural properties that is crucial for meeting the requirement for tissue engineering and wound dressing purposes. The mechanically poor chitosan material was found to exhibit shape memory properties for bone regenerative purposes. This can be seen when chitosan is paired with a biomaterial such as bioactive glass, which aids in the mineralization and osteoblastic differentiation processes. Chitosan has been found to be highly potential for wound healing purposes due to its antibacterial and coagulating capabilities (Azuma et al. 2015; Correia et al. 2015; Madhumathi et al. 2009). Electrospun carboxymethyl chitin and poly(vinyl) alcohol blend scaffolds have shown to be suitable for human mesenchymal stem cell adhesion and proliferation throughout a three-dimensional scaffold (Shalumon et al. 2009). Chitosan-based tubular scaffolds for vascular tissue engineering introduce a specified route for cells to distribute and grow. Simultaneously, the chitosan material will swell and resist a blood pressure of 4000 mmHg (Zhang et al. 2006).

10.1.2 Cellulose

Synthetic and natural polymers have been widely used in nanomedical application because of its valuable properties (e.g. biocompatibility, biodegradability, mechanical properties, etc.). Figure 10.2 illustrates a schematic diagram of polymer applications in nanomedicine. Cellulose is known as a natural polysaccharide that is present in plant walls, bacteria, and tunicate. It is considered the most abundant and inexhaustible material. It consists of D-glucose units linearly joined by β-1,4-glycosidic bonds, which make it indigestible. Cellulose can be functionalized into multiple derivatives, including carboxymethyl cellulose, cellulose acetate, methylcellulose etc. that has potential use in nanomedicine. Cellulose exists in a microscale, known as microfibril cellulose, which consists of stacked cellulose chains held together by van der Waals and inter- and intra-chain hydrogen bonds between the oxygen and hydroxyl groups with crystalline and amorphous regions. However, this paragraph mainly focuses on nanoscale cellulose options. The crystalline segments resulted in a nanoscaled version known as nanocrystalline cellulose, which is subdivided into celluloses of nanocrystals, nanocrystallites, and nanowhiskers. Cellulose nano-based materials are inclusive of nanofibrillated cellulose, nanofibrils, nanocellulose, and bacterial nanocellulose (Klemm et al. 2011). The ability to utilize hydrophilic property and chemical-modification characteristics of cellulose on a nanoscale of a broad surface to size ratio enables an enormous potential for function optimization and application of these materials. Nanoscaled cellulose can also undergo carboxylation, oxidation, sulfonation, and acetylation, as well as grafting onto other polymers. Smaller crystallites have a better reactivity and solubility. In this manner, self-aggregation and phase transformations also need to be considered (Klemm

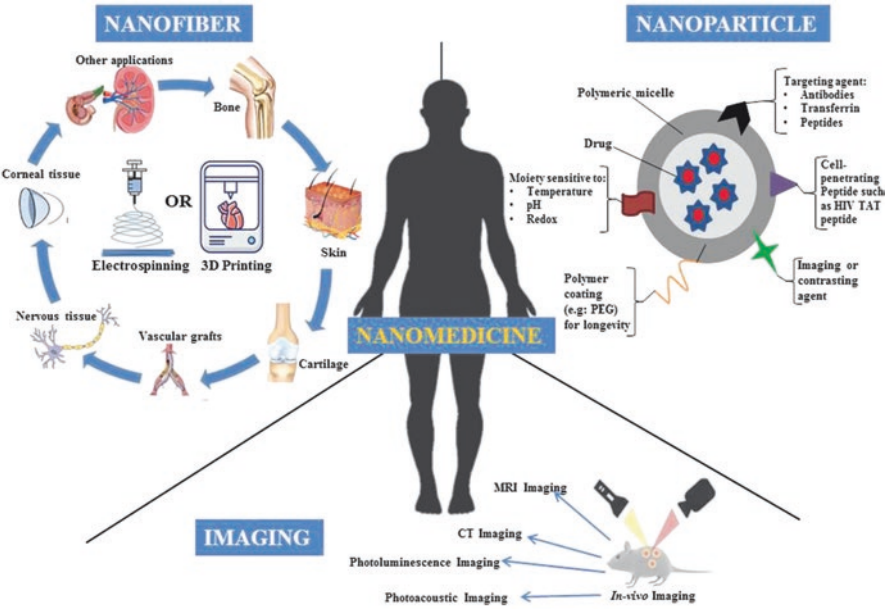

Fig. 10.2 Schematic representation of polymer application in nanomedicine

et al. 2011). Above-mentioned nano-scaled cellulose types find their nanomedical applications in tissue engineering, drug delivery, and bioimaging.

The following paragraph will elaborate on some examples of bioimaging using cellulose nanoparticles. Fluorescent polymer-based nanoparticle imaging is a medical diagnostics tool that is being actively explored. Nanocrystal cellulose has been well studied as a drug carrier targeting cancer and antibacterial therapies with a variety of water-soluble and insoluble drugs (Seabra et al. 2018). It has been used for targeted immunofluorescent imaging of brain cancer cells. Nanocrystal cellulose has been conjugated to folic acid to successfully prove that it functions as a drug delivery system as the overexpressed folate receptors on the cancer cells readily uptake the folic acid conjugated cellulose nanocrystal (Dong et al. 2014). Despite the lack of cytotoxicity of cellulose nanocrystals over the dermal and oral route of administration, the intravenous administration has yet to be cleared as such (Seabra et al. 2018).

Cellulose nanofibers exhibit a very foamy structure with high porosity since they are transformed into aerogel while they uptake the drug to act as a drug carrier. The cellulose nanofibres originated from bacteria or plant have the same chemical properties. This cellulose nanofibers have inherent mucoadhesive and tuneable swelling properties that can be exploited as drug release features (Bhandari et al. 2017). A forerunner for tissue engineering and wound dressings is filament-structured bacterial nanocellulose, which has been used in wound dressings consisting of bacterial cellulose and antibacterial acting zinc to support the healing progress of burn wounds (Khalid et al. 2017). Bacterial cellulose with high crytallinity has been

found to be very suitable for bone regeneration due to its characteristic of easing the mineral deposition within the scaffold and capability of enhancing the osteoblastic differentiation when incorporated with osteogenic growth factors and collagen or attachment associate (Saska et al. 2017).

10.1.3 Fibrin and Fibrinogen

Fibrin is a fibrous, non-globular protein chain that plays a crucial role in hemostasis and is formed as a result of the fibrinogen-thrombin reaction in the coagulation cascade. Advances in nanotechnology-based fibrin site-specific delivery can enhance the control of cardiovascular-related morbidity, mortality, and social impacts. This enhancement achieved by effectively binding nanomaterials to multiple constituents of atherosclerotic plaque, particularly fibrin, which remains a crucial part of the thrombus. With the availability of nanoscale investigative and treatment options to monitor blood clotting and fibrinolysis, indicating the thrombin binding and fibrin binding factor XIII activity, atherosclerosis as one of the leading causes of cardiovascular diseases can be detected promptly and contribute to a better prognosis. In atherosclerosis, the deposition of plaque on the artery walls can lead to narrowing and thrombi formation, which impose a considerable risk of suffering from myocardial infarction, emboli followed by ischemic stroke and other peripheral arterial diseases. Utilizing fibrin site-specific nanoparticles allows control over the interrelationship of anti-angiogenic drug delivery and the targeted angiogenesis of atherosclerosis. Lanza et al. (2006) utilized such fibrin ligand-directed perfluorocarbon nanoparticles, which are lipid-encapsulated emulsions of 200–250 nm used as a non-nephrotoxic molecular imaging agent. The nanoparticle can be used as a nanocarrier to anti-restenotic and anti-angiogenic drugs (Lanza et al. 2006). The resulting accessibility of thrombi detection and quantification via magnetic resonance and fluorine imaging and local anti-angiogenic therapy gives the ability to noninvasively magnetic resonance image and therapeutically manage the thrombi and related physiological complications (Morawski et al. 2004). The targeted intra-arterial thrombolytic activity has been investigated using perfluorocarbon nanoparticle coated with anti-fibrin monoclonal antibodies and fibrinolytic or thrombolytic enzymes, including urokinase and streptokinase (Marsh et al. 2007, 2011). Thrombi localization and related image specificity with reduced side effects has been observed through the utilization of copper oleate nanocolloids (Pan et al. 2011) or manganese oleate lipid emulsions (Pan et al. 2009) compared to the conventional gadolinium-based magnetic resonance imaging. Since thrombosis is a cancer-associated problem, fibrin-targeted imaging of cancer cell aggregates that manifest fibrin (Abdalla et al. 2014). Cancer can be targeted using fibrinogen-based nanoparticles through the two-step coacervation based calcium chloride crosslinked fibrinogen nanocarriers containing the anticancer drug 5-fluorouracil. Fibrin and fibrinogen are actively involved in angiogenesis and progression of the tumor growth (Rejinold et al. 2011). Thus adequate drug delivery to the tumor site is

expected due to the selectivity through manipulation of the nanoparticle size to meet the porosity of the endothelial permeability of blood vessels surrounding the tumor vasculature and increased vascular circulation duration.

Restoration of different types of tissue has been shown to be possible via fibrin and fibrinogen nanoscaffolds or scaffolds consisting of drug or growth factor loaded and unloaded electrospun nanofibers (Rajangam and An 2013). An increased cell seeding efficiency throughout a three-dimensional nano-textured fibrin or fibrinogen scaffold idealy mimics the natural cellular matrix to permit the regenerating cellular interactions within. The inclusion of specific growth factors widens the applicability of such scaffolds to aid in nerve tissue regeneration by filling nerve-conduits with uniformly distributed seeded cells to yield an increased distance of regenerate nerve length (Kalbermatten et al. 2008), bone and cartilage regeneration with improved adhesion and decreased loss of blood. Additionally, possible in delivering osteogenic growth factors (Marimuthu and Kim 2009), and vascular regeneration and wound healing through the incorporation of vascular endothelial growth factors incorporated in fibrin scaffolds with a sustained release to enable persistent angiogenesis (DeBlois et al. 1994; Sahni and Francis 2000).

10.1.4 Gelatin

Gelatin, a natural protein derived from partial hydrolysis of collagen, is extracted mainly from animal by-products such as pig skin (46%), bovine hides (29.4%), pig, and cattle bones (23.1%) and fish scales (≤1.5%). It has attracted a great interest due to being cheap, readily available, highly biocompatible and biodegradable, thus widely used in pharmaceutical and medical applications. Gelatin is manufactured from hydrolysis process of collagen. Collagen is known to have a high antigenicity due to its animal origin. Depending on the raw material used (source and age of the animal), collagen and gelatin does not have the same structure, composition, and properties. Hence, the complexity of the molecular heterogeneity of gelatin has caused a significant challenge in preparing a highly homogenous polymer formulation. Gelatin has extensively used in the bio-nano application due to its gelation properties. Gelatin can be used alone or either blended with different polymers for various uses, although ionic strength and pH play a significance in the mixture.

Gelatin has been used in drug and gene delivery in the form of microspheres and nanoparticles due to a high number of sites that can bind various target moieties. Numerous techniques have been employed in nanoparticles preparation with gelatin such as emulsification, solvent extraction, coacervation-phase separation, desolvation, nano-percipitation, layer-by-layer coating, reverse-phase micro emulsion and self-assembly (Berenstein and Russell 1981). Anjali and team demonstrated the preparation of gelatin grafted poly(acrylic acid) (gelatin-*g*-PAA) nanoparticles through polymerization process and encapsulating paclitaxel using microemulsion/nanoprecipitation method (Pal et al. 2018). In another study, iron oxide (Fe_3O_4) nanoparticles successfully fabricated with gelatin for paclitaxel, an anticancer drug delivery. The

Fe_3O_4 nanoparticles were coated with gelation conjugate by co-precipitation method. The paclitaxel was effectively loaded into the nanoparticles and showed a high drug loading efficiency (86.7 ± 3.2%) and a sustained release up to 5 days, indicating a possible potential application in drug delivery systems for cancer therapy (Nguyen 2017).

In a similar study, the Fe_3O_4 nanoparticles were synthesized using chemical co-precipitation in a simple single step. They utilized gelatin as a biopolymer coating to achieve hydrophilicity and conjugating capability with an anti-cancer drug, mercaptopurine (Sirivat and Paradee 2019). Li et al. (2013), investigated core-shell nanoparticles prepared based on gelatin with Fe_3O_4 and calcium phosphate for magnetic resonance imaging and anti-cancer drug delivery. The encapsulation of doxorubicin was performed by forming a shell over gelatin-Fe_3O_4 core using electrolytic co-deposition of calcium phosphate method. The formed nanoparticles delivered a favorable multifunctional nano-depot for therapeutics and diagnostics (Li et al. 2013). Another technique to prepare nanocomposites is by electrostatic interaction that involves interaction of two oppositely charged molecules, in which case would be negatively charged gelatin with positively charged molecules. Gaowa et al. (2014), studied the nanoparticle hydrogel that formed through electrostatic interaction with cationic epidermal growth factor receptor (EGFR)-targeted hybrid peptide (EGFR2R-lytic), which has an exceptional antitumor and cytotoxic activities and anionic gelatin. This complex nanoparticle displayed a more extensive circulation time-release kinetic studies and higher antitumor activity in treated mice. It revealed that the presence of gelatin has attributed in slow and controlled release of the peptide from the nanoparticle hydrogel (Gaowa et al. 2014).

10.1.5 Collagen

Collagen is the main structural and most abundant extracellular matrix animal protein found in various connective tissues in the body. The molecular structure of collagen mainly made up of amino acids that are bound together to form triple helix of elongated fibril known as collagen matrix. It generally found in fibrous tissues (tendons, ligaments, and skin). The biochemical features and the molecular structure of collagen have extensively reviewed in nanomedicine applications (Chattopadhyay and Raines 2014). Collagen increases the wound healing process by promoting the deposition of nascent large-diameter collagen fibers parallel to the fibers in the matrix leading to an increase in tensile strength in open dermal wounds. In a study, collagen nanofibers incorporated with silver nanoparticles were produced to evaluate the efficacy of wound healing on rat models (Rath et al. 2016). The results revealed an accelerated reepithelization, collagen production and better wound contraction with the nanofiber composites. Injectable collagen formulations vastly used in delivering growth factors for cellular regeneration and tissue repair. According to Huang and team, an injectable nano scaffold of hydroxyapatite/collagen incorporated with chitosan/β-glycerophosphate was investigated (Huang et al.

2009). The scaffold exhibited a good cell proliferation on bone marrow derived mesenchymal stem cells indicating a promising injectable device for bone tissue engineering. An extensive study of collagen has led to further understanding of the mechanism and kinetics of transports, cellular function, migration, proliferation, and differentiation of cells.

In a study, curcumin was incorporated in fish scale collagen/hydroxypropyl methyl cellulose nanogel for wound healing process (Pathan et al. 2019; Mitra et al. 2015). The ex-vivo permeation experiment exhibits that the nanogel has a higher percent contraction value compared to control group concluding the possibility of nanogel to be used in nanomedicine applications. The biodegradation of collagen has broadly examined by using metalloproteinases (MMP-1, MMP-2, MMP-8, MMP-13 and MMP14) and the high biodegradability of collagen makes it an excellent biomaterial for a variety of nanomedicine applications (Chattopadhyay and Raines 2014). Collagen has vastly been used in both its native and with other synthetic polymers to produce nanofibers using electrospinning process. A study of electrospun recombinant human collagen peptides (produced in lab, M_w 112 kDa, no source of the peptides mentioned) with chitosan nanofibers were prepared and revealed better fibroblast activities compared to control treatment in *in vitro* cell proliferation studies. Importantly, a rapid epidermidalization and angiogenesis were seen in a rat scalding model after treating with nanofibers. All these results showed that nanofibers of collagen could be an ideal candidate that can be used for wound healing applications (Deng et al. 2018). Another research was conducted on collagen with poly(epsilon-caprolactone) biomimetic nanofibrous scaffolds with crossed fiber organisation via electrospinning process for wound healing. The regulation of crossed nanofibrous scaffolds showed enhanced wound repair, which was evidenced with the accelerated migration of fibroblasts and keratinocytes that have promoted angiogenesis in diabetic rats. These outcomes revealed that the collagen biomimetic crossed nanofibrous scaffolds have potential for the repair of chronic wounds (Sun et al. 2018).

10.1.6 Casein

Casein is a family linked to phosphoprotein that commonly used in drug delivery applications in the form of micelles (100–200 nm). Casein usually found in mammalian milk, and there are four different casein phosphoprotein identified (αS1, αS2, β, and k-casein) (Zittle and Custer 1963). Casein can be bind to many different polymers due to the presence of several functional groups such as phosphate, carboxyl, and amino groups in the structure. The presence of hydrophobic and hydrophilic domains in the casein structure allows the opposite charge polymers to form nanoparticles, hydrogels, micelles, which utilized in nanomedicine applications (Lohcharoenkal et al. 2014). A recent work, casein nanoparticles were prepared using a simple coacervation method to evaluate the capability as an oral carrier and enhance the bioavailability for resveratrol (Peñalva et al. 2018). The

report says that according to *the in-vitro* study, the resveratrol release from casein nanoparticles was not affected by the pH conditions and followed a zero-order kinetic. When nanoparticles were administered orally to rats, the oral bioavailability of resveratrol from casein nanoparticles found to show a ten times higher than the control. In the field of nanotechnology, bioimaging based on nanoparticles is an added vital application. Bare iron oxide nanoparticles have revealed to be a superb magnetic resonance imaging (MRI) contrast agents, but unfortunately, the toxicity impedes its use in the biomedical field. Hence, casein has been considered just like other biopolymers to explore as a coating material of iron oxide for MRI contrast enhancement and efficient cellular targeting. Huang and co-workers prepared nanoparticles casein with polymaleate and octadecene co-polymers and encapsulated iron oxide together with doxorubicin (Huang et al. 2015). As a result of functionalization of casein over magnetic nanoparticles, it showed enhanced permeability compared with uncoated particles in *ex vivo* experiments. This could be attributed to the lack of a three-dimensional rigid structure of casein that causes changes in geometry and size that leads to energy-independent penetration of the plasma membrane by casein molecules.

Casein, as a versatile protein polymer, has been prominently examined for the use in the oral delivery of drugs due to its high affinity for binding ions and molecules plus its capability to self-assemble as micelles. However, the use of casein in the form of nanofibers for tissue engineering is relatively new. This offers a thought-provoking opportunity for scientists to formulate nanofibrous scaffolds for bone tissue regeneration purposes. Somya Selvaraj and the team have explored casein and polyethylene oxide to serve as a nanofibrous scaffold with silver nanoparticles for wound healing applications (Selvaraj et al. 2018). The fabrication of the nanofibers is achieved by using the co-electrospinning method. The results revealed that the presence of silver nanoparticles incorporated in casein nanofibers showed an excellent antibacterial property, and the nanofiber matrix itself exhibited outstanding biocompatibility with fibroblast cell proliferation. Casein phosphopeptide has been used in a study to prove that it could be used as nucleation sites for calcium ion binding to aid the formation of hydroxyapatite nanoparticles for bone tissue engineering (Fan et al. 2013). The outcome of this study discovered that the 3D scaffold has an exceptional osteoblastic performance due to the excellent biological properties of casein. The α-isoform of casein protein itself has acted as a tumor suppressor function by triggering the STAT1 signaling pathway and therefore preventing breast cancer (Bonuccelli et al. 2012). This shows that casein plays a vital part in the cellular homeostasis by sustaining cell growth and apoptosis.

10.1.7 Poly(Lactic Acid) (PLA)

Poly(lactic acid) or also known as polylactide (PLA) is a hydrophobic thermoplastic aliphatic polyester that is derived from renewable resources. The PLA exist in two types of optical forms: L-lactide and D-lactide. PLA can be prepared from lactide by ring-opening polymerization (Middleton and Tipton 2000). The physical properties and the biodegradability of PLA can be controlled by employing a hydroxyl acid co-monomer component or by racemization of the D- and L-isomers (Vroman and Tighzert 2009). PLA has widely used in nanomedicine applications due to its biodegradable and bioadsorbable properties. PLA nanoparticles developed as an anti-inflammatory drug vehicle for skin diseases such as psoriasis and atopic dermatitis (Boisgard et al. 2017). The best formulation is chosen depending on their physicochemical properties, penetration, and permeation capability into healthy and inflammatory skin. In another study, PLA nanoparticles were prepared to encapsulate bovine seminal ribonuclease using the adsorption method and demonstrated aspermatogenic and antiembryonal efficacy *in vivo* for the treatment of leukemia (Michaelis et al. 2000).

PLA been also widely used in theranostics applications. The nanoformulations could be used as MRI, optical imaging, photoacoustic imaging contrast agents. It is also concurrently be used as drug carriers, protecting the active ingredient from degradation, increasing tumor uptake through improved permeability, and enhancing the therapeutic benefits (Fernandez-Fernandez et al. 2011). These inventions tend also to enhance *in-vivo* cell marking, early diagnosis of disease, and image-guided therapy (Nottelet et al. 2015). In a study, theranostic nano-micelles prepared using PLA–poly(ethylene glycol)–poly(L-lysine)-diethylenetriamine pentaacetic acid (PLA–PEG–PLL–DTPA) and PLA–PEG–PLL-biotin with paclitaxel in the cores of the micelles and gadolinium ions chelated to the DTPA moieties for imaging (Liu et al. 2015). Biotinylated alpha-fetoprotein antibodies were linked to the surface of the micelle by a biotin-avidin reaction. These micelles ranging around 147.50 ± 4.71 nm, revealed a better result in cytotoxicity test and antitumor efficiency compared to free paclitaxel. The micelles showed a better imaging intensity (increased by 3×) and prolonged imaging time (from 1 to 6 h) compared to the control indicating a great potential in hepatocellular carcinoma theranostics (Liu et al. 2015). Artificial conduits restricted in peripheral nerve regeneration due to long lesion gaps that lead to scar formation, possibilities to collapse, and early resorption (Belkas et al. 2004). PLA has been widely explored in synthetic conduits due to their flexibility of mechanical properties, bioabsorbable and lack of antigenicity. A recent study demonstrated a nanofibrous conduit scaffold using PLA with single or multiple microchannels (Sun et al. 2012). The *in-vitro* result reveals a better protein absorption and cell adhesion with the nanofibrous conduit. An increase in the adhesion of PC12 rat cells and rabbit patellar fibroblast reveals the possibility to use for recovery of peripheral nerve damage. Many researchers have discovered the scaffolds utilisation as a substrate to support the differentiation of stem cells.

PLA nanofibrous mat were functionalized with peptide Tyr-Ile-Gly-Ser-Arg (YIGSR) and evaluated (Callahan et al. 2013). Several formulations of electrospun mats were produced (aligned-untreated, aligned-functionalized with peptide, random-untreated, random-functionalized with peptide). The aligned-functionalized with peptide nanofibrous scaffold increases the differentiation of mouse embryonic stem cells by expressing the neuron-specific class III β-tubulin, neurite extension, and gene expression for neural markers and increased the differentiation of mouse embryonic stem cells. Similarly, Wang and team has investigated the significance of aligned PLA nanofibrous scaffolds on embryonic E9 chick dorsal root ganglion cells and rat Schwann cells. The result shows that aligned scaffolds promoted the neurite extension parallel to fibers and aligned Schwann attachment and cell growth (Wang et al. 2008).

10.1.8 Poly(Glycolic Acid) (PGA)

Poly(glycolic acid) or polyglycolide (PGA) is aliphatic polyester that is vastly used in nanomedical applications due to its biodegradable and biocompatible properties. PGA is usually prepared using glycolic acid by ring-opening polymerization (Ikada and Tsuji 2000). PGA scaffolds could be a perfect model for the regeneration of vascularization, cartilage, and blood vessels in tissue engineering applications. Kobayashi and team investigated the nanocomposites of PGA and collagen and proved that within 5 days the nanocomposites were vascularized and completely occupied after the implantation in animal experiment (Kobayashi et al. 2013). Scientists have urged to seek out the potential use of PGA nanoparticles for drug delivery and drug targeting due to their high biocompatibility and biodegradability. Hsin and colleagues have investigated functionalized polymer nanoparticles of poly(γ-glutamic acid) with poly(lactic-co-glycolic acid) (γ-PGA-g-PLGA) incorporated with doxorubicin and indocyanine to overcome multidrug resistance (MDR) breast cancer in chemotherapeutic treatments (Chen et al. 2019a). The *in-vitro* study shows the multidrug resistance (MDR) cancer cells improved due to the cellular uptake of nanoparticles and this is due to the inhibition of P-glycoprotein (P-gp) activity by γ-PGA receptor-mediated endocytosis. After the photo-irradiation experiment, the nanoparticles show a synergistic effect of chemo and thermal therapy by reducing the MDR tumor growth in mice stating the effectiveness of the nanoparticle for human MDR breast cancer. Tomoaki has studied on PGA nanoparticles as a potential vaccine carrier to deliver antigenic proteins to antigen-presenting cells and inducing immune responses based on antigen-specific cytotoxic T lymphocytes (Yoshikawa et al. 2008). The subcutaneous immunization with PGA nanoparticles is capable of inhibiting the growth of ovalbumin transfected tumor in mice by entrapping the ovalbumin antigenic proteins into antigen-presenting cells more efficiently than control groups. There were no histological changes observed after the subcutaneous injection of the nanoparticles. The nanoparticle system

proved for possible antigen delivery that would able to progress for vaccines against cancer treatment (Yoshikawa et al. 2008).

10.1.9 Polycaprolactone (PCL)

Polycaprolactone (PCL) is biodegradable polyester that is synthesized by ring-opening polymerization of ε-caprolactone using tin (IV) oxide as a catalyst and heat (Middleton and Tipton 2000). PCL have been used in tissue engineering, drug delivery and medical implants. Jean and team have used polycaprolactone nanoparticles as an intracameral injection device for glaucoma treatment in rabbit model. The outcomes disclose that the device reduced intraocular pressure in normotensive rabbits for 23 weeks indicating an efficacious long-term glaucoma treatment and aid patience compliance rather than using eye drops multiple times in a day (Kim et al. 2018). Electrospun PCL with europium hydroxide nanorods was conducted by Augustine et al. (2017) for promoting vascularization. The scaffolds exhibited good endothelial cell adhesions in cell culture studies (Augustine et al. 2017). Enhanced angiogenesis was also noticed in the *in-vivo* chick embryo angiogenesis experiment. This was proven by protein expression study, where the phosphorylations of protein kinase B (Akt) and vascular endothelial growth factor receptor 2 (VEGFR2) proteins were increased indicating possible angiogenesis signalling mechanism. PCL scaffolds with europium hydroxide nanorods were effective to promote angiogenesis/vascularization in tissue engineering application (Augustine et al. 2017). In a study, a carboxylated PCL was synthesized and encapsulated exemestane for sustained targeted drug delivery through intravenous pathway. The nanoparticles shows a high percentage of drug entrapment and the drug release mechanism showed a sustained release, following Korsmeyer-Peppas model, demonstrating Fickian drug release (Kumar and Sawant 2013). This shows PCL nanoparticles are plausible for providing passive drug delivery.

10.1.10 Poly(Vinyl Alcohol) (PVA)

Poly(vinyl alcohol) (PVA) is a biodegradable, thermoplastic polymer that results from the alkaline hydrolysis of polyvinyl acetate. Its hydrophilicity, which is based on the distribution of hydrogen bond donating hydroxyl groups and crystallinity, allows PVA to be employed as swelling and pH-independent solubility agent. These characteristics enable nanoparticles containing either water-soluble and insoluble drugs to be tuned for intended drug release profiles (Brough et al. 2016). PVA is a non-toxic, non-carcinogenic synthetic polymer that has found several applications in nanomedicine.

Nanoparticles containing PVA have been used for targeted drug delivery. PVA is implemented as a coating material to nanoparticles for the improved delivery of

nanocarriers such as magnetic iron oxide nanoparticles containing the anti-cancer drug doxorubicin (Kayal and Ramanujan 2010; Nadeem et al. 2016). Here, nanocarriers with a controlled percentage of PVA coating yield the desired surface functionalization for prolonged circulation, and increased drug loading efficiency can be achieved without compromising the magnetically targeted drug delivery. In a water-based medium such as blood, the inert backbone and the abundant hydroxyl groups found in PVA polymers allow them to act as a protective capping agent of the biologically active drug from breakdown or early absorption as the reticuloendothelial system would shorten the nanoparticle half-life. PVA coated nanoparticles are able to disguise to these natural defence mechanisms of the human body by mimicking carbohydrate surfaces through the non-crosslinked hydroxyl groups found on the synthetic PVA polymer (McBain et al. 2008). This aspect aids in overcoming the changes that the nanoparticles otherwise may undergo while traveling through the human body to the target site of the drug delivery system. Another advantage of including PVA in a therapeutic nanocarrier is its mucoadhesive nature. Considering that nanoparticles that are utilized to target mucosal surfaces for rapid drug absorption, a PVA coating can aid in a rapid absorption to yield uniform drug distribution with an extended retention time within the mucus. This applies particularly to partially hydrolyzed PVA (Popov et al. 2016). In the utilization of a synthetic polymer such as PVA nanoparticles, biocompatibility plays a crucial role in their application, whether for bioimaging for diagnosis or therapeutic purposes. By coating superparamagnetic iron oxide nanoparticles with PVA, diagnostic imaging of the autoimmune disease; rheumatoid arthritis has shown that there is no overall cytotoxic effect on human immune cells, but had a change in cytokine secretion (Strehl et al. 2015). When designing a nanoparticle-containing PVA, the possible physiological responses towards it should be put into consideration when intending its application despite the overall biocompatibility of PVA.

PVA exhibits the tensile strength, flexibility, and degradation stability required for the application of fibrous scaffolds in several disciplines (Kumar and Han 2017). It has been suitable for the production biocompatible artificial cornea due to its transparency allowing the transmittance of light to allow the capability of sight while providing fitting mechanical properties (Bakhshandeh et al. 2011). Furthermore, PVA electrospun nanofibers show satisfactory parameters of biocompatibility, growth factor inclusion, mechanical stability, and porosity for bone, cartilage, skin, nervous, and vascular tissue engineering (Teixeira et al. 2020). In regards to cardiac tissue engineering, three dimensional PVA scaffolds have not yet proven to mimic the cardiac extracellular matrix perfectly. Yet, they do provide the required conditions for cardiomyocytes adherence, differentiation, and growth since PVA has elevated cytocompatibility (Dattola et al. 2019).

10.2 Conclusion

The utilization of polymer-based nanoparticles opens a new frontier into research and nanomedical applications. The science of building nano sized materials is changing the diagnostic and therapeutic approaches on cellular and genetic level for a variety of diseases including cancer, or even cardiovascular related conditions. Polymer-derived nanoparticles and nanofiber-based scaffolds or tissue grafts are found to exhibit improved safety levels for the application on the human body through precise bioimaging, tissue regeneration and drug delivery applications with enhancement of the drug distribution and bioavailability, and minimized adverse effects of the treatment. Aside from ongoing research on polymer-based nanoparticles more than 40 nanomedicines of the categories nano crystals, nanoparticles, virosomes, etc. have been proved by the FDA. The utilization of polymeric nanomaterials combines the known attributes of polymers and nanoscale medicine together to stride towards specific and personalized medical approaches.

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Chapter 11
Delivery of Drug Payloads to Organs and Organ-Systems

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11.1 Introduction

The advancement of nanotechnology has boosted the development of new and modified medicine in treating various human diseases and disorders, particularly in cardiovascular diseases and cancers, which are the two main death-causing killers globally. Nanomaterials are defined as a material with sizes of 1 and 100 nm. Owing to the diverse properties and types of nanomaterials, there is a huge potential utilising them to modify and improve the current drugs and therapeutic strategies. These include enhancing drug effectiveness, increasing target specificity, improving drug stability and bioavailability, increasing body absorption, and minimising off-target cytotoxicity. To achieve the abovementioned drug activities, the responses of the targeted cells, tissues, organ and subsequently the organ system play important parts. Unquestionably, the drug responses could also largely be affected by the pathophysiology and surrounding micro-environments of the diseased models or organs. *Vice versa*, the drug payloads may also contribute to the alterations in the routine biological processes of the organs or may render toxicity to the target organ or organ systems. This chapter aims to discuss the current nano-systems used for drug delivery and target specificity as well as to provide insights to improve the drug selectivity, safety profile and therapeutic efficacies. The overview of this chapter is depicted in Fig. 11.1.

11.2 Utilization of Nanosystems for Drug Delivery

Nanomaterials have been extensively studied for their biological and pharmacological activities including their use as anti-cancer and anti-microbial agents, and as drug enhancers (Barabadi et al. 2017; Ogunsona et al. 2020; Patra et al. 2018). Another prominent use of these nanomaterials is to serve as a nano-carrier for drug delivery for treating various diseases. Generally, the nano-based delivery system can be categorised into several types: metallic, polymeric and biodegradable delivery systems (Patra et al. 2018). This section discusses different type of nano-systems based on their potential advantages and limitations.

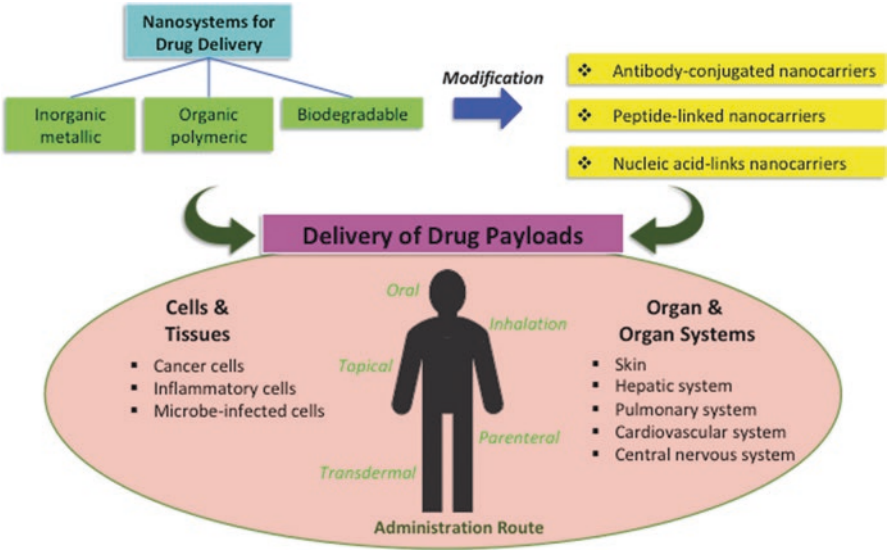

Fig. 11.1 Overview of drug payloads delivered by modified nanosystems into various tissues and organ systems through different routes

11.2.1 Inorganic Metallic Nanosystems

Inorganic nano-systems are generally comprised of metallic and derivatives of silica compounds. Metals in the form of nanoparticles (NPs) are good targeted drug delivery systems (DDS) as they can be modified with different functional groups, allowing them to bind to drugs, molecules, and antibodies (Patra et al. 2018). Silver, copper, and iron are the common types of metal NPs, with gold being the most extensively studied NPs. Other metals such as zinc oxide, titanium dioxide, platinum and palladium are among the many to be explored as NPs in biomedicine (Patra et al. 2018).

Gold (Au) and silver (Ag) NPs are known to be the most important in biomedical applications. AuNPs and AgNPs are extensively used in medicine owing to their inherent optical, chemical, physical, and electrical properties (Lee et al. 2020; Burdusel et al. 2018). The high versatility and biocompatibility allow their uses as anti-microbials, anti-cancers, and drug delivery vehicles (Lee et al. 2019; Teow et al. 2018). One of the most common application of these nanoparticles is in cancer therapy. In many cases, chemotherapeutic drugs are administered systematically due to tumour metastasis and the lack of efficient drug delivery system. This has led to the study of site-targeted anti-cancer drugs delivery (ACD) using AuNPs and AgNPs. Examples of the success of ACD delivery using AuNPs (Farooq et al. 2018; Brown et al. 2010) and AgNPs (Naz et al. 2017; Benyettou et al. 2015) have been recently reported. Similarly, targeted antimicrobial therapy using these metallic NPs conjugated to drugs have also shown to be effective against a wide range of bacteria

(Yahyaei and Pourali 2019; Kaur and Kumar 2019). Contributions have also been made in delivering anti-inflammatory molecules and cytokines to targeted site (Roome et al. 2019; Baganizi et al. 2017).

Despite the wide range of biological and pharmacological activities of metallic NPs, numerous studies have reported the cytotoxicity of these nano-systems. A review by Yao et al. (2019) demonstrated the liver damage caused by various metallic NPs at cellular and subcellular levels. Similarly, Pacheco and Buzea (2018) highlighted the biomedical use of various metallic NPs which could penetrate into the cells and adversely affect the immunomodulatory cellular processes and hence the immune system.

11.2.2 Organic Polymeric Nanosystems

The term organic in biology and chemistry refers to molecules or compounds constituting carbon and hydrogen, and are often accompanied by oxygen or nitrogen. Organic compounds include important building blocks of biological structures such as lipids, nucleic acids, proteins, and carbohydrates. Hence, the use of organic nanocarriers (ONs) as DDS offers high biocompatibility. Furthermore, the complex chemical and physical properties of ONs allow simultaneous loading of hydrophilic and hydrophobic drugs (Xu et al. 2016). Conventionally, ONs can be produced through the self-assembly or synthesis routes. The self-assembly route generally produces polymeric amphiphilic systems such as micelles and liposomes and vesicle formation (Lombardo et al. 2019). The synthetic route produces dendrimers, nanogels, carbon nanotubes and hyperbranched polymers (Ali et al. 2020; Singh et al. 2019). Currently, the new generation ONs are constructed through a combination of the two methods through supramolecular chemistry (Lombardo et al. 2019).

Several ON-based platforms for drug delivery have been approved by Food and Drug Administration (FDA) for the treatment of cancers such as leukaemia, metastatic pancreatic cancer, and ovarian cancer (Vieira and Gamarra 2016). Other applications such as delivery of drugs against various infections such as bacterial, fungal and viral infections have also been FDA-approved (Ventola 2017). Owing to the success of liposomal drug delivery since the 1980s, many clinical trials are currently ongoing (NCT03076372, NCT03168061, NCT02833766, NCT03669562).

Compared to metallic NPs, some polymeric NPs can be more cytotoxic depending on the synthesis method and the choice of stabilizers such as poly(vinyl alcohol) and solvents such as dichloromethane and acetone. Hence, less toxic reagents are usually prioritized to construct synthetic or natural polymeric NPs such as the use of chitosan and gelatin. Some of the toxicities exhibited by polymeric NPs are toxic degradation, toxic monomers aggregation, and residual material associated with them (Yang et al. 2017).

11.2.3 Biodegradable Nanosystems

Biological nanoparticles and polymers are biodegradable systems based on naturally occurring structures involved in the physiology of the host organism. The intricacy and physiological functions of such complexes are stringently conserved by evolutionary stress, thereby making them ideal vehicles for drug delivery in living systems (Fathi and Barar 2017). Among the synthetic biopolymers used as DDS, hydrophobic polymers such as poly(lactic acid) (PLA) and poly(lactic-co-glycolide) (PLGA) paired with the hydrophilic polyethylene glycol (PEG) to form amphiphilic copolymers are widely used due to their inherent features such as high biocompatibility, tunability of biodegradation, and low toxicity (Su and Kang 2020). This has led to FDA-approval for the use of PLA and PLGA as drug nanocarriers (Ventola 2017). Currently, the effectiveness of PEG-PLGA amphiphilic copolymer in particular as an effective and circulation-stable DDS in humans is extensively explored, where therapeutic agents encapsulated in the PLGA hydrophobic core is protected from cellular assaults such as phagocytes. This is exemplified in a recent study where therapeutic molecules and macromolecules encapsulated by PLGA maintained colloidal stability even in the stringent microenvironment of the diseased tissues (Castillo-Santaella et al. 2019).

In recent years, the use of natural biopolymers as DDS include chitosan, alginate, pectin, cellulose, xanthan gum, dextran, hyaluronan, and heparin (Lombardo et al. 2019). Chitosan-based DDS is one of the most commonly investigated and is generally used for the slow release of drugs particularly in tight epithelial junctions, which includes organs such as the intestine, eye, ocular, nasal, and buccal region (Li et al. 2018; Duttagupta et al. 2015). Chitosan-based DDS have been shown to be more effective when they are prepared in conjunction with other biopolymers such as alginate, cellulose, folic acid and xanthan (Abd Elgadir et al. 2015). Like other DDS, natural biopolymers are effective at delivering anti-cancer, antimicrobial, and other therapeutic agents while being eco-friendly to reduce pollutions to the environments (Patra et al. 2018). Comparatively, they are less toxic to the target cells or organs than metallic and organic polymeric nanosystems due to the biodegradable nature.

11.3 Modified Drug Nanocarrier for Targeted Delivery

In general, unmodified nanomaterials do not have specific targets, and same applies to the nano-based drug carrier. Cumulative evidences showed that depending on the administration route, the nano-carriers are delivered through blood stream or tissues and eventually cleared in the kidney after the absorption into the body (Longmire et al. 2008). To ensure that the drug is pharmacologically active and remain circulating in the blood, higher dosage will be required to compensate the loss of the nanocarriers with drugs from the complex biological processes in the human body. This

may lead to the unnecessary off-target cytotoxicity (Din et al. 2017). On the other hand, nanocarrier which possess high kidney retention could also be toxic to the kidney and other organs of human body (Kamaly et al. 2016). To circumvent this problem, target-specific nanomaterials have been made by various methods to deliver the drugs to the target tissues or organs (Patra et al. 2018; Attia et al. 2019). This section focuses on the development of modified nanomaterials to allow target-specific drug delivery. Some of the molecules used for cell targeting include antibodies, site-specific peptides, nucleic acid and other ligands and small molecules (Din et al. 2017).

11.3.1 Antibody-Conjugated Nanocarriers

Antibodies play vital roles in fighting pathogens and are present in high-level organisms with an adaptive immune system. Other than neutralizing pathogens and abhorrent cells, antibodies also mediate the recruitment of effector immune cells. The application of antibodies is invaluable by virtue of the high structural integrity and versatility of antibodies in binding to a wide range or extremely narrow range of targets. Antibody-mediated therapies are currently in trend for the treatment of cancers (Cruz and Kayser 2019), autoimmune diseases (Hafeez et al. 2018) and infectious diseases (Awi and Teow 2018; Che Nordin and Teow 2018) as antibodies are manufactured specifically by living cells against very specific antigens, resulting in high biological specificity by acting as 'homing missiles'. With this high specificity and affinity, antibody has also been adopted along with drug-loaded nanocarrier to improve the therapeutic efficacy and to enhance target specificity primarily in cancer therapy (Din et al. 2017).

Pirollo et al. (2007) utilised an anti-transferrin receptor (TfR) antibody-modified nanoimmunoliposome to deliver the therapeutic small interfering RNA (siRNA) to specifically target and inhibit tumour growth in pancreatic cancer. In another study, Heister et al. (2009) engineered a single-walled carbon nanotubes (SWCNTs) with doxorubicin, a carcinoembryonic antigen (CEA)-targeting antibody and a fluorescent marker to improve the specificity and therapeutic effect in colon cancer cells. Using the same approach, prostate-specific membrane antigen (PSMA)-targeting antibody was also conjugated with apoferritin nanocarrier loaded with doxorubicin for prostate cancer treatment (Dostalova et al. 2016). More recently, Xu et al. (2018) constructed a programmed death-ligand 1 (PD-L1) antibody-conjugated docetaxel-loaded NPs for *in vitro* gastric cancer cell treatment. In addition to targeted cancer therapy, antibodies have also been conjugated to nanocarrier or nanoparticles to treat microbial infection although most of the antibody-modified nanosystems are used to enhance the detection sensitivity of the microorganisms for diagnostic application (Tripathi and Driskell 2018). For example, intercellular adhesion molecule 1 (ICAM1) antibody was conjugated to an antibiotic nanocarrier to treat drug-resistant *Klebsiella pneumoniae*-infected pneumonia mice (Kang et al. 2019).

11.3.2 Peptide-Linked Nanocarriers

Peptides and proteins are structures of linked amino acids via peptide bonds, with peptides generally being shorter in length with approximately 2–50 amino acids. Both have been actively used for nanocarrier conjugation to improve diagnostic and therapeutic applications. Comparatively, peptides have several advantages over antibodies or proteins which are generally limited by their high molecular weight to effectively penetrate into the cells as well as shorter lifespan owing to enzymatic degradation and immune response (Solaro et al. 2010). Hence, the therapeutic use of antibodies and large proteins are only restricted to vascular targets on the luminal side of tumour vessel endothelia or haematological cancers (Zhang et al. 2012a, b). In addition to their small size, peptide can be easily synthesized and selectively deliver the conjugated 'cargoes' coupled with the nanocarriers to the target cells or organs. Other advantages of using peptide-based nanocarriers include high biocompatibility, high drug loading capacity and high chemical diversity (Tesauro et al. 2019).

For target-specific therapy against glioblastoma, Agemy et al. (2011) constructed a nanosystem using tumour-homing and proapoptotic peptides and iron oxide nanoparticles. While enhancing the tumour-penetrating efficiency, the nanosystem successfully delivered the payloads to mitochondria of brain tumour endothelial cells and induced apoptosis (Agemy et al. 2011). Following this study, the same research group identified a new modified peptide designated as Lin TT1 which could escape the non-target-specific entrapment (Sharma et al. 2017). Lin TT1 was able to deliver the nanoparticles to the tumour cells and effectively kill the cells in breast cancer mice model (Sharma et al. 2017). In a diabetes study, insulin-loaded nanoparticles were conjugated with a targeting peptide known as CSK followed by a pharmacological study using diabetic rat models (Jin et al. 2012). The modified nanosystem enhanced the cellular uptake and bioavailability of the nanoparticles, and resulted in higher level of inhibition in the blood glucose level (Jin et al. 2012). Although peptides could enhance various properties and pharmacological effect of nanocarrier, some limitations remain. These include the poor stability, short half-life and susceptibility to enzymatic degradation like proteins (Zhang et al. 2012a, b).

11.3.3 Nucleic Acid-Linked Nanocarriers

Over the years, nucleic acid including DNA, RNA and aptamers-oligonucleotide molecules that bind to specific target molecules, have also been utilised to modify and improve the functionality of nanocarriers. Some of these molecules possess therapeutic activity such as gene silencing and regulation which result in combination effect after their conjugation with the target nanosystems while others are responsible for the target specificity and cell-penetrating capacity of the nanosystems (Gracyzk et al. 2020). For instance, the gold nanoparticles conjugated with

RNA successfully penetrated into the blood-brain-barrier (BBB) and resulted in tumour inhibition in glioblastoma (Jensen et al. 2013). In another study, Zheng et al. (2012) conjugated the gold nanoparticles with siRNA targeting epidermal growth factor receptor (EGFR) which resulted in the accumulation of nanoparticles on the skin followed by regression of the skin diseases using the mouse model.

Similarly, aptamers which are short single stranded DNA have also been used to guide the nanocarriers to target cells. For example, aptamer-conjugated doxorubin-loaded DNA nanoparticles have demonstrated potent inhibition on cancer cells (Chang et al. 2011). Kim et al. (2010) also constructed aptamer-conjugated gold nanoparticles for both imaging and treatment of prostate cancer. In another study, Xiao et al. (2012) conjugated a polymeric nanoparticle with RNA aptamers to enhance the target specificity and improve the cancer growth inhibition in prostate cancer.

With advent of current nanotechnology, nanocarriers can also be linked with other types of ligands such as ascorbate/vitamin C, folate, transferrin, carbohydrate and glycoprotein to improve their functionalities. For example, ascorbate-conjugated nanocarrier have been reported to improve targeting and inhibition in glioma cells (Salmaso et al. 2009) as well as protecting cells from oxidative stress at low doses (Chakraborty and Jana 2017). Folate-conjugated nanocarrier have also shown improved cellular uptake and therapeutic efficacies in breast cancer (Hemati Azandaryani et al. 2017), cervical cancer (Wei et al. 2017) and lung cancer (Tan and Wang 2018). Similarly, transferrin-conjugated nanosystems have also shown promises in various cancers including breast (Soe et al. 2019) and lung cancers (Tan and Wang 2018).

11.4 Delivery of Drug Payloads to Various Cell Types and Organs

In addition to the therapeutic capability of nanocarrier, the drug efficacy is also largely affected by the drug payloads and host response. As different cells, tissues and organs function differently, their responses towards the nano-carriers may also vary. As the nano-systems are seen by the host as ‘foreign intruder’, immune activation of the host system depending on the type of nanomaterials is anticipated. This immunogenicity may lead to several complications including cell apoptosis, tissue necrosis and even organ failure (Yang et al. 2017). In general, drug payloads can be delivered to the target mainly through energy-dependent endocytosis which include phagocytosis, micropinocytosis, clathrin- and caveolae-mediated endocytosis (Nelemans and Gurevich 2020). This section summarises the drug payloads delivery into various types of diseased and infected cells as well as in different organs and organ systems with reported non-specific toxicities.

11.4.1 Cells and Tissues

11.4.1.1 Cancer Cells

As abovementioned, drug nanocarriers have been extensively studied for cancer therapies (Vieira and Gamarra 2016) and many have been approved by FDA for clinical use (Ventola 2017). As highlighted in the previous section, target specificity of nanocarrier has been greatly improved through conjugation with various molecules including antibodies, homing peptides and aptamers. Anticancer drug payloads such as doxorubicin (Soe et al. 2019; Wei et al. 2017), oxaliplatin (Brown et al. 2010), letrozole (Hemati Azandaryani et al. 2017), paclitaxel (Yao et al. 2018), cisplatin (Tan and Wang 2018) and so on have been successfully delivered to the target cells and resulted in enhanced therapeutic efficacies in various solid tumours. Yao et al. (2018) demonstrated that the paclitaxel-loaded nanocarrier entered the lung cancer cell line A549 via lipid rafts in energy-dependent manner. Similarly, paclitaxel was also delivered by polymeric micelles into Caco-2 colon cancer (Huo et al. 2010), A2780 ovarian cancer (Xiao et al. 2011) and MCF7 breast cancer (Wang et al. 2017) cell lines via energy-dependent clathrin- and caveolae-mediated endocytosis.

11.4.1.2 Inflammatory Cells

Drug-loaded nanocarriers have also been shown to treat arthritis which is associated with inflammation of joints and cartilages. Zhou et al. (2010) demonstrated that the fumagillin nanoparticles could enhance the anti-inflammatory effects of methotrexate when they were used in combination in treating arthritis in mice. Similarly, Jeon et al. (2016) reported a methotrexate-loaded hyaluronic acid-5β-cholanic acid nanocarrier that was delivered to inflamed joints in collagen-induced arthritis mice to treat the inflammation. In another study, PLGA/PLA/PEG copolymers were loaded with betamethasone phosphate and was shown to successfully reduce the inflammatory arthritis in rat models (Ishihara et al. 2009). In osteoarthritis treatment, a PEGylated dendrimer nanocarrier conjugated with insulin-like growth factor 1 (IGF-1) have also shown success in rescuing cartilage and bone in rat models (Geiger et al. 2018).

11.4.1.3 Microbe-Infected Cells

The success of utilising nanocarrier in delivering drugs toward cells infected by various pathogens has also been proven. Clemens et al. (2012) demonstrated the delivery of rifampin using mesoporous silica nanoparticle to *Mycobacterium tuberculosis*-infected macrophages which successfully killed the pathogens. Ranjan et al. (2009) reported a nanostructure which was loaded with gentamicin effectively

killed *Salmonella enterica*-infected macrophage cells. In addition to targeting bacterially infected cells, nanosystems have also been used to target virus-infected cells. For instance, Dutta and Jain (2007) reported that a lamivudine-loaded mannosylated poly (propyleneimine) dendrimer was able to enhance the cellular uptake of lamivudine and antiviral inhibition in HIV-1infected MT2 leukemic T-cells. Similarly, PEG-based polymeric carrier loaded with saquinavir was delivered to HIV-1 infected MT-2 cells and significantly improved the cellular uptake and antiviral action (Gunaseelan et al. 2004; Wan et al. 2006).

11.4.2 Organs and Organ Systems

11.4.2.1 Skin

Skin is the major organ of human beings, nanosystems have been reported to deliver into various disease skin models such as melanoma, microbially infected and chronically inflamed skins (Gupta et al. 2012). The application of nanocarrier on skin has been extensively studied at dermal, follicular and transdermal drug delivery (Vogt et al. 2016). For instance, poly(lactide-co-glycolide) nanoparticles was shown to deliver flufenamic acid to the human skin and improved penetration efficacy was observed (Luengo et al. 2006). In another study, a microemulsion-based hydrogel (MBH) loaded with itraconazole was shown to improve the skin permeation through *in vitro* permeation assay using Franz diffusion cells and hairless mouse skin (Lee et al. 2010). Patel et al. (2011) reported that a microemulsion loaded with antifungal ketoconazole could effectively penetrate into a rat skin and exert inhibitory action against *Candida albicans*. More interestingly, the microemulsion was stable up to 3 months. Bacchav et al. (2011) constructed azole-based micelle nanoformulation and successfully showed enhanced delivery into human skins. In another study, micelle carrying nadifloxacin was shown to effectively deliver the drug into Yucatan micropig skin (Inoue et al. 2017).

11.4.2.2 Pulmonary System

Various nanocarrier have been shown to target human lung for treating cancers or other pulmonary diseases. For example, the use of nanoformulation loaded with doxorubicin (Otterson et al. 2010), docetaxel (Ernsting et al. 2012), cisplatin (Wittgen et al. 2007) and paclitaxel (Latimer et al. 2009) for lung cancer therapy has been previously reported. Most of these are being evaluated in clinical trials (Van Rijt et al. 2014). To further improve the target specificity, EGFR-targeting ligands have been conjugated with the nanosystem for drug delivery as EGFR is highly expressed in lung cancer tissues. For example, it was shown that EGFR ligand conjugated gelatin-based nanosystem (Tseng et al. 2009) and PEG-based carbon nanotube (Bhirde et al. 2010) could deliver cisplatin effectively to the target site using

mice model. In addition to lung cancers, similar therapeutic strategy has also been employed against lung diseases and infections. For instance, polymeric micelles containing budesonide were able to reverse the inflammation in bronchoalveolar lavage fluid in asthmatic rat model (Sahib et al. 2011) while polymeric nanoformulation containing steroid was able to reverse the airway inflammation (Matsuo et al. 2009). In targeting and controlling microbial infection in lungs, nanocarriers containing various drugs such as ciprofloxacin (Bruinenberg et al. 2010), amikacin (Okusanya et al. 2009), amphotericin B (Slobbe et al. 2008) and bortezomib (Vij et al. 2010) have shown promises using animal models.

11.4.2.3 Hepatic System

Liver plays an important role in detoxification and drug metabolism. To treat liver cancer, doxorubicin was delivered using gold nanocage-based nanosystem and antitumour effect was observed both *in vitro* and *in vivo* in mice models (Ji et al. 2018). Similarly, Tian et al. (2019) also co-delivered polyethylene glycol-polyetherimide (PEG-PEI) based nanosystem containing doxorubicin and Bcl-2 siRNA to liver which exhibited improved cellular uptake, higher cellular apoptosis and higher antitumour effect. In another study, Wu et al. (2018a) constructed a SP94 peptide-conjugated PEGylated liposomal doxorubicin for liver cancer treatment in xenograft mouse models. Recently, Chakraborty et al. (2020) conjugated a nanocarrier containing paclitaxel with aptamer L5 and killed liver cancer cells via mitochondrial-dependent apoptosis. The nanocarrier internalised into the cells via clathrin-mediated endocytosis and more importantly, the nanocarrier did not show any toxic effects in healthy hepatocytes. Drug nanocarrier have also successfully delivered to treat other liver diseases such as liver injury and fibrosis (Bartneck et al. 2014). For instance, a dexamethasone-loaded liposome could reduce the liver injury and fibrosis as well as hepatitis by mainly reducing the immune active T-cells in the mice models (Bartneck et al. 2015). On the other hand, Yang et al. (2014) loaded oxymatrine which has anti-hepatic fibrosis effect into RGD peptide-modified poly(ethylene glycol)-b-poly(ε-caprolactone) (PEG-b-PCL) to treat hepatic fibrosis. Interestingly, the nanocarrier was able to suppress the hepatic stellate cells and the effect of hepatic fibrosis was attenuated in rat models. In another study, a micelle containing berberine was shown to accumulate in liver and was able to ameliorate the metabolic disease using mice models (Guo et al. 2019).

11.4.2.4 Cardiovascular System

Advantageous properties of nanocarrier particularly on target specificity has also benefited treatment of cardiovascular diseases such as atherosclerosis, hypertension and myocardial infarction (Deng et al. 2020). For instance, cyclodextrin-based nanocarriers loaded with rapamycin were shown to have potent anti-atherosclerotic activity by inhibiting the macrophage proliferation and suppressed the foam cell

formation in mice models (Dou et al. 2016). In another study, anti-atherosclerotic action was also seen in simvastatin-loaded polylactic co-glycolic acid (PLGA) nanocarrier by targeting the macrophages (Zhang et al. 2017). In hypertension treatment, various drug-loaded nanocarriers such as aliskiren-loaded PLA (Pechanova et al. 2019), lacidipine-loaded niosomes (Qumbar et al. 2017) and valsartan-loaded liposome (Ahad et al. 2016) have been successfully delivered to hypertensive rats and potent protective effect was observed. Other than that, several studies have also reported the great potential of using nanocarrier for myocardial infarction (Deng et al. 2020). For example, methotrexate-loaded lipid core nanoparticles and VEGF (vascular endothelial growth factor)-loaded liposome were reported to have protective action on myocardial infarction using the rat models (Maranhao et al. 2017; Scott et al. 2009). Similar effect was also observed when IGF-1-loaded PLGA and VEGF-loaded PLGA NPs were tested using infarcted mice models (Chang et al. 2013; Oduk et al. 2018).

11.4.2.5 Central Nervous System

Over the years, discovery of novel therapy against central nervous system (CNS) disorders has always been challenging, particularly on the brain in which the drug delivery is highly limited by the blood-brain barrier (BBB). Recently, the emergence of various type of nanocarrier has given hope as one of the therapeutic modalities to overcome this challenge (Alexander et al. 2019). For example, Wohlfart et al. (2011) demonstrated that doxorubicin-loaded PLGA nanocarrier could penetrate through the BBB and exerted its anticancer effect in rat models. In another study, Nance et al. (2014) showed that paclitaxel-loaded PLGA-co-PEG nanocarrier could effectively delay the growth of brain tumour through a potent brain tissue diffusion. Zhang et al. (2012a, b) also conjugated a paclitaxel-loaded polyphosphoester hybrid micelle with transferrin which enhanced the BBB uptake followed by potent anti-tumour effect in brain cancer. Other than brain tumour, other neurodegenerative diseases such as stroke, Alzheimer's and Parkinson's disease could also be targeted by drug-loaded nanocarriers primarily through enhanced uptake in BBB (Saraiva et al. 2016).

11.5 Advanced Modifications of Nanocarrier and Potential Challenges

While utilisation of target-specific molecules and ligands could improve the target specificity and therapeutic efficacy, there are still several other challenges in utilizing the nanocarriers for clinical use such as non-uniform size, lack of stability, inconsistent drug loading and release, and safety profile (Patra et al. 2018). To these, numerous strategies can be adopted to circumvent these hurdles such as

ligand-modifications, stimuli-responsive modifications, and generation of nanocomposites (Patra et al. 2018; Din et al. 2017; Lombardo et al. 2019). Many of these have been discussed in the previous section to improve the target specificity to the targeted tissues or organs. This section will emphasize on various types of modifications on drug nanocarrier and their corresponding effects.

11.5.1 Ligand Modifications

As discussed in Sect. 11.4, nanocarriers can be modified to improve the target specificity by conjugating them with other site-specific molecules such as antibodies, peptides, aptamers and other molecules. The modified nanocarriers are made to escape endosomal sequestration and specifically target different cells and other cellular components such as nucleus, mitochondrion and endoplasmic reticulum (Parodi et al. 2015). This has posed a significant impact on the therapeutic efficacy in various diseases especially on cancers (Mi et al. 2020). In addition to target specificity, ligand and surface modifications of nanocarrier could also improve their stability in the presence of various biological fluids (Guerrini et al. 2018).

11.5.2 Stimuli-Responsive Modifications

As mentioned earlier, one of the major drawbacks of drug nanocarrier is the inconsistent drug release. To this, various stimuli-responsive nanocarriers have been constructed to enhance the release of drugs in a controlled manner. These stimuli include pH, redox, light, temperature, ultrasound, magnetic, enzyme, hypoxia and in combination (Qin and Li 2020). For example, Hu et al. (2019) constructed a pH-responsive acetylated β-cyclodextrin-based nanocarrier loaded with camptothecin was able to exert its anticancer action against liver cancer cells. In this study, phthalocyanine was used as a photosensitizer which was affected by the pH change, thereby improving the release profile of camptothecin and the biological effect. Wang et al. (2019a, b) constructed a thermoresponsive mitochondria-targeted nanocarrier which was loaded with paclitaxel for cancer cell therapy. The release of paclitaxel was improved at high temperature followed by the enhanced cellular uptake and anti-tumour inhibition. In another study, a doxorubicin-loaded hypoxia-responsive nanocarrier could selectively release the drug in hypoxic environments. This nanocarrier was found to be stable in blood at a prolonged period of time and resulted in improved *in vivo* anticancer effect with reduced off-target toxicity (Zhang et al. 2020).

More importantly, dual-responsive nanocarrier have also been developed to further facilitate the controlled release of drug (Vijayakameswara Rao et al. 2018; Alsehli 2020). For instance, redox- and pH-responsive glycopolymer-drug conjugate was shown to have enhanced stability under physiological condition while

releasing the drug in a rapid manner in hepatocarcinoma cells (Wu et al. 2018a, b). Magnetic- and pH-responsive silica-based nanocarrier loaded with doxorubicin could effectively kill the drug-resistant breast cancer cells in both *in vitro* and *in vivo* models (Wang et al. 2019a, b). In another study, Huong et al. (2018) loaded doxorubicin into an enzyme- and redox-responsive mesoporous silica based nanocarrier which showed potent anti-tumour action against murine mammary carcinoma cells and *in vivo* model. Interestingly, Gao et al. (2019) demonstrated a dual (doxorubicin and bortezomib) drug-loaded nanocarrier which showed improved cellular uptake and enhanced cytotoxicity in breast cancer cells. Further, it was shown that the release of drug can be induced by pH and irradiation in a controlled manner. Similarly, Zhang et al. (2019) loaded celecoxib and doxorubicin into a pH and redox-responsive nanocarrier for a drug-resistant breast cancer treatment. The nanocarrier showed improved stability, controlled drug release followed by remarkable anticancer effect.

11.5.3 Limitation of Drug Nanocarrier Development

While a few of modified drug nanocarriers have been approved by FDA and many are undergoing clinical trials (Ventola 2017), most of the potential nanocarriers are being evaluated using animal models (Yang et al. 2017). There are certainly differences between the human body and these animal models in terms of susceptibility and toxicity (Yang et al. 2017). This is mainly caused by the variation of structural barriers, drug release profile, cellular uptake mechanisms, endosomal entrapment and lysosomal degradation in the different biological systems (Reinholz et al. 2018; Wacker et al. 2016). Consequently, this makes the translational impact of the research challenging which subsequently increase the failure of clinical trials.

In order to improve the functionalities of nanocarriers, various modifications can be made by multiple processes. These modifications increase the manufacturing steps hence the cost of product is also elevated. This can be a potential challenge in scaling up the product for commercial use in the market (Hare et al. 2017). Compared to other controlled drugs under trials, the guideline given by the regulatory authorities for the approval of drug nanocarrier seem to be lacking. This might further slow down the progress of the development and approval of new drug nanocarrier (Hare et al. 2017; Patra et al. 2018).

11.6 Challenges of Nanodrug Delivery by Different Routes

In clinical application, nanodrugs can be administered into human body via several routes: oral, inhalation, transdermal, parenteral and topical routes similar to how conventional drugs are delivered. In general, non-invasive routes such as oral, transdermal and topical administration are more preferred than the invasive routes such

as parenteral method. The determining factors for the different routes primarily depend on the characteristics of nanodrugs such as solubility, stability, safety profile, bioavailability, and renal clearance rate. The optimum route is chosen to effectively cross the tissue barrier and deliver the nanodrug to the targeted disease site. Each route has its own advantages and limitations which will be discussed in this section.

11.6.1 Oral Route

Out of all routes, oral route is the most convenient and safest method for nanodrug delivery. Through this route, the nanodrug is mainly adsorbed from the small intestine and partly adsorbed in mouth and stomach. The nanodrugs pass through the intestinal wall followed by liver before being circulated via bloodstream to target site. Due to the drug metabolism processes in liver, decreased amount of drug will reach the bloodstream (Reinholz et al. 2018). In addition to liver metabolism, the major drawback is that the drug typically moves through digestive tract, hence the success of delivery is highly limited by the conditions such as acidic pH and temperature, and other components in the intestinal mucus layer such as proteolytic enzymes, microbiome, epithelial cells and cellular debris (Bose et al. 2014). To overcome these barriers, drugs are typically loaded into a colloidal carrier to prolong the interaction between the nanodrug system and epithelial layer in the digestive tract (Reinholz et al. 2018). For example, a liposomal nanoformulated insulin—hepatocyte-directed vesicular (HDV) insulin was developed to improve the pharmacokinetics and route of drug administration (Caster et al. 2017). Similarly, chitosan-modified NPs could improve the oral delivery of albumin *in vivo* using rabbit and rat models (Nashaat et al. 2019). To overcome the extreme acidic pH in the digestive system, Wang et al. (2013) developed the 5-fluorouracil-loaded PLGA NPs which showed no or limited release at pH 1.2–6.8, but a stable and slow release of drug at pH 7.4 using *in vivo* models.

11.6.2 Inhalation route

After atomization, nanodrug can be administered by inhalation through mouth or nose into the lungs followed by adsorption into the bloodstream. Nanodrug inhalation provides a faster and more potent delivery route than oral administration. Unlike in oral or other routes, inhalation route can solve the problem of drug degradation in digestive tract and difficulty in crossing barriers such as mucus and epithelial cell layers (Bose et al. 2014). This administration route is commonly targeting respiratory diseases such as asthma and lung cancer. The drugs are directly delivered to lung alveoli which have less stringent environment compared to other organs, including larger surface area for drug absorption and lower proteolytic

enzymatic activity (Bose et al. 2014). For example, Arikayce which is an inhalable liposomal formulation of amikacin designed for lung infection treatment has completed phase 1, 2 and 3 trials (Caster et al. 2017). In another study, Tseng et al. (2009) developed a biotinylated gelatin NP carrier which enhanced the delivery of cisplatin in lung cancer via inhalation. It has been previously shown that the particle size is one of the key factors in optimizing the nanodrug administration by inhalation route and therapeutic efficacy (Bose et al. 2014). Hence, the particle size must be put into consideration for the development of inhalable nanodrug. Other challenges of drug delivery through inhalation route are that meticulous and convenient monitoring is required to ensure the right dosage at specified time is given as well as specific equipment known as inhaler is required for the administration of such aerosolized nanodrugs.

11.6.3 Topical Route

Topical administration of nanodrugs general refers to the application of nanodrugs on the skin just like any topical agents. The main obstacle of topical route is the three main layers of skin: the epidermis, dermis and hypodermis which comprise of keratin, structural and immune cells. The nanodrugs need to cross these barriers in order to reach the target site (Bose et al. 2014; Lakhani et al. 2018). However, it is important to note that when there is an open wound, the skin barriers are compromised and this will facilitate the entry of nanodrug. Balguri et al. (2016) showed the potent delivery and accumulation of indomethacin through nanostructured lipid carriers into ocular tissues following the topical application. In another study, Iwaszkiewicz and Hua (2014) demonstrated that topical application of loperamide HCl-encapsulated liposomal gel exerted effective and prolonged anti-inflammatory action in the rodent models.

11.6.4 Transdermal Route

Other than topically applying the nanodrug on the skin, they can also be delivered through a patch which results in a continuous and slow release of drug in a controlled manner. A combination of nanoformulation in the patch is usually designed for this purpose in order to constantly maintain the drug level in the blood for target site delivery. This is particularly useful for drugs that are less stable and can be rapidly eliminated from the body. For example, Zhang et al. (2014) demonstrated an effective transdermal delivery of aconitine via solid lipid nanoparticles and microemulsion systems which enhanced the properties of nanosystems, reduced the toxicity of aconitine and improved the anti-inflammatory action. When N-palmitoylethanolamine, another anti-inflammatory agent was delivered through nanostructured lipid carriers transdermally and intravenously, it was found that the

transdermal delivery sustained the drug release up to 24 h and remained biologically active while the biological effect started to decrease after 3 h following the intravenous administration (Tronino et al. 2016). More interestingly, Anirudhan et al. (2016) demonstrated the effective release (80%) of lidocaine, an anti-pain topical agent from the hyaluronic acid microparticles in the transdermal patch matrix at pH 7.4 but no drug was detected at pH 1.2. This was due to the reduced hydrogen bond dissociation energy between the matrix and lidocaine in the increasing pH (Anirudhan et al. 2016). Other than pH-responsive nanodrugs, the use of other stimuli-responsive nanosystems can also be applied. In general, transdermal administration of nanodrug shared the same challenges faced by those delivered by topical route due to the complexity of keratinous layers of skin. On top of that, this administration route cannot be utilised for those treatment that require quick response and high load of drugs. Similar to the inhalation route, the amount of drug release into the blood might be more troublesome to monitor. Moreover, the site where the patch is adhered to may also cause discomfort or irritation on individual with sensitive skin.

11.6.5 Parenteral Route

Despite being an invasive route, parenteral administration of nanodrug can circumvent various limitations encountered by oral, topical and other routes. This mode directly delivers the nanodrugs to the target site or systemic circulation bypassing the administration barriers such as those in digestive tracts and skin. In general, the parenteral route can be divided into intravenous, intramuscular and subcutaneous categories (Bose et al. 2014). For example, dexamethasone-loaded polymeric NPs was demonstrated to effectively inhibit leukaemia following a parenteral administration (Krishnan et al. 2013). Similarly, tamoxifen-loaded solid lipid nanocarrier could also kill leukemic cells through parenteral route (Mudshinge et al. 2011). Another study by Bisht et al. (2010) showed the effective delivery of curcumin with polymeric NPs through subcutaneous route followed by potent anticancer action. The challenges in parenteral route are the immune responses of the host system following the drug entry into the blood circulation, either through protein destabilisation by plasma proteins and proteolytic enzymes or targeting by immune cells such as monocytes and macrophages through opsonization (Bose et al. 2014). Hence, it is crucial to take these issues into consideration during the construction of these nanocarriers. There are several ways to design the nanosystems that are capable of escaping the immune surveillance. For example, nanosystem coated with PEG can protect the nanodrug from opsonization and increase the blood circulation time. Interestingly, the effect of immune protection also depends on the size, shape, molecular weight, surface density and hydrophilicity of PEG on the surface of nanoparticles (Bose et al. 2014). This partly explains the large number of FDA-approved PEGylated nanosystems for clinical use (Ventola 2017).

11.7 Concluding Remarks

The discovery of nanocarrier and the capacity of nanocarrier modifications has unquestionably revolutionized the therapies of various diseases and infections. The two major prominent advantages of using nanocarriers over other naked drugs or compounds are the cell-penetrating capability and target specificity. These enhanced features of nanocarrier could deliver the drugs to various organs and organs systems including BBB which is extremely difficult to target. The field of utilising drug nanocarrier for treating various diseases and infections is currently saturated. However, important issues such as the safety profile, optimum administration route and mechanisms of nanodrug must be evaluated in order to increase their chances of FDA approval for clinical uses.

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Chapter 12
Drug Delivery Towards Cancer

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12.1 Introduction

Some special characteristics like evading growth suppressors, sustaining proliferative signaling, enabling replicative immortality, activating invasion and metastasis, inducing angiogenesis, and resisting cell death have made cancer cells very challenging to destroy (Hanahan and Weinberg 2011). Cancer cells have high level of telomerase enzyme and they are mostly unresponsive to cellular growth regulators and apoptosis. These challenging factors help the cancer to maintain its DNA integrity and allow them to replicate infinitely. The capability of inducing angiogenesis promotes rapid formation of new blood vessels in the cancerous tissue and thus the cells remove their toxic waste and get sufficient nutrients for their rapid growth. It has also capability to migrate and penetrate into the other parts of the body and thus

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forms new, secondary tumors. Besides, cancer cell has overexpressed glucose transporter which leads to increased glucose uptake into the cancer cells. They can also perform aerobic glycolysis which acts as metabolic switch and may allow to nucleosides and amino acids anabolism for additional growth and proliferation. Moreover, as the cancer cells are derived from own host body cells, the markers for immune recognition are not well expressed in the cell surfaces which makes it less susceptible to T-lymphocyte recognition and thus allowing them to avoid elimination by the immune system (Tran et al. 2017). All of these characteristics have made cancer targeting and treatment very challenging which ultimately fueled the demand of special type of drug delivery systems.

Nonspecific tissue and cell biodistribution of the conventional drug delivery system often leads to altered drug efficacy as some drugs undergo rapid catabolism to produce inactive materials and are excreted from the body of the subject. In these regards, delivery system comprising advanced chemotherapeutics formulation, enabling induced selectivity with site specific binding can make the cancer treatment much safer and more efficient (Kanamala et al. 2016; Wicki et al. 2015). The research to develop a suitable delivery vehicle which has the capability to specifically target the cancerous tissue leaving the healthy parts of the body is one of the key interests among the scientists worldwide.

12.2 Designing the Effective Drug Delivery Vehicle

To be an ideal drug delivery vehicle (DDV), the designed carrier should have capability to overcome the biological barriers. It should also have a targeting system which can guide the drug carrier to the cancer tissue/cells. There are various approaches to achieve these objectives which are discussed below.

12.2.1 Biological Barriers

A designed DDV can only be useful if it is designed in such a way so that it can travel to the target tumor site intact form. So, for being a successful nanocarrier, the fabricated nanoparticles should able to pass through the biological barriers to reach the target sites as these barriers are responsible and sensitive to eliminate the nanocarriers before reaching to the target site.

12.2.1.1 Reticuloendothelial System

The reticuloendothelial system (RES) consists of both noncellular and cellular components. It is also known as the mononuclear phagocyte system (MPS). Phagocytic cells bind with the DDV and trigger release of cytokines, thereby increasing clearance of the vehicle and loaded drug from local inflammatory tissue and bloodstream

(von Roemeling et al. 2017). Besides, random binding with blood and tissue proteins and lipids can disrupt the functionality of the DDV and may also trigger the recognition by the immune system and thus induce the clearance (García et al. 2014; Miele et al. 2009). In these regards, surface of the DDV is often modified by zwitterionic ligands such as cysteine and glutathione or PEGylation. These modifications may lead to less recognition by the RES and thus increase the availability of the DDV in the circulation and thus induce bioavailability of the loaded drug into the targeted cancer tissue (García et al. 2014; Locatelli and Franchini 2012). Size and shape of the DDV is very important for binding and clearance. Spherical DDVs are usually aggregate in the center of the blood vessel and show poor binding with endothelial cell whereas disc-like DDVs may possess enhanced endothelial cell interactions and thus increase their ability to extravasate into tumor tissues (Toy et al. 2011, 2014).

12.2.1.2 Renal System

Kidney is responsible for filtering circulating blood and thus removing unwanted and toxic elements from the body. If a DDV comprises favorable size, shape and charge for being easily pass through the glomerular membrane of the kidney it usually cleared very rapidly by the kidney. Spherical nanoparticles with diameters less than 6 nm were shown to have higher renal clearance than those with diameters higher than 8 nm (Cho et al. 2008). Besides, as the glomerular basement membrane is negatively charged, cationic nanoparticles of 6–8 nm diameter showed higher clearance than those negatively charged or neutral of the same size (Liu et al. 2013). If a DDV has high renal clearance the bioavailability of the loaded drug may be found to be very low. On the other hand, if its size, shape and charge do not favor the kidney clearance it may show long-term toxic effect. So, there should be a balance between the rate of kidney clearance and targeted tissue uptake of the loaded drug. It was found that DDVs which had initially unfavorable characteristics for kidney clearance but became favorable to clearance after systemic degradation, were the best in terms of performance (Stylianopoulos et al. 2012).

12.2.1.3 Blood–Brain Barrier

One of the main challenges of treating brain cancer is the blood–brain barrier (BBB) as it only permits passage for less than 2% of molecules, including nutrients, ions, specific proteins and peptides, and leukocytes (Pardridge 2005). This BBB is made by tightly bound endothelial cells and enclosed by basal lamina, astrocytic cells, pericytes, and microglia. So, as there is a lacking of pours to facilitate the drug transfer. So, receptor mediated endocytosis may be main pathway to deliver drugs into the brain. In this regard, DDV designated to treat brain cancer are designed so that it can bind to specific cell surface receptor of the BBB to induce endocytosis (Ulbrich et al. 2009). To fulfill the purpose, the DDV is often conjugated by targeting ligands or peptides such as transferrin, lactoferrin, and low-density lipoprotein receptors etc. (Hu et al. 2009; Kim et al. 2007). Covalent attachment of apolipopro-

teins to human serum albumin-coated DDV were found to be improved their ability to cross the BBB (Michaelis et al. 2006). Size of the DDVs is also an important factor. It was found that DDVs with diameters of 20–70 nm were preferential for transporting through the BBB (Shilo et al. 2015).

12.2.1.4 Pathophysiological Barriers in Cancer

Tumor microenvironment is highly heterogenous and dependent on cancer type, location, and progression state, along with patient-specific characteristics. This heterogenicity often acts as a potent barrier for the DDVs and deeper penetration into the tumor is restricted. It was found that smaller particle size of the DDV might lead to increased penetration into the cancer tissue. DDVs which had higher degradation rate inside the tumor microenvironment were found more effective to transport the loaded drug in the center of the tumor (Stylianopoulos et al. 2012). In these regards, particle size, its degradation pattern and binding affinity with body protein and lipids are critically should be considered to fabricate the DDVs.

12.2.2 Cancer Targeting

Drug carriers facilitate to increase bioavailability of the administrated drug in the targeted sites by active targeting, passive targeting or triggered release behavior (Fig. 12.1). It also reduces the drug leak out from the targeted cells so that drug concentration in the cells increased (Cho et al. 2008). Passive targeting is a mechanism by which the drug carriers can penetrate through the larger pores of the leaky blood vessels of tumor tissues but cannot pass through the comparatively tight blood vessels of healthy tissue/organ. Besides, cancer tissue also has poorly developed lymphatic system. This lymphatic system usually is a drainage system for the tissue

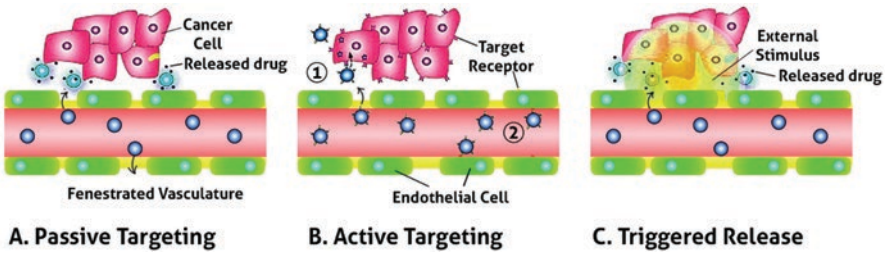

Fig. 12.1 Three types of targeting can be adopted by the drug delivery vehicle. (**a**) Passive targeting which relies on size dependent permeability. (**b**) Active targeting which uses ligand-receptor interaction for target specificity (target receptor can be found in both cancer cell (1) and endothelial cell (2) but overexpressed in cancer cell). (**c**) Triggered release which is mediated by external or internal stimuli like heat, light, infrared radiation, ultrasound, magnetic force etc.

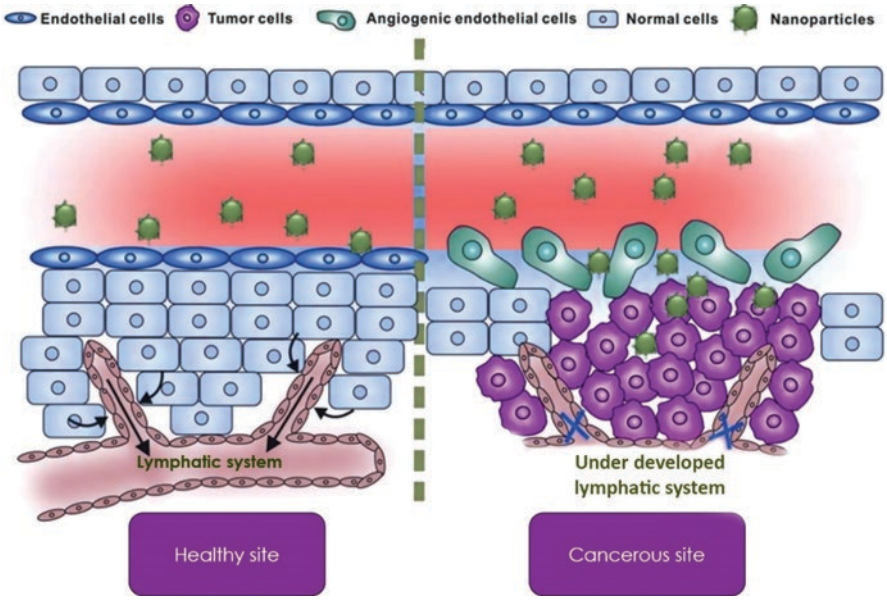

Fig. 12.2 Schematic diagram of enhanced permeability and retention (EPR) effect

which removes unwanted elements from the tissue. So, if a drug carrier enters to a healthy tissue/organ, lymphatic system rapidly clears it out. But as the cancer tissue has underdeveloped lymphatic system, causes the entered drug carriers cannot be cleared out. So, the carrier gets enough time to release its drug load to the targeted cancer tissue (Albanese et al. 2012; Bregoli et al. 2016). This mechanism is known as enhanced permeability and retention (EPR) effect (Fig. 12.2).

However, this passive targeting is not enough for target specificity as some other organs like liver, spleen also has larger pores in their blood vessels. That is why active targeting is required. Active targeting comprises a (or more than one) targeting ligand(s) which will be conjugated on the surface of the drug loaded nanocarrier. Targeting ligands are moiety, which has selective affinity to bind with any specific receptor, which is unique/overexpressed in the cancer cells. This binding enhances concentration of the nanocarrier into the tumor tissue compared with the other healthy tissues and organs; therefore, it increases drug accumulation in the cancer cell and reduces side effects (Wüstemann et al. 2019; Ding et al. 2016). There are a variety of molecules have been identified as cancer targeting ligands and more are adding to the list due to the extensive cancer research worldwide. A targeting ligand can be protein or small peptides, vitamins like folic acid, glycosaminoglycan like hyaluronic acid, antibodies or antibodies fragments, aptamers or even carbohydrates or polysaccharides.

In case of stimuli-responsive system, both external and internal stimuli can be used to trigger the drug release through stimuli induced change of the drug carrier. Changes in pH, ionic strength, or stress in target tissues are some examples of

internal stimuli (Wicki et al. 2015). There is a difference between the pH of blood stream and intracellular organelles which is the key factor to design a pH responsive drug carrier. Cho et al. (2008) showed pH responsive properties of hydroxyapatite and sodium alginate bi-coated iron oxide drug delivery vehicle. This vehicle was capable to release hydrophobic drugs curcumin and 6-gingerol to the cancer micro environment triggered by comparative low pH of the tumor microenvironment.

External (physical) stimuli include light, temperature, ultrasound, electric fields or magnetic force. A size dependent drug carrier can be developed which will have increased permeability to the targeted area and can be mediated by increasing temperature of the target site which ultimately increases the pour size of its blood vessels (Chen et al. 2013). On the other hand, carriers responsive to magnetic or electric filed can be designed where external magnetic or electric field are used to aggregate the drug carrier to specific sites (Guduru et al. 2013).

12.3 Different Types of Drug Delivery Vehicle

Considering the huge potential for safer cancer treatment, a vast types of drug delivery system have been investigated and these are discussed below.

12.3.1 Quantum Dots

Quantum dots (QDs) with diameter of 2–10 nm of inorganic nanocrystals, which show high fluorescence intensity and photostability. A QD usually consists of a semiconductor core covered with a shell. The shell protects the core from oxidation and enhances quantum yield. Although QDs are usually used for bioimaging and labeling, their applications in targeted drug delivery for cancer have also being explored in the recent years (Bilan et al. 2016).

12.3.1.1 Photoluminescent Semiconductor Quantum Dots

Photoluminescent semiconductor quantum dots are known for their distinct photochemical properties. Quantum dots (QDs) like Cadmium Sulfide (CdS) and Cadmium telluride (CdTe) nanoparticles have been widely investigated. However, there are still some challenges to overcome to use these QD for cancer targeted drug delivery *in vivo*. Firstly, there is a tendency of these QDs to aggregate in physiological fluids which reduces their functionality *in vivo*. Besides, many reports have been reported that most of these semiconductor QDs are toxic to animals (Cai et al. 2016). So, reasonable modifications and stabilizations are required to use these photoluminescent semiconductor QDs as cancer targeted DDVs.

12.3.1.2 Zn Quantum Dots

ZnO QDs are inexpensive and low-toxicity nanomaterial, which have made these very suitable for designing DDVs (Hahn et al. 2012; Tripathy et al. 2015). In comparison with traditional drug delivery systems, ZnO based QD DDVs have many distinct advantages. These are low cost, easy to prepare, benign and less toxic. Besides, these have ability to rapid dissolution at cancer microenvironment pH and can exhibit significant cytotoxic effect (Nel et al. 2009) which have made them pH responsive DDVs. Cai et al. (2016) prepared a water dispersible, luminescent ZnO QD with surface modified by amino groups and conjugated with PEG. Hyaluronic acid was bound to the ZnO-PEG QD to introduce cancer targeting behavior. An anticancer drug doxorubicin (DOX) was bound to the ZnO-PEG QD not only by covalent interactions but also by formation of Zn^{2+}–DOX chelate complex. It was found that the prepared QD biodegraded completely in the acidic environment of tumors and could induce apoptosis. Besides, loaded DOX could be released under acidic intracellular conditions and further enhanced the anticancer activity effect.

12.3.1.3 Graphene Quantum Dots

Due to their extraordinary chemical properties and single atomic-layered structure graphene and graphene oxide (GO) have been caught significant interest in the recent years among the scientists all over the world (Zhang et al. 2012; Iannazzo et al. 2015). Graphene quantum dots (GQD), are single-layer two-dimensional graphene having enormous potential in biomedical field and thus considered as the next generation of carbon-based nanomaterials (Shen et al. 2012). If we compare between graphene and GQDs, GQDs are more hydrophobic and less toxic. Besides, it possesses stable strong fluorescence (Zhu et al. 2013). The size and shape of GQDs can be tailored easily and it has strong ability of drug loading by π–π interactions. Thus, the DDVs made by GQDs can selectively target cancer cells via ERP effect which can be easily tracked for their intrinsic fluorescence effect. Moreover, coupling with cancer targeting ligands enables active targeting in the GQD DDVs. Iannazzo et al. (2017) has developed a biocompatible and cell traceable GQD DDV to deliver DOX into cancer cells. The GQD was water soluble and highly dispersible and tagged with tumor targeting module biotin (BTN). The developed GQD-BTN was found to recognize biotin receptor which was over-expressed in the cancer cell surface. The formulation exhibited considerable high cytotoxic effect compared to free DOX *in vitro*. Javanbakht and Namazi (2018) reported a novel hydrogel nanocomposite formed by incorporating GQD into carboxymethyl cellulose (CMC) hydrogel. DOX was loaded into the hydrogel and evaluated for anticancer properties against blood cancer cells (K562). The experimental results suggested that the prepared GQD-CMC-DOX hydrogel possessed pH-sensitive anticancer properties.

12.3.2 Engineered Peptides

In recent years, peptides and peptidomimetics have gained attention as an alternative to popular antibodies and affibodies as cancer targeting ligands. Peptide-based drug conjugates offer multiple advantages. These are less immunogenic and as they are small in size, they can penetrate deep into the cancer tissue easily. Peptide based drug carriers are also cost effective as these peptides can be easily synthesized using automated solid phase methodology. They can be also easily conjugated with the anticancer drugs with high repeatability. Moreover, these peptides are comparatively more stable in different storage conditions (Ladner et al. 2004). However, fast renal clearance, poor enzymatic stability and difficulty of maintaining secondary structure etc. drawbacks should be also considered while designing the peptide based DDVs (Soudy et al. 2017).

Peptides are designed so that they can bind with the cell surface receptors, growth factor receptors or cell adhesion molecules which are overexpressed in the cancer cells. Zhang et al. (2001) screened against WAC2 neuroblastoma cells using an *in vitro* phage-display peptide library and discovered a linear peptide named as p160. This newly made peptide was found to cluster at the MDA-MB-435 cell membrane and eventually internalized. In another research, ^{131}I-labeled p160 was injected intravenously in mice and found to accumulate in the breast tumor xenografts specifically compared to other healthy organs like liver, heart, spleen and brain (Askoxylakis et al. 2006). Another analog of p160 (70 linear peptide sequences) developed by Ahmed et al. (2010) showed threefold better binding compared to p160 for the MDAMB-435 and MCF-7 cancer cells.

A diverse variety of drugs such as chemotherapeutic agents, antisense oligonucleotides, toxins and proapoptotic peptides have been conjugated with this cancer targeting peptides for site-specific delivery. For an example, anti-tumor cytokine like tumor necrosis factor (TNF) was conjugated with RGD (Arg-Gly-Asp) and NGR (Asn-Gly-Arg) peptides to guide the TNF to the cancer cells. Study suggested that peptide guided TNF was similarly effective at 1000-fold lower dose compared to TNF alone. This lower dose requirement improved the anticancer effect of TNF and also reduced the side effect to a great extent (Nagy et al. 1996). In another study, Doxorubicin (DOX) was conjugated to Luteinizing-hormone-releasing hormone (LHRH) agonist and antagonist peptides for selectively targeting cancer cells. Anticancer effect of the LHRH-DOX conjugate was studied on various cancer cell types i.e., breast, prostate, and ovarian cancer cells and was compared with corresponding free drugs. *In vivo* studies suggested that the drug-peptide conjugate was more effective in cancer regression compared to free DOX. The conjugate also showed less toxicity compared to the free drug (Nagy et al. 1996).

It has been shown that polycationic peptides have very high cell membrane permeability and they are able to carry hydrophilic drugs into the cells by creating a passage through the lipid bilayer if covalently bound with the drugs. These peptides are known as cell penetrating peptides (CPP) which are 9–32mers of cationic and/or amphipathic peptides mostly composed of arginine, lysine and histidine. Recent

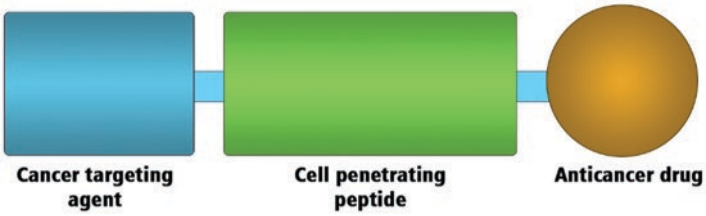

Fig. 12.3 Chimeric cell penetrating peptides (CPP) bound with chemotherapeutic agent for cancer targeting

research suggested that these CPPs are not only facilitate the hydrophilic drug transportation, they can also be tagged with cancer targeting ligands to deliver the anticancer drug to the specific cancer microenvironment (Bolhassani 2011). A chimeric CPP can be developed by conjugating cancer targeting ligands which can be further covalently bound with chemotherapeutic agent (Fig. 12.3). The resulting formulation then can be used as a cancer selective DDV.

12.3.3 Nanocarriers

Currently, due to the huge prospects, a wide variety of platforms are being explored and myriad of nanostructures have been used to deliver the anticancer drugs in controlled manner. The mostly studied nanostructures are as nanocarriers for cancer treatment, including liposome, exosomes, bionanocapsule (virus), cyclodextrin, dendrimers, inorganic compounds, polymers, polymer micelles, organic-inorganic hybrids as well as other nanomaterials (Fig. 12.4).

12.3.3.1 Liposome

Liposomes are spherical nanoparticles made by lipid bilayers. These liposomes are spontaneously form when an amphiphilic lipid is mixed with water or other hydrophilic liquids. The size of the liposomes usually lies between 50 and 500 nm. It is possible to encapsulate hydrophilic drugs inside the liposomes while preparing it simply by dissolving the drug in the liquid used for formation of the nanostructure (Sun et al. 2014). Liposome is firstly used for DDV by Gregoriadis and Ryman in 1971. Since then a wide range of liposome-based drug carriers have been studied and reported. The first FDA approved liposome nanocarrier for cancer treatment was Doxil®. This was approved in 1995 and indicated to use for AIDS-related Kaposi's sarcoma, myeloma, ovarian cancer and breast cancer (Liu et al. 2016). Recently many other types of liposomal nano carriers have been reported and some of those are in clinical trials or in commercialization stage (Wakaskar 2018; Yue and Dai 2018; Pillai 2019).

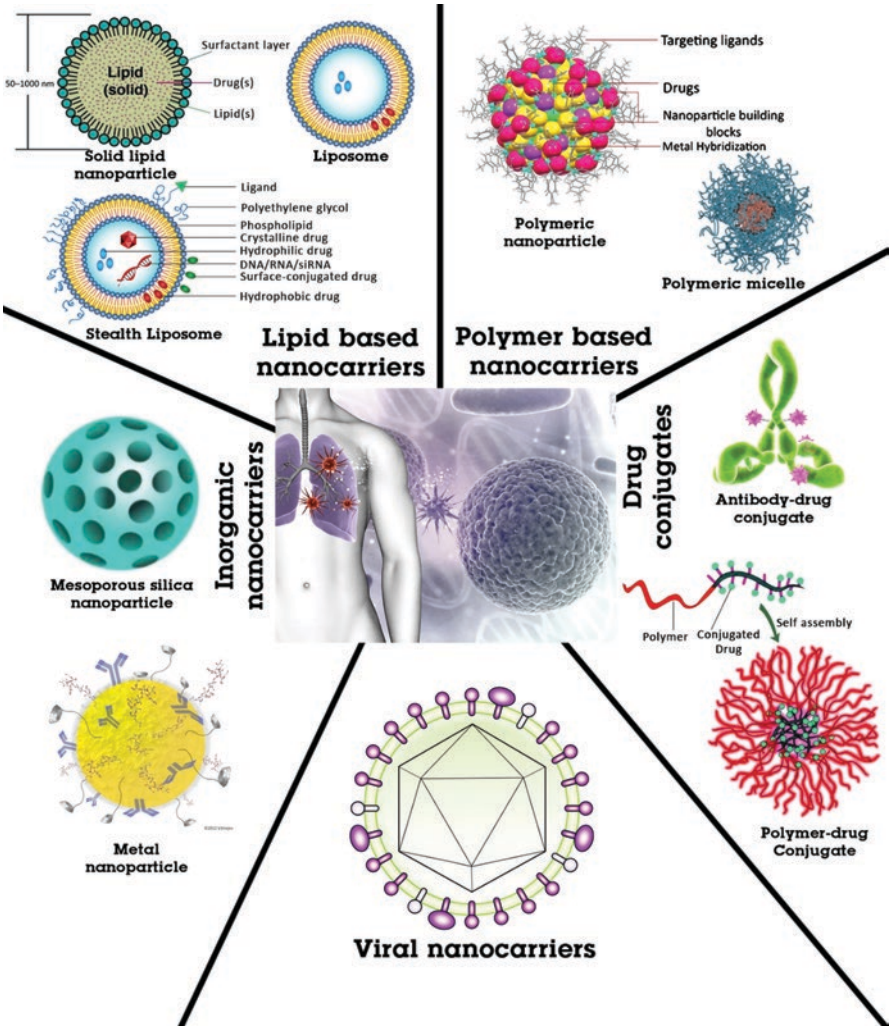

Fig. 12.4 Different types of nanocarriers used as DDVs for cancer treatment

12.3.3.2 Bionanocapsule or Virus-Based Delivery Vehicles

Virus-based DDVs is a viral structure, which is missing its pathogenic machineries. This nanovehicles contain viral proteins which can target specific cells or tissues and unload the encapsulated drugs in the targeted tissue. One of the most common virus-based nanocapsules is hepatitis B virus bionanocapsule which is a hollow nanostructure made of hepatitis B surface antigen (HBsAg), L protein (HBV) and a lipid bilayer. Particle size of the carrier is about 70 nm and can entrap drugs, proteins etc. It can also be conjugated by therapeutic genes (Jung et al. 2008; Kang et al. 2010). Because of the L protein, this nanocarrier can specifically target

hepatocytes to deliver genes or drugs in to it (Kuroda et al. 1992; Yamada et al. 2003; Yu et al. 2005, 2006).

12.3.3.3 Cyclodextrin and Dendrimers

Cyclodextrins are cyclic sugar molecules having hydrophobic cavity interior and hydrophilic exterior. For the hydrophilic exterior, cyclodextrin is highly water soluble but its hydrophobic cavity allows it to encapsulate hydrophobic moieties. Because of the sugar structure, cyclodextrin possesses good biocompatibility and the inner hydrophobic core enables excellent stability and bioavailability (Davis et al. 2010; Sivasubramanian et al. 2013). Drugs that have poor bioavailability and water solubility can be encapsulated and transported by the cyclodextrin. Ghasemali et al. (2013) reported that helenalin anticancer agent found from *Arnica chamissonis* and *Arnica montana* can be encapsulated in the α-cyclodextrin to form α-cyclodextrin-helenalin complex. The nanocomplex showed significantly better inhibition of T47D breast cancer cell growth compared to free helenalin.

On the other hand, dendrimers are hyper branched spherical macromolecules emerging from a central point. These nanoparticles are formed layer by layer. The diameter of the dendrimers is typically 100–200 Å. Degree of branching and size of the dendrimer can be easily controlled by using specific initiator cores. Depending on the initiator core and branches structure the internal void space of the dendrimer can be surrounded by hydrophilic or hydrophobic moieties. So, dendrimers can be used to encapsulate either hydrophilic or hydrophobic agents depending on the structure. These dendrimers are proved as a carrier for large numbers of drugs and siRNA and thus improved solubility and bioavailability of poorly soluble agents (Svenson and Tomalia 2012; Kaminskas et al. 2012; Navarro and de ILarduya 2009).

12.3.3.4 Metal Oxides and Other Inorganic Nanoparticles

Nanocarriers made from carbon nanotubes, graphene, fullerenes, metal or metal oxides like, zinc, iron, calcium, titanium, silver, gold etc., clay, silicone and similar materials are known as inorganic nanocarriers. These nanocarriers possess special and enhanced physical and chemical properties compared to their building blocks depending on the particle size, shape and bond energy. In the recent years, inorganic nanoparticles gained a significant attention in the field of oncology for a variety of applications, tumor specific drug delivery, including tumor imaging, or enhancement of radiotherapy. Inorganic nanoparticles are highly stable compared with organic materials. Besides these can be designed to have hydrophilic or hydrophobic properties. However, immune system often recognizes the inorganic nanoparticles as a threat to human body and are cleared by the reticuloendothelial system. For this reason, these nanocarriers are mostly subjected to surface treatment for better immune compatibility (Olov et al. 2018).

12.3.3.5 Polymer and Other Organic Nanoparticles

Although a large number of inorganic nanocarriers are developed, their application is limited in many cases for toxicity or rapid clearance. As most of the inorganic nanocarriers are hydrophobic in nature, reticuloendothelial system often opsonizes the nanoparticles which ultimately lead to degradation of it and eventually cleared from the body (Chakraborty and Parak 2019). Concerning the problem, many researchers are working to mask the inorganic nanocarrier with water soluble polymers like PEG, proteins, organic acids etc. (Simon-Yarza et al. 2018; Bharathiraja et al. 2018; Sharma et al. 2018). There is also a growing research emphasizing to develop nanocarriers from organic materials especially from proteins and polysaccharides to eliminate rapid removal and being more biocompatible.

Polymer based nanoparticles have been widely explored as DDVs. There are a wide variety of polymers have been proved as highly promising for being nanocarriers such as polyethylene glycol (PEG), poly(lactic-co-glycolic acid) (PLGA), poly-L-arginine (PLA), poly-L-lysine (PLL), graphene, nanotubes and many more. Beside these, biopolymers like proteins and polysaccharides have also been extensively studied in the recent years. The advantages of polymer carriers over other material choices include biodegradability, tailorability and ease of synthesis. Moreover, bioactivities like the ability to facilitate cell uptake, to overcome certain biological barriers, and endosomal escape, that have made these polymer-based nanocarriers highly effective from therapeutic aspects.

12.3.3.6 Organic-Inorganic Nanohybrids

Combining organic and inorganic components to form nanostructures is the new smart technology to create entirely unique nanocarriers with added advantages. It is possible to induce both of the *in-situ* stimuli responsive (one of the key advantages of organic nanocarriers) and remote stimuli responsive (one of the key advantages of inorganic nanocarriers) properties in the DDVs through this organic-inorganic hybridization (Tamayo et al. 2019; Li et al. 2016; Zimpel et al. 2019). So, the resulting hybrid nano carriers can be pH, stress in target tissues, and ionic strength responsive (host stimuli) as well as magnetic field, heat, near infrared, or ultrasound (external stimuli) responsive and thus they offer a better control over the drug release in the targeted tissue.

12.4 Conclusion

In the recent couple of decades, there are extremely varied drug delivery approaches have been studied to selectively target cancer cells. Although, many of them have reported very promising anticancer effects, however there are many existing challenges to overcome. Special consideration should be given to the active targeting

system which is mainly mediated by the overexpressed surface receptors of the cancer cells. But unfortunately, these receptors are also found to be expressed on healthy cell surface. This is why overabundance of a specific receptor may not enough to guarantee the selectivity and some of the drug may end up off-target, affecting non-cancerous cells. However, it can be concluded that the discussed DDVs may not totally eliminate the side effects of the chemotherapeutic agents but will definitely reduce the toxicity which will lead to better cancer survival rate. Overall, the targeting system should be guided by more than one mechanism for more accurate targeting and being a biocompatible and safer DDV. In these regards, the most suitable DDV should have both active and passive targeting mechanisms as well as stimuli-responsive properties while comprising nontoxic and non-immunogenic properties.

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Chapter 13
Nanotechnology in Tissue Engineering and Implant Development

Hui-Li Tan, Nuraina Anisa Dahlan, and Pushpamalar Janarthanan

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13.1 Introduction

In the early 1990s, tissue engineering has emerged to overcome the drawbacks of organ transplantation, such as donor shortage and demand for immunosuppressive therapy (Caddeo et al. 2017). The concept of tissue engineering involves three important elements: biomaterial scaffold, living cell/tissue, and growth factors/bioreactor (Tran et al. 2018). Regardless of the type of tissues to be engineered, an ideal scaffold should be biocompatible and biodegradable, to allow the cells to synthesize

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their extracellular matrix (ECM), eventually replace the implanted scaffold (O'Brien 2011). Besides, optimum mechanical strength of scaffold is also needed to facilitate tissue regeneration (Alvarez and Nakajima 2009). Other than scaffold tissue engineering, biomedical implants are also extensively studied as another major area of biomaterials. The implants are typically used to replace or support the damaged or lost tissues. In contrast with the tissue engineering scaffold, the implants could stay in the living system permanently (Li and Mai 2016). The examples of implants are artificial joints and dental implants, as hard tissue replacement (Li et al. 2017).

In recent years, with the advances in nanotechnology, nanomaterials are commonly employed in different biomedical applications, including the design of biomaterials. Nanotechnology in tissue engineering and implant involves the synthesis, design and application of biomaterials at nanometer scale achieved through physical, chemical, and biological routes. The ECM within the natural three-dimensional (3D) cell microenvironment assists in providing structural support, biochemical and biophysical cues to regulate cell behavior (Prasopthum et al. 2019). Therefore, the utmost aim of nanotechnology in tissue engineering is to mimic or recapitulate the ECM function within the biomimetic scaffolds for cell growth, migration, and proliferation. This chapter discusses on several recent nanotechnologies that contribute to the development of nanomaterials for tissue engineering and implants applications.

13.2 Nanoparticles for Tissue Engineering and Implant Development

13.2.1 Nanoparticles

Nanoparticles are particles with sizes ranging from 10 to 1000 nm, which can be prepared in solid and colloidal forms. Nanoparticles are attractive for numerous applications due to the advantageous properties such as high penetration ability, large surface area, and tailorable surface properties (Fathi-Achachelouei et al. 2019). Nanoparticles can be categorized based on the materials, such as metals, ceramics, polymers, and carbon-based materials (Makarov et al. 2014; Singh et al. 2016a; Bennet and Kim 2014; Kokorina et al. 2017).

13.2.2 Nanoparticle-Based Composite Materials

Materials containing nanoparticles can be developed in different forms and the examples are presented in this section.

13.2.2.1 Nanoparticle-Hydrogel Composites

Hydrogel is a popular type of tissue engineering scaffold with high water content and soft consistency, which makes it resembles natural tissues (Dutta et al. 2019). As the crosslinked 3D polymer network, hydrogel possesses porous structures that allow diffusion of various solutes and nutrients (Vega et al. 2017). There is a wide range of synthetic and naturally derived materials that can be used to form hydrogels for tissue engineering applications (Drury and Mooney 2003). Nanoparticles can be incorporated into tissue engineering scaffolds to form composite materials (Tan et al. 2019). The resulted nanocomposite hydrogels could exhibit enhanced intrinsic and extrinsic properties as compared to conventional hydrogels (Vashist et al. 2018).

13.2.2.2 Nanoparticle-Polymer Porous Composite Scaffolds

Tissue engineering can also be achieved using a polymeric porous scaffold or matrix (Freyman et al. 2001). As compared to hydrogels, porous scaffolds have higher porosity levels, with homogenous interconnected pore networks (Singh et al. 2016b), as illustrated in Fig. 13.1. The interconnected spaces are desirable because the 3D microenvironments could promote a wide range of cellular activities such as cell migration, cell-cell interactions, tissue growth and eventually vascularization (Lemos et al. 2016; Jiang et al. 2019). Over the years, attention has also been given composite porous scaffold containing nanoparticles because the interaction between nanoparticles and polymeric porous scaffold has resulted in improved properties, such as mechanical property (Sun et al. 2014) and biological property (Abdal-hay et al. 2017).

13.2.2.3 Implant

Surgical implants, prostheses, and medical devices are essential for the replacement of missing body parts, which may be lost due to accident, trauma, disease, or congenital condition. Metallic implants materials made of stainless steel, cobalt-based alloys, and titanium-based alloys are the common biomaterials for these applications

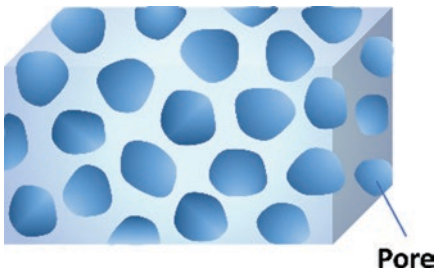

Fig. 13.1 3D porous scaffold with high porosity

(Zaman et al. 2015). In recent years, research has been conducted to modify the surface of existing implants using nanomaterials to achieve enhanced biological and mechanical performance (Parnia et al. 2017; Sivolella et al. 2012).

13.2.3 Roles of Nanoparticles

Nanoparticles can find different roles in tissue engineering scaffolds and medical implants. In terms of biological property, the incorporation of nanoparticles can affect the differentiation of cells and provide antimicrobial activity. Besides, nanoparticles could alter the electrical property and mechanical property of the biomaterials. In addition, nanoparticles could act as an excellent carrier for the delivery of biomacromolecules, such as growth factors and genetic materials.

13.2.3.1 Stem Cell Differentiation

In tissue engineering and regenerative medicine, stem cells play an essential role due to the ability of self-renewal and differentiate into multiple lineages with proper stimuli (Zhao et al. 2013a). Over the years, various nanoparticles were reported to moderate the differentiation of various types of stem cells via different mechanisms (Wei et al. 2017).

Titanium and its alloy are the common biocompatible materials used in bone implants but their surfaces are inert (Hou et al. 2013). Hence, surface engineering of titanium implant has been carried out to improve the cell behavior. In a study, the surface of the titanium dental implant was silanized by chemical treatment and immobilized with gold nanoparticles. The titanium implants surface functionalized with gold nanoparticles enhanced the osteogenic differentiation of human adipose-derived stem cells, with increased expression of osteogenic differentiation specific genes (Heo et al. 2016). Based on the potential of gold nanoparticles in inducing stem cell differentiation, gold nanoparticles were added to advanced-platelet-rich fibrin to enhance the osteoblastic differentiation of human mesenchymal stem cells (Ghaznavi et al. 2019). Metal oxide nanoparticles are also utilized to enhance the bioactivity of tissue engineering scaffolds. Xia et al. prepared iron oxide nanoparticles-calcium phosphate cement scaffold for bone tissue engineering, for differentiation of human dental pulp stem cells (Xia et al. 2019).

Besides, the potential of bioceramics was also studied. Patel et al. have reported the enhanced osteogenesis of human mesenchymal stem cells treated with nanocrystalline hydroxyapatite synthesized from biowaste eggshells (Patel et al. 2019). Besides, hydroxyapatite nanoparticles were incorporated into photo-cross-linkable poly(trimethylene carbonate) scaffold. The results of the *in vitro* study demonstrated the improved differentiation of human bone marrow stem cells osteogenic differentiation (Guillaume et al. 2017). The potential of alumina nanoparticles was also revealed. Zafar et al. studied the effect of nanocomposites of silk fibroin and alumina

nanoparticles on the osteogenic differentiation of rabbit adipose-derived stem cells. The cells have expressed the osteogenic gene markers and osteocalcin protein, as an evidence of the initial stages of osteogenesis (Zafar et al. 2020).

13.2.3.2 Antibacterial Property

Infection caused by opportunistic microorganisms can be a severe threat to organ transplantation, prosthetic grafts, and tissue engineering constructs. Therefore, antimicrobial agents are loaded into scaffolds to overcome this issue. However, the scaffolds may lose the antimicrobial activity after the complete release of antimicrobial agents due to the emergence of resistance among the various strains of microorganisms (Zhao et al. 2015). Over the past decades, nanotechnology-based technology has received remarkable attention in order to overcome the multidrug resistance in microorganisms. Among the nanomaterials, silver nanoparticles hold promising results as they are found to show high toxicity to Gram-negative and Gram-positive bacteria, including multidrug-resistant bacteria (Losasso et al. 2014). Until today, the generally accepted mechanism of toxicity of silver nanoparticles is the attachment of silver nanoparticles onto the cell wall and membrane, followed by the damages of intracellular biomolecules and structures induced by silver nanoparticles and silver ions. Besides, oxidative stress induced by silver ions also taking place (Tang and Zheng 2018). In a study, silver nanoparticles were added into macroporous gelatin/bioactive glass scaffold for bone tissue engineering. Dose-dependent antibacterial property against gram-negative *Escherichia coli* and gram-positive *Staphylococcus aureus* was reported (Yazdimamaghani et al. 2014). Besinis et al. developed titanium alloy medical implants surface with dual-layered silver-hydroxyapatite nanocoatings. Bacterial growth and biofilm formation were successfully inhibited (Besinis et al. 2017). A co-dispersing graphene oxide-silver nanosystem was developed and introduced into poly L-lactic acid/polyglycolic acid scaffold. Interestingly, a synergistic effect on antibacterial activity against *Escherichia coli* was observed (Shuai et al. 2018b). Besides, an antibacterial scaffold was fabricated by incorporating magnesium oxide nanoparticles to poly(3-hydroxybutyrate-co-3-hydroxyvalerate) scaffold. An effective antibacterial effect against *Escherichia coli* was reported. It was suggested that magnesium oxide nanoparticles caused oxidative damage and mechanical damage to bacteria via generation of reactive oxygen species, as well as direct contact action, respectively, eventually caused damage to structures and functions (Shuai et al. 2018a).

13.2.3.3 Electrical Property

For tissue regeneration of electrogenic tissues such as cardiac and neuronal tissues, the transfer of electrical signals between cells and throughout the tissue is crucial. Therefore, conductive scaffolds are desirable to promote efficient cell-cell electrical interaction, tissue regeneration, and differentiation of electrogenic cells (Yadid et al.

2019). In order to fulfill the demand, different types of conductive nanomaterials are widely explored for their potential in conductive scaffolds (Min et al. 2018). One of the popular examples of metal nanoparticles is gold nanoparticles. Shevach et al. have synthesized conductive cardiac patch for cardiac tissue engineering, by depositing gold nanoparticles on fibrous decellularized omental matrices (Shevach et al. 2014). Recently, it was highlighted that selenium nanoparticles could be a potential alternative to produce conductive material for cardiac tissue engineering (Kalishwaralal et al. 2018).

Besides, iron oxide nanoparticles have also attracted many interests due to the advantageous physicochemical properties (Wu et al. 2016). An electroconductive biocompatible collagen films was obtained due to the incorporation of polyethylene glycol capped paramagnetic iron oxide nanoparticles, showing the potential for tissue engineering application (Bonfrate et al. 2017). Iron oxide nanoparticles were also utilized to form nanocomposites with polyurethane. The electrical conductivity and hydrophilicity of the nanocomposites were improved; the viability and attachment of fibroblasts were supported (Shahrousvand et al. 2017). Due to the attractive properties, graphene-based materials have also emerged as the popular nanomaterials for tissue engineering (Shin et al. 2016). A conductive injectable hydrogel was prepared by introducing graphene oxide (GO) into oligo(poly(ethylene glycol) fumarate) hydrogel. The hydrogel has improved the heart function in the rat model with myocardial infarction (Zhou et al. 2019).

Other than metal-based and carbon-based nanoparticles, studies have also been conducted on conductive polymer-based nanoparticles. For example, poly(3,4-ethylenedioxythiophene) nanoparticles were assembled on the chitosan/gelatin porous scaffold. The composite scaffold has shown an increase in electrical conductivity. Based on the results of *in vitro* studies, there was higher neuron-like rat pheochromocytoma (PC12) cellular neurite growth, with increased protein and gene expression, showing the potential for neural tissue engineering (Wang et al. 2017).

13.2.3.4 Mechanical Property

In tissue engineering, the mechanical properties of a scaffold should match with the native tissues to be repair or regenerated (Prasadh and Wong 2018). Therefore, nanoparticles also play the roles as strengthening agent and crosslinker (as illustrated in Fig. 13.2), through the incorporation into the scaffold to form nanocomposite

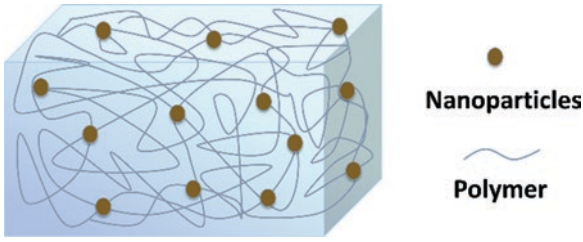

Fig. 13.2 In nanoparticle-hydrogel composite, nanoparticles could act as crosslinkers of the hydrogel, as well as fillers that strengthen the matrix

scaffold. In the system of nanocomposite materials, the physical or chemical interaction between the nanoparticles and polymer chains could lead to a robust hybrid polymer network (Chen et al. 2019). Kim et al. have synthesized a composite 3D scaffold comprised of silk fibroin and titanium oxide nanoparticles. It was shown that titanium oxide nanoparticles acted as nano-sized fillers for silk fibroin scaffold, caused the improvement in the mechanical property (Kim et al. 2014). Nair et al. developed a composite hydrogel composed of chitosan–poly(hydroxybutyrate-co-valerate) and chondroitin sulfate nanoparticles. The composite hydrogel was able to withstand the stress corresponding to regular human activities, showing the potential for nucleus pulposus tissue engineering (Nair et al. 2015).

The development of scaffolds with superior mechanical properties for bone and cartilage engineering is often challenging because the implanted scaffold requires sufficient integrity from the time of implantation until the end of tissue remodeling (O'Brien 2011). Therefore, many efforts have been made to improve the mechanical strength of scaffolds. For example, silica nanoparticle-alginate-polyacrylamide nanocomposite hydrogels were thoroughly tested to evaluate the potential for cartilage replacement. It was suggested that the mechanical improvements were due to the strong interfacial binding between the nanoparticles and the polymer matrix, causing effective stress transfer between the two main constituents (Arjmandi and Ramezani 2019).

Bioactive nanohydroxyapatite particles were used to reinforce poly(L-lactide) films and scaffolds as potential biodegradable scaffolds for bone implants. There were increased elastic modulus and yield stress of the composite materials, which was probably due to the restricted C-C bond rotations and polymer sliding (Díaz et al. 2019). Bioactive glass nanoparticle is also an attractive material for bone tissue engineering. El-Fiqi et al. have developed the collagen hydrogel incorporated with surface-aminated mesoporous nanobioactive glass nanoparticles. It was suggested that the chemical interactions that occurred in the composites have led to an increase in resistance to loading and stiffness (El-Fiqi et al. 2013). Besides, various types of metal-based nanoparticles such as gold nanoparticle (Russo et al. 2017), iron oxide nanoparticles (Yang et al. 2019), copper oxide nanoparticles (Sahmani et al. 2019), zinc oxide nanoparticles (Sahmani et al. 2018), and aluminum oxide nanoparticles (Wei et al. 2019) were incorporated into bone scaffolds and implants for mechanical enhancement.

13.2.3.5 Growth Factor Delivery

In tissue engineering, one of the main strategies is scaffold-based delivery of signaling molecules such as growth factors (Lee et al. 2011). However, growth factors are highly sensitive to heat, pH, and proteolytic degradation. Therefore, the application of delivery systems using carrier such as nanomaterials is crucial to maintain stability, bioactivity, and controlled delivery (Lim et al. 2010).

Rajam et al. have developed a porous collagen-chitosan scaffold by the freeze-drying method. Epidermal growth factor (EGF) and fibroblast growth factor (FGF)

were encapsulated in chitosan nanoparticles and impregnated in the porous scaffold for dual growth factor delivery. Controlled release of growth factors and significant enhanced cell viability and activity of fibroblast cells were reported (Rajam et al. 2012). The bladder acellular matrix allograft was modified with vascular endothelial growth factor (VEGF)-loaded PLGA nanoparticles. Long-term sustained release of VEGF was demonstrated as a potential solution to overcome the issue of insufficient angiogenesis in bladder tissue engineering (Jiang et al. 2015).

Besides, nanoporous silica nanoparticles are the attractive nanomaterials due to the large specific surface area, controllable particle size, large pore volumes, biocompatibility, and availability for surface modifications (Neumann et al. 2013). Schmidt et al. have tested the long-term delivery of brain-derived neurotrophic factor (BDNF) from nanoporous silica nanoparticles. The release of BDNF over 39 days has shown to improve the survival rate of spiral ganglion neurons, showing the potential of BDNF-releasing nanoporous silica nanoparticles in the development of cochlear implant-based growth factor delivery system (Schmidt et al. 2018). Metal-based nanoparticles were also studied for growth-factor delivery. For example, basic fibroblast growth factor (bFGF) was conjugated to magnetic iron oxide nanoparticles. The adult nasal olfactory mucosa cells that were seeded in fibrin hydrogel scaffold containing of bFGF-conjugated nanoparticles exhibited enhanced migration, growth, and proliferation as compared to the treatment of same or higher concentration of free bFGF (Ziv-Polat et al. 2012).

13.2.3.6 Gene Delivery

Despite the attractive potential of growth-factor delivery for tissue regeneration purposes, the approach is limited by some challenges such as high toxicity due to explosive release, the short half-life of growth factors, high costs of purified growth factors (Hadjizadeh et al. 2017). As an alternative, localized gene delivery can be conducted to provide a continuous expression of specific growth factors (Krebs et al. 2010). Generally, gene delivery involves viral and non-viral vectors as carriers to transfer genetic material into the cells (Hadjizadeh et al. 2017). Encapsulation of genes in the delivery carrier is crucial to protect the gene before it reaches the target. Although viral vectors show high efficiency, there are concerns such as virus replication and inflammatory reactions (Zhu et al. 2005). Therefore, there is an increase in the studies on non-viral vectors such as nanoparticles, due to the easy in preparation, controllable properties, absence of recombination potential, and low immunogenicity (Lin et al. 2018).

In a study on bone tissue engineering, plasmid DNA encoding for bone morphogenetic protein-2 (BMP-2) was complexed with calcium phosphate, and the calcium phosphate-DNA nanoparticles were incorporated in alginate hydrogel. The bone formation capacity of transplanted MC3T3-E1 pre-osteoblast cells was enhanced (Krebs et al. 2010). On the other hand, hyaluronic acid/chitosan/plasmid-DNA

nanoparticles encoding transforming growth factor (TGF)-β1 were embedded in porous chitosan scaffold. A sustained release of plasmid DNA over 120 days was reported, and the cultured chondrocytes exhibited enhanced expression of TGF-β1, showing the potential of this system for cartilage tissue engineering (Lu et al. 2013). The importance of scaffold composition for this delivery approach was highlighted as it was found to affect the transfection efficiency (Raftery et al. 2015). Recently, an innovative approach was conducted by loading the nanoparticle/gene complexes onto the surgical suture to reduce the possibility of leakage during the injection. Based on the results of *in vitro* and *in vivo* studies, bFGF and VEGF-gene loaded nanoparticle/coated sutures were shown to be promising for the healing of injured tendons (Zhou et al. 2019).

13.3 Nanofibers for Tissue Engineering and Implant Development

In most cases, scaffolds with nanofibrous structures resemble the native ECM. Therefore, nanofabrication approaches such as electrospinning, phase separation and self-assembly have progressed significantly to the development of nanoscale biomimetic scaffolds (Fig. 13.3).

13.3.1 Methods of Preparation

13.3.1.1 Electrospinning

The discovery of electrospinning technology was traced back in 1600. According to Tucker and co-authors, William Gilbert started the study on electrostatic of a liquid, which eventually led to technological evolutions on electrostatic production and electrospinning of solution to form nanofibers (Tucker et al. 2012). Currently, electrospinning is the most effective approach to produce controllable highly nanofibrous membranes from polymer solutions. High voltage was applied to the polymer solution to form a Taylor cone at the tip of the needle. With enough applied voltage, the polymer solution was then elongated to charged fluid jet. In the process, the solvent was evaporated, and nanofibers were collected at the grounded collector (Law et al. 2017). Fabrication of nanofibers using electrospinning technique produces the nanofibers with diameters ranging between 50 and 5000 nm. Furthermore, electrospinning offers the flexibility to produce various types of nanofibers, for example, 3D nanofibers, hybrid nanofibers, core-shell nanofibers, hollow and porous nanofibers (Cao et al. 2017; Nadim et al. 2017; Park et al. 2016).

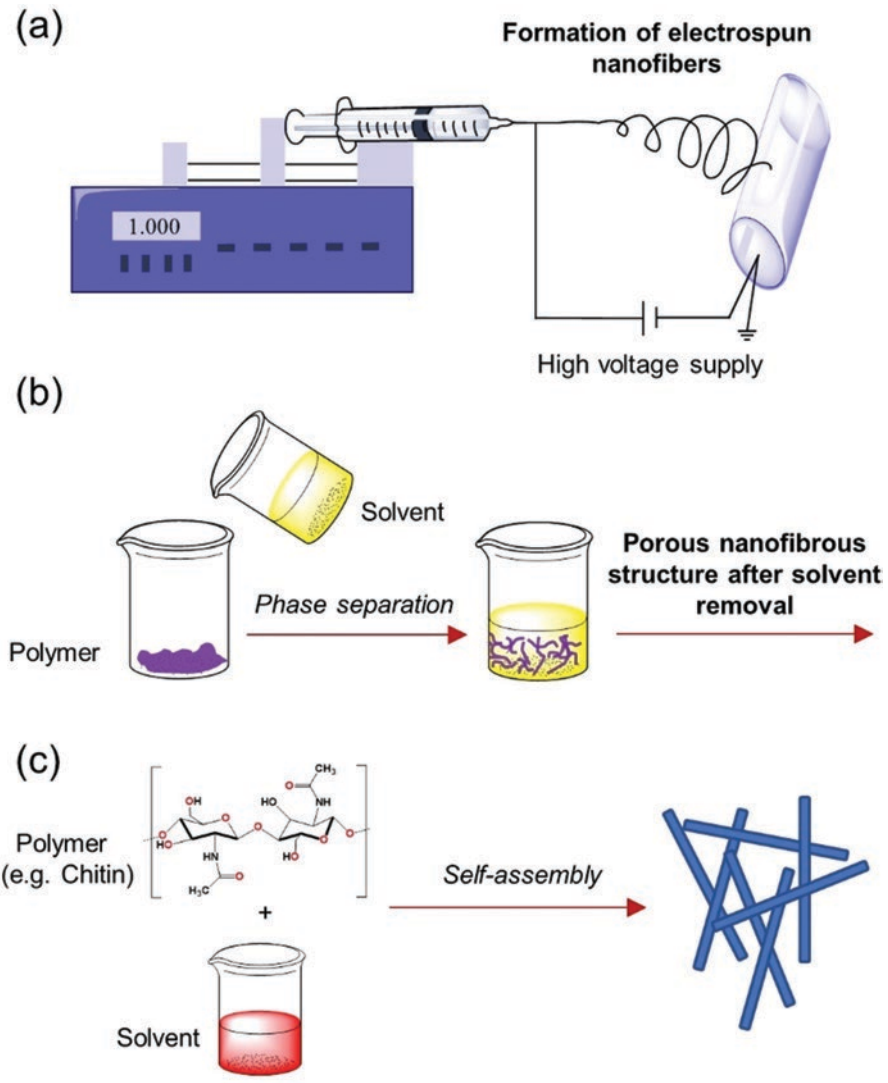

Fig. 13.3 Illustration of nanofibers fabrication technique namely (**a**) electrospinning, (**b**) phase separation and (**c**) self-assembly

13.3.1.2 Phase Separation

Phase separation occurs by removing the thermal energy of homogenous polymer solution at a certain temperature to trigger phase separation followed by the formation of two distinct liquid phases namely polymer-rich and polymer-lean (contains non-solvent system). The solvent was then removed by freeze-drying (sublimation). The polymer-rich liquid phase organized into solidified skeleton monoliths while

the polymer-lean phase flows within the skeleton monolith to form micro- and nanoporous structures (Kim et al. 2016; Zhao et al. 2011; Kang et al. 2016). Phase separation approach can be divided into four main types namely thermally induced phase separation (TIPS), non-solvent induced phase separation (NIPS), vapor induced phase separation (VIPS) and solvent evaporation. However, TIPS and NIPS are commonly used methods for the creation of nanofibrous porous structure (Kim et al. 2016; Jung et al. 2016). Studies reported TIPS method produced nanofibrous structures with 50–500 nm in diameter and microporous structures favored for cell adhesion (Zhao et al. 2011; Chen et al. 2018). Liu et al. (2014) developed 3D PCL nanofibrous scaffolds using liquid-liquid TIPS method from a PCL/dioxane/water (polymer/solvent/non-solvent) system. The porosity and nanofibrous structure can be controlled through the gelation temperature (T_{gel}), volume ratio of dioxane:water and composition of sugar (porogen). Furthermore, the fabricated scaffolds demonstrated the ability to modulate the formation of a uniform bone-like apatite layer. Chen and colleagues introduced a modified TIPS technique called cloud point-TIPS (CP-TIPS) reported in 2018 to develop poly l or d (L-lactic acid) (PLLA)-based scaffolds. Cloud point is defined as the temperature when clear polymer solution forms cloudy appearance. In the CP-TIPS method, the PLLA/dioxane/water system was maintained 1–2 °C above the cloud point before the phase separation process. As a result, the PLLA scaffolds formed macroporous structure of 300 μm (average diameter) with pore wall composed of 250 nm (average diameter) nanofibers. Chitosan was uniformly dispersed within the highly porous PLLA structure to improve the scaffold's hydrophilicity, degradation and mechanical properties for bone regeneration (Chen et al. 2018). Zhao et al. (2011) prepared chitosan acetate nanofibers by solid-liquid phase separation method. In this approach, Zhao and colleagues tested three different cooling temperatures of −18 °C, −80 °C and instantaneous freezing in liquid nitrogen (2 h) as well as different chitosan and acetic acid concentrations. They found that at optimal conditions of 0.05% (v/v) chitosan, 0.025% (v/v) acetic acid and instantly cooled in liquid nitrogen resulted in a low crystal growth rate of chitosan acetate during the solid-liquid phase separation process. As a result, chitosan acetate nanofibers with diameters ranging between 50 and 500 nm were formed.

13.3.1.3 Self-Assembly

Likewise, self-assembly is another common nanofibrous scaffold fabrication method. This bottom-up approach involves the spontaneous interaction between pure molecules in aqueous environments that results in the formation of insoluble nanofibrous structure. The nanofibrous structures were stabilized by non-covalent bonds, for example, intermolecular hydrogen bonds, electrostatic and van der Waals forces (Sukegawa et al. 2017; Zhong et al. 2010). Zhong et al. (2010) studied the self-assembly of chitin nanofibers in two different environments. First, chitin nanofibers with an average diameter of 10.2 ± 2.9 nm were generated by dissolving the chitin in LiCl/N,N-dimethylacetamide (DMAC) to destabilize the hydrogen bonds,

followed by the addition of water. On the other hand, ultrafine chitin nanofibers (2.8 ± 0.7 nm) were produced by breaking the hydrogen bonds of starting chitin materials in hexafluoro-2-propanol (HFIF), followed by the evaporation of the solvent to obtain the insoluble nanofibers. In another study by Hassanzadeh et al. (2016) reported one-pot self-assembly of ultrafine chitin nanofibers within crosslinked gelatin methacryloyl (GelMA). The elastic modulus of the crosslinked GelMA hydrogel increased significantly by 1000-fold after chitin nanofibers reinforcement. The co-culture of human umbilical vein endothelial cells (HUVECs) and human mesenchymal stem cells (HMSCs) exhibited 90% viability over 5 days of incubation. Further vascularization study proved the expression of vasculogenic markers suggesting the cell proliferation and formation of primitive vascular structures.

13.3.2 Potential Applications of Nanofibers

Nanofibers-based scaffolds expressed the most distinctive characteristics of the native ECM due to their high surface-to-volume ratio, mechanical properties, porosity, and flexibility. Therefore, the recent application of nanofibers-based scaffolds for bone, cartilage, skin and cardiovascular tissue engineering is discussed in this section.

13.3.2.1 Bone Regeneration

Natural human bone composed of 70 wt% hydroxyapatite nanocrystals and 30 wt% of collagen fibrils (Fan et al. 2010). Typically, bone defects caused by traumatic injuries, bone-related diseases, congenital bone disorders, and infections may result in a slower healing process that hugely affecting the musculoskeletal health. In the current clinical treatments, bone implants and grafts are promising treatments for partial recovery of the defected sites compared to the healthy bones. Several factors such as surface chemistry, physical, mechanical properties, and surface topographies influenced the quality of the implants and biological response of surrounding tissues for successful implantations (Martínez-Calderon et al. 2016). Above all, surface topographies at the nanoscale level provide higher surface energy and a conducive environment to enhance the adsorption of matrix proteins such as fibronectin and vitronectin to trigger cellular migration and proliferation and eventually osseointegration process (Martínez-Calderon et al. 2016; Oliveira et al. 2017). Therefore, surface modification of biomedical implants using nanotechnology approach has sparked the interest of the research community to improve the biocompatibility and mechanical properties. Chozhanathmisra and co-workers studied on the bilayer coating of titanium alloy with nanomaterials coating composed of zinc/halloysite nanotubes (Zn-HNT)/ strontium (Sr^{2+}), samarium (Sm^{2+}) substituted hydroxyapatite

(M-HA). The bilayer coated-titanium alloy demonstrated excellent antibacterial and bioactivity properties for orthopedic applications (Chozhanathmisra et al. 2016). Most commonly, bone tissue engineering scaffolds were designed with bioactive inorganic molecules such as nano-hydroxyapatite (nHAP) that stimulate calcium phosphate mineralization to promote osteogenic differentiation process (Khan et al. 2019).

13.3.2.2 Cartilage Regeneration

Cartilage composed of specialized cells called the chondrocytes. Cartilage is commonly present in the joint areas that connect the bones to prevent friction and provide flexibility to movements. As chondrocytes are confined in tight cavities called lacunae with limited blood vessels for nutrient transports, therefore, cartilage has very slow growth and regeneration rate as opposed to rapid-healing organs such as the skin (Sharifi et al. 2020). However, the regeneration and repair of the damaged cartilages due to rheumatoid arthritis, osteoarthritis and injury are achievable using tissue engineering approach. Currently, studies are focusing on adapting the nanotechnology to fabricate scaffold-based nanofibers suitable to support the cartilage regeneration. Studies have reported various nanofiber composite scaffolds aimed for cartilage regeneration such as small heterocyclic compound-nanofibers, co-electrospun composite nanofibers, hybrid nanofibers and modified natural-based nanofibers (Silva et al. 2020; Agheb et al. 2017; Cao et al. 2017; Sharifi et al. 2020).

13.3.2.3 Skin Regeneration

Skin is the largest human organ of the integumentary system. Structurally, the skin is made up of two specialized layers called, the epidermis and dermis. Besides, the skin's structure contains many specialized cells, nerves, blood vessels, and receptors to protect against pathogens and regulate body temperature to maintain the organs' function. Furthermore, the skin could sense various types of sensations such as cold, warm, pain, and physical pressure (Khalili et al. 2019). However, severe skin defects due to burns, congenital defects, chronic diseases, and accidents can cause infections, disability and many negative effects (Park et al. 2016). Current clinical treatments are expensive especially in the case of prolonged hospitalization. Apart from shortage of skin donor, skin transplantation could cause uneven skin texture, reduced skin sensation and limited joint movements due to scar contracture (Chouhan et al. 2018; Min et al. 2014). Therefore, attaining full-recovery and scar-free of healed skin are still the main challenges in skin tissue engineering considering the complex structure of skin tissue. A work by Park et al. (2016) compared the co-culture characteristics of human dermal fibroblast (HDF) and human skin keratinocytes (HaCAT) to form artificial bilayer skin substitutes on silk-fibroin scaffolds fabricated using electrospinning, freeze-drying and salt leaching techniques. Based

on histology examination, they found that the electrospun silk-fibroin nanofibers showed prominent HDF proliferation in a deeper part of the nanofibers followed by differentiation of HaCAT in the superficial layer.

13.3.2.4 Vascular Tissue Engineering

Cardiovascular diseases (CVD) are among high-risk diseases due to the dysfunction of heart and blood vessels of the cardiovascular system. CVD includes coronary heart disease, arterial disease that affects the blood supply to the heart, brain (cerebrovascular disease) and peripheral regions, as well as strokes (Cheng et al. 2020; Frayn and Stanner 2018). In fact, 23 million mortality is expected worldwide due to cardiovascular diseases by 2030 (Frayn and Stanner 2018). CVD accounts for more than 30% of death among elderly patients. The risk factors associated with CVD include patients with a history of glucose imbalance, elevated low-density lipoprotein cholesterol (LDL-C), hypertension, aging and unhealthy lifestyles (Kankala et al. 2018; Gao et al. 2019). These days, vascular bypass grafting is commonly used to treat ischemic heart disease and peripheral artery disease. However, the commercial vascular bypass grafts made of either polyester (Dacron®) or expanded polytetrafluoroethylene (ePTFE) (Teflon®) are known to work effectively for large arteries with an inner diameter of more than 6 mm (Hu et al. 2010; Kannan et al. 2005). The grafting of small-diameter arteries (<6 mm) with the commercially available grafts could increase the occurrence of occlusion, restenosis and infections eventually causing the bypass graft failure (Liu et al. 2020). A recent study by Liu et al. (2020) reported the fabrication of poly(L-lactide-co-caprolactone) (PLCL) nanofibers using the core-spun electrospinning technique followed by grafting with tussah silk fibroin (TSH). They further report the successful fabrication of biomimetic PLCL/TSF nanofibers as potential small-diameter vascular graft with an inner diameter of less than 1.5 mm. Besides, PLCL/TSF nanofiber vascular scaffolds promoted the *in vitro* adhesion and proliferation of vascular endothelial cells (VECs) along the axial direction of the nanofibers.

13.4 Nanotubes for Tissue Engineering and Implant Development

Nanomaterials such as nanotubes showed similar physicochemical properties to the native human tissues, thus gaining tremendous attention as reinforcement agents and carriers to deliver therapeutic agents for various biomedical applications (Kumar et al. 2020).

13.4.1 Carbon Nanotubes Nanocomposite Scaffolds/Implants

Carbon nanotubes (CNTs) are made of carbon and its allotropes. CNTs are sheets of 2D graphene rolled up into 3D cylindrical shapes. The length of hollow CNTs can reach up to several micrometers with an approximate diameter of 100 nm. CNTs are categorized based on the number of carbon layers namely single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) (Edwards et al. 2009; Anzar et al. 2020). The rod-like shape of CNTs resembles the fibrillar proteins of native ECM, which match the requirements of excellent scaffolds for tissue engineering applications. Although the toxicity of CNTs for biomedical applications is still unknown, however, it is clear that the toxicity levels vary depending on the nanotubes size and amount of impurities formed during the CNTs synthesis (Venkatesan et al. 2012). Therefore, researchers found that alternatively the biocompatibility of CNTs towards living cells can be enhanced by incorporating the nanomaterials within biomaterials matrices or functionalization of CNTs surfaces with functional groups such as carboxylic acid group (-COOH), hydroxyl (-OH) and thiol (-SH) (Ravanbakhsh et al. 2019; Robati et al. 2016). For example, Ravanbakhsh et al. (2019) observed the average pore size of glycol chitosan/glyoxal composite hydrogels reinforced with 250–750 μg/ml of carboxylic acid functionalized-CNTs (COOH-CNTs) increased significantly between 30% and 120%. Furthermore, they reported the composite hydrogels reinforced with higher concentration of COOH-CNTs showed higher swelling ratio, which is significantly important to induce cell migration and proliferation. Studies reported significant growth and proliferation of cells on scaffolds reinforced with CNTs compared to scaffolds without CNTs.

13.4.2 Halloysite Nanotubes Nanocomposite Scaffolds/Implants

Halloysite nanotubes (HAL) are naturally occurring aluminosilicate with a chemical formula of $Al_2Si_2O_5(OH)_{4\cdot n}H_2O$. Naturally, HAL dimensions vary between 100 and 1500 nm in length, an outer diameter of 20–200 nm and inner lumen diameter of 10–70 nm (Govindasamy et al. 2014; Zheng et al. 2019). The charge polarity of nanotubular HAL consist of negatively charge external surface and positively charged inner lumen (Yendluri et al. 2017). It was reported that HAL could effectively be incorporated into the scaffolds as fillers to improve the mechanical properties. Therefore, in tissue engineering field, the nanotubes are at an advantage compared to nanoparticles due to their uniform distribution within polymers, hydrophilicity, drug entrapment and non-toxic properties (Liu et al. 2015; Yendluri et al. 2017). Studies reported the entrapment of drugs such as paclitaxel, doxorubicin, ibuprofen, dexamethasone, diphenhydramine hydrochloride and diclofenac sodium *via* adsorption, intercalation and tubular entrapment mechanism (Yendluri et al.

2017; Wu et al. 2018; Ghaderi-Ghahfarrokhi et al. 2018). The abundant hydroxyl groups (-OH) on the external and internal surfaces of HAL allows for surface functionalization with bioactive molecules such as folate for targeting cancer therapy (Wu et al. 2018). Besides, the -OH groups modification of HAL to carboxylic acid (-COOH) and amine ($-NH_2$) demonstrated sustained release of drug molecules and improved cell attachment (Ghaderi-Ghahfarrokhi et al. 2018). Moreover, HAL demonstrated ideal nanomaterials to induce nutrient transportation such as protein absorption for better cell attachment and proliferation (Zhao et al. 2013b; Cai et al. 2015). For example, Cai et al. (2015) observed uniform HAL distribution of co-electrospun HAL/PLLA nanofibers with straight and aligned nanotubes along the fiber axis. Instantly, the uniform distribution of HAL improved the mechanical properties of the organic/inorganic hybrid nanofibers system.

13.4.3 TiO_2 Nanotubes Nanocomposite Scaffolds/Implants

Titanium alloys are commonly used in high loadbearing orthopedic and dental applications due to excellent mechanical strength and resistance against corrosion. However, titanium alloy-based implants are inert, non-biocompatible, which could lead to poor osseointegration at the implantation sites (Chozhanathmisra et al. 2016; Oliveira et al. 2017). Nanostructured titanium dioxide (TiO_2) forms spontaneously on the surface of titanium alloys when exposed to air or aqueous electrolytes. The nanostructured TiO_2 in various forms, for example, nanotubular, honey-combs, wires and rods can be fabricated using hydrothermal, sol-gel and electrochemically by anodization (Neupane et al. 2011; Oliveira et al. 2017). Additionally, the biocompatibility of nanotubular TiO_2 can be enhanced through surface modification technique as reported in several literatures. Predominantly, the surface modification could also improve the bone-implant integration and minimize post-implantation complications which eventually improve the recovery time post-surgery (Gunputh et al. 2020; Neupane et al. 2011). Neupane et al. (2011) studied the surface modification of nanotubular TiO_2 with gelatin stabilized gold nanoparticles (AuNPs gelatin) for biomedical implants. They observed uniform distribution of gelatin stabilized gold nanoparticles on the TiO_2 surfaces due to the effective interaction between the positively charged gold nanoparticles and the negatively charged TiO_2 nanotubes surface. Finally, *in vitro* assay confirmed the biocompatibility of TiO_2 nanotubes-AuNPs gelatin surface modified with MC3T3-E1 osteoblast cells. Gunputh et al. (2020) reported the uniform distribution of silver nanoparticles within the nanotubular TiO_2 matrix that spontaneously grown on the titanium alloys. In addition, the TiO_2 nanotubes loaded silver nanoparticles with an average diameter of 47.5 ± 1.7 nm demonstrated excellent antibacterial activities against *Staphylococcus aureus* (gram positive). They further concluded that the coating with nHAP maintained the slow release of silver nanoparticles, which is important to prevent bacterial infections during long-term bone regeneration.

13.5 Industrial Perspective of Nanotechnology

Over the years, patents on nanomaterials that are potential for tissue engineering and implant development are available. The examples of inventions are presented in Table 13.1.

Table 13.1 Recent granted patents for the use of nanotechnology in tissue engineering and implants development

Patent number	Type of nanomaterials	Description of Invention	References
WO2009006905	Nanoparticle	Synthesis of dehydrated chitosan-based siRNA nanoparticles, as potential nanoparticles for transfection of cells on tissue engineering scaffolds.	Kjems et al. (2009)
WO2010087912A1	Nanoparticle	Synthesis of hydrogels crosslinked with gold nanoparticles for tissue and organ engineering.	Prestwich et al. (2010)
DE102007029672	Nanoparticle	Methods of implant surface coating with nanoparticles using "mild" process conditions.	Barcikowski and Schuessler (2009)
US20100255447A1	Nanoparticle	Methodologies and compositions of bio-compatible polymer surface coatings for implants and tissue engineering scaffolds.	Biris et al. (2010)
US20080112998A1	Nanofibers	The invention describes the deposition of electrospun micro or nanofiber layers (several layers consist of fibers loaded with bioactive molecules) and layers of living cells to create bioengineered 3D tissue.	Wang (2008)
WO2013109642A1	Nanofibers	A method of fabrication nanofibers as scaffolds with basketweave configuration to resemble the native cardiac tissue includes electrospinning of biodegradable polymer to create the electrospun mats.	Xie (2017)
US20160067375A1	Polymer nanofibers, nanotubes–carbon nanotubes (CNTs)	Fabrication of 3D biomimetic scaffolds loaded with CNTs using electrospinning and 3D printing for osteochondral regeneration.	Holmes and Zhang (2016)
US9440003B2	Nanotubes—carbon nanotubes (CNTs)	Implantable and insertable medical devices coated with porous diamond-like coatings comprised of CNTs.	Weber (2016)

13.6 Challenges and Future Perspectives

In conjunction with the fast development of nanotechnology in regenerative medicine research, the safety issues of nanomaterials have also received considerable attention. As a result, a branch known as nanotoxicology has emerged to investigate the health effects of nanomaterials (Jia et al. 2017). Owing to the tiny size of nanomaterials, they can penetrate the biological membrane easily and enter the cells. Also, they can access the circulatory system through multiple routes, such as inhalation, ingestion, oral routes, as well as skin penetration. Therefore, the translocation to body and tissues can occur easily (Das et al. 2016). One challenge of the research in this area is the limited physiological relevance of conventional *in vitro* testing methods (Fröhlich 2018). Inconsistent toxicological data was obtained due to the unique physicochemical properties of nanomaterials (Bahadar et al. 2016). By considering the promising potential of nanomaterials in the biomedical field, more advanced testing systems such as 3D culture should be utilized for a better understanding of the mechanism of toxicity (Elje et al. 2020).

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