Chapter 2
Discrete Nano Biomaterials

Polymeric Nanoparticles as Nano Biomaterials

2.1 Introduction

Polymeric nanoparticle-based therapeutic systems are significantly impacting the future of biomedicine. The last few decades have seen a rapid rise in the development of a variety of polymer-based nanoparticle systems targeting disease diagnosis and drug delivery (Brannon-Peppas and Blanchette 2004). These polymeric therapeutic agents predominantly focus on the treatment of cancer, diabetes, asthma, and infectious diseases etc. Polymeric nanoparticles also show great promise as efficient nanocarriers for controlled drug delivery applications. Some of the significant benefits of polymeric nanoparticles include biocompatibility and biodegradability; sustained release of drugs; simultaneous delivery of multiple drugs; delivery of less soluble drugs; and easy functionalization for targeted drug delivery (Singh and Lillard 2009). Further, the non-toxic nature of polymers such as poly-(D, L-lactide-co-glycolide), poly(lactic acid), poly(glutamic acid), poly-(amino acids), and poly(Ɛ-caprolactone) makes them safe for in-vivo administration of drugs (Acharya and Sahoo 2011). Recent innovations in design and synthesis methods of nanoparticles has facilitated advanced functionalities such as specific targeting of disease, temporal drug release, stimuli responsiveness, and multiple drug encapsulation (Hu et al. 2014). With significant developments in polymer engineering and synthetic techniques, polymeric nanoparticles are now consistently produced with targeted application (Kim et al. 2012). These advances significantly improve the chances for polymeric nanoparticles towards clinical translation. More such clinical studies will unravel less known interactions between the polymeric nanoparticles and biological systems. With increased focus, research has now shifted towards producing polymeric nanoparticles that can evade the multi-faceted nature of immune clearance and deliver better therapeutic efficiency.

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2.2 Synthesis Approaches

Polymer nanoparticle therapeutics refers to a mixture of polymer and active drug components that are formed in different ways. For instance, drug components can be incorporated into the polymer during or after the polymer is formed. Active drug components are often encapsulated within the polymer to create nanocapsules, but active drug components can also be absorbed on the surface of the polymeric nanoparticles to create nanospheres (Fig. 2.1). Similarly, polymeric nanoparticles can be formed in two different ways. (a) In the bottom-up approach, monomers of respective polymers react in an emulsion/micelles, resulting in the formation of the polymer known as latex. (b) In the top-down approach, preformed polymers are used to generate nanoparticulate polymers.

2.2.1 The Bottom-up Approach

In the bottom-up approach, polymeric nanoparticles are formed from monomers. The formation of polymers is often catalyzed by suitable agents and performed in an aqueous system and aided by emulsion/micelles. This emulsion polymerization-based synthesis approach is widely used for the preparation of polymer nanoparticles and was first used to prepare biodegradable polyalkylcyanoacrylate nanoparticles for drug delivery (Couvreur et al. 1979). In this process, the monomers react at the interface of the hydrophilic and lipophilic phases and form oligomers, which further form the desired polymers on continued reaction. The

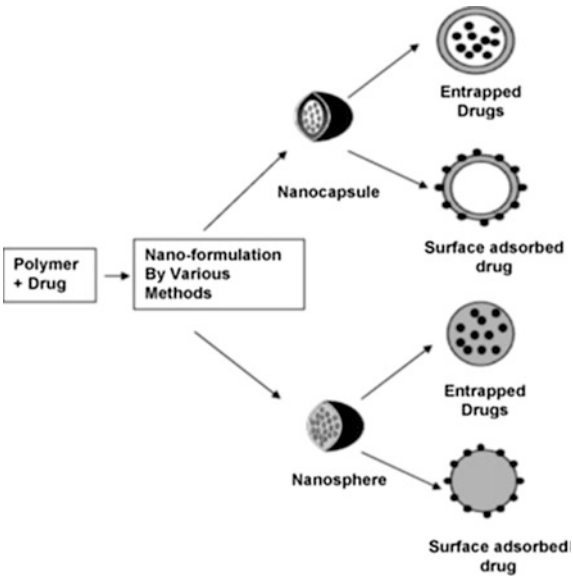

Fig. 2.1 Type of polymeric nanoparticles based on the presence of drug (Kumari et al. 2010)

polymerization reactions generally occur spontaneously but are sometimes aided by an external energy source, such as heat or light. The polymerization reaction typically continues until all the monomers are exhausted or until reactive groups are present on the products. The reaction can be controlled by the monomer concentration or by external factors, such as the temperature and pH of the medium. The encapsulation of the active drug within these polymeric particles depends mainly on their solubility in the aqueous or the organic phase. Sometimes, the active drug molecules may also interact with activated monomers during the polymerization reaction leading to their inactivation (Grangier et al. 1991). The most commonly used techniques in bottom-up approaches are (a) Emulsion, (b) Mini emulsion, (c) Micro emulsion, (d) Interfacial polymerization, and (e) Controlled/Living radical polymerization(C/LRP) (Fig. 2.3).

2.2.2 The Top-down Approach

Not all polymeric materials can be prepared from the bottom-up using emulsion polymerization. For instance, nanoparticles of polyesters, epoxy-ethers, polyurethanes, and also semi-synthetic cellulose polymers cannot be obtained using the emulsion approach and are not dispersible in water. To overcome this limitation, preformed polymeric materials are utilized to develop polymeric nanoparticles. The physicochemical and biological properties of polymers formed using conventional methods can be effectively controlled. Thus, they have to be adapted and optimized to obtain nanoparticles for desired applications. The polymeric colloidal nanoparticles are formed by dissolving the preformed polymer in an organic or supercritical fluid. The solution is then emulsified using water, followed by solvent evaporation or controlled desolvation. The polymer's nanoparticles are then obtained by decreasing the droplet size of the emulsion to the nano-size. Methods such as sonication, high-pressure homogenization, or microfluidics are utilized to accomplish this process (Nagavarma et al. 2012; Karnik et al. 2008). Industrial methods to obtain polymeric nanoparticles from water-insoluble polymers are mostly generic and follow processes such as (a) solvent evaporation from emulsions, (b) solvent displacement through diffusion or nanoprecipitation, (c) salting-out, (d) dialysis, and (e) supercritical fluid technology (SCF) (Fig. 2.2). While these methods are commonly used, there are also other, less commonly used methods for polymeric nanoparticle preparation.

2.3 Materials for Polymeric Nanoparticles and Their Applications

The chemistry of nanoparticulate polymers may also provide suitable structures for applications such as encapsulation, drug delivery, controlled drug release, and lower molecular weight drugs. However, a major concern in using polymers is their

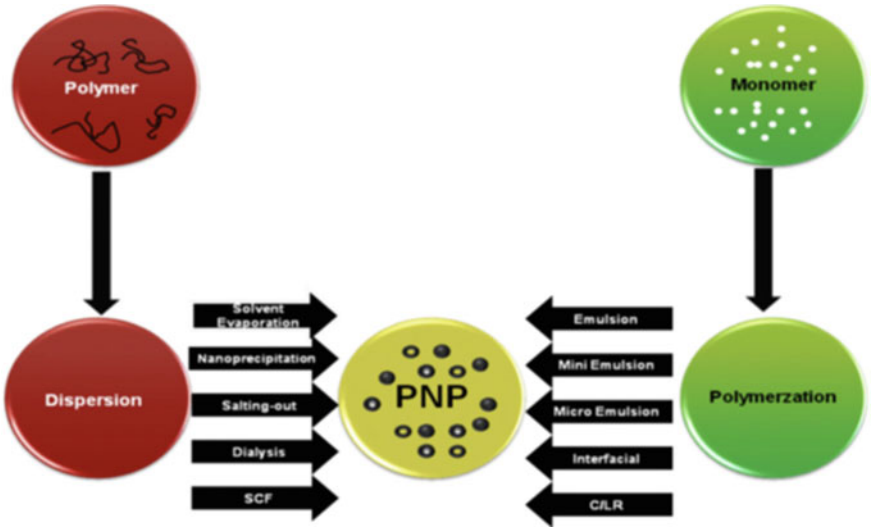

Fig. 2.2 Schematic representation of different techniques for the preparation of polymer nanoparticles (Rao and Geckeler 2011)

toxicity; therefore, polymeric nanoparticles must also be biocompatible and biodegradable. Initially, polyacrylates (Vert et al. 1994) and polyesters (Schmidt and Lamprecht 2009) were explored for this reason. Later, many other polymers, both naturally occurring and synthetic, were investigated for the development of drug delivery systems (Lai et al. 2014).

2.3.1 Naturally Occurring Polymers

Some of the main benefits of using natural polymers (such as chitosan, gelatin, sodium alginate, and albumin) is their biocompatibility, low cost, and water solubility. Alternatively, some of their limitations include low hydrophobicity, which hinders the lipophilic drug entrapment, and the presence of varying amounts of extraneous contaminants between batches. Generally, natural polymers are well-known for their safe use in humans and for their compatibility with both the human body and with drugs. In addition, natural polymers are also water-soluble, but they can be converted into nanoparticles by denaturation processes, which lead to reduced water-solubility. Nanoparticles can be formed from natural polymers by coacervation, which occurs when oppositely charged counter-ions combine by electrostatic neutralization. One of the well-known protein substitutes for human use, albumin, is completely compatible even at higher quantities and has surface properties that suit the stabilization of polymeric nanoparticles (Lai et al. 2014). Further, albumin has also shown stabilization of paclitaxel drugs during its

preparation (Desai et al. 1999). Drug nanoparticles that are stabilized by protein denaturation are often covered by layers of albumin. The denaturation of protein usually occurs by cross-linking aldehyde groups, but it can also be triggered by shear forces from the evaporation from emulsions.

Another naturally occurring polymer, gelatin, is extensively used in the pharmaceutical industry (Elzoghby 2013). Gelatin is a protein that is commonly obtained from the hydrolysis of collagen. This biodegradable material is attractive for use in polymeric nano-therapeutics. Gelatin can either be used as the major polymer component to encapsulate the drug, or it can be deposited on the surface of the nanoparticle that contains the drug. Different types of gelatin provide many options for forming suitable polymeric nanoparticles. Because of their biocompatibility and biodegradability, gelatin nanoparticles have been widely utilized as drug and gene carriers to targeted tissues for treating cancer, HIV infection, and tuberculosis. They have also been used in the treatment of vasospasm and restenosis (Tian et al. 2013). Gelatin polymer is also used as a coating around quantum dots (QDs) to reduce QD cytotoxicity (Byrne et al. 2007). Additionally, gelatin nanoparticles can cross blood brain barriers, which may also assist in treating brain disorders (Tian et al. 2012). Gelatin nanoparticles are also reported to target macrophages and treat different ailments (Nahar et al. 2010). Gelatin, additionally, is actively used in tissue and bio-engineering to construct three dimensional scaffolds to generate artificial tissues and organs.

Chitosan is a naturally occurring linear polysaccharide with randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-D-glucosamine (acetylated unit). The deacetylated chitin has a great potential for use in biomedicine. For instance, chitosan has been reported to increase paracellular permeability of intestinal epithelia, where the property of transmucosal absorption enhancement has been attributed to chitosan polymers (Artursson et al. 1994). Chitosan is also an excellent material for vaccine delivery research because of its biocompatibility, low production costs, and low toxicity. One significant advantage of using chitosan micro- or nanoparticles is their water-based preparation and loading, which helps avoid the use of organic solvents that may alter the immunogenicity of antigens (Balan and Verestiuc 2014).

2.3.2 Synthetically Obtained Polymers

Most polymeric nanoparticles are based on synthetic or semi-synthetic polymers due to their high stability and reproducible preparation methods (Balan and Verestiuc 2014). The synthesis methods can be tailored to get polymeric nanoparticles' desired chain lengths and types, co-polymer compositions, and molecular weights, which enhances the polymer's performance for the desired application. Further, by tailoring the chemical composition and molecular structure of the nanoparticles, their physical properties (e.g. their temperature, pH sensitivity, and response to other stimuli) can be controlled. Such control over their properties

enables targeted drug delivery to specific sites (Balan and Verestiuc 2014). However, one of the major drawbacks of synthetic polymer nanoparticles is their sparing solubility in water. They are usually hydrophobic, soluble in mostly organic solvents, and often require surfactants to form stable dispersions in water (Singh and O'Hagan 1998). Some commonly used synthetic biodegradable polymers include Poly glycolic acid (PGA), Polylactic acid (PLA), and the copolymer of the two, PLGA with various ratios and molecular weights. Other examples of synthetic polymers include polyanhydrides, polyorthoesters, polycyanoacrylates, polycaprolactone, polyglutamic acid, polymalic acid, poly(N-vinyl pyrrolidone), poly(methyl methacrylate), poly(vinyl alcohol), poly(acrylic acid), polyacrylamide, poly(ethylene glycol), and poly(methacrylic acid) (Nagavarma et al. 2012). The advantage of using a PLGA copolymer is that it gets degraded easily within the body through the hydrolytic ester bond cleavage of the PLGA into individual units of glycolic and lactic acids. These acids are further metabolized in the Kreb's cycle and are eliminated as water and CO_2 from the body (Panyam et al. 2003). Biodegradable implants were first made from polylactic acid (PLA), where the polymer chains get hydrolyzed by water leaving lactic acid as the byproduct. For drug delivery, polylactide-coglycolide (PLGA) is more suitable because of its biocompatibility, cost efficiency, ease of preparation, and versatile application. Further, the hydrolysis products of PLGA within the body are also biocompatible. For these reasons, PLGA has been approved by FDA as well as by the European regulatory authorities (Edlund and Albertsson 2003).

Another high molecular weight synthetic polymer, Poly(Ɛ-caprolactone) (PCL), is also widely used due to its non-toxicity and biodegradability (Wei et al. 2009). PCL is commonly used for long-term drug delivery (sustained release) applications owing to its higher molecular weight, resulting in slower hydrolysis within the body. The degradation products from the hydrolysis are neutral, so it does not affect the physiological pH. Further, PCL is compatible with other polymers making it easier to form mixtures with different formulations for desired applications.

Another category of synthetic polymers is polyalkylcyanoacrylate nanoparticles, which constitute core-shell nanospheres and oil and water containing nanoparticles. Their properties mainly depend on the side chains that are introduced into the polymers. For instance, introduction of a longer alkyl chain increases the life-time of the particles towards degradation with the body. The hydrophobic nature of the alkyl groups can also affect their degradation behavior and, thus, a drug's release performance (Kreuter 1983).

2.4 Applications of Polymeric Nano-Particles/Biomaterials

2.4.1 Drug Delivery and Transfection

Biodegradable nanoparticles (NPs) have been studied as drug carrying devices for quite some time (Soppimath et al. 2001). Polymers, particularly, have been used

because they can efficiently carry drugs to exact physiological locations, which maximizes therapeutic benefits and results in fewer side effects (Soppimath et al. 2001). Liposomes, too, have been used because they prevent drug deterioration; they carry a drug to target areas; and they decrease a drug's harmful side effects (Soppimath et al. 2001). However, liposomes are also problematic such that they have low encapsulation efficiency; they leak water-soluble drugs when combined with blood; and they do not store stably (Soppimath et al. 2001). Because of these limitations, researchers are investigating biodegradable polymeric NPs, which provide more drug and protein stability and have more control release (CR) properties (Soppimath et al. 2001). In addition, biodegradable polymeric NPs can identify specific organs and tissues that carry DNA and allow proteins, peptides, and genes to be taken orally (Soppimath et al. 2001). These NPs range from 10 to 1000 nm and deliver drugs in different ways (Soppimath et al. 2001). A drug can be dissolved in, attached to, or encased within the NP matrix (Soppimath et al. 2001). Drugs can also be delivered via nanocapsules and nanospheres based upon the technique of drug encapsulation (Soppimath et al. 2001). Nanocapsules are vesicular systems that encase the drug in a hollow space enclosed by a polymer membrane (Soppimath et al. 2001). Nanospheres use a system of matrices to equally disperse the drug (Soppimath et al. 2001). Examples of polymeric NP's include polyalkylcyanoacrylate nanoparticles which have been investigated as a way of delivering cancer chemotherapy drugs throughout the body (Douglas et al. 1986).

Cancer nanotherapeutics are also being used to overcome some of the deficiencies of traditional drug delivery systems. These deficiencies include nonspecific biodistribution and targeting, lack of water solubility, poor oral bioavailability, and low therapeutic indices (Cho et al. 2008).

Nanoparticles, however, have been used to increase biodistribution, to improve active targeting strategies, and to minimize drug resistance (Cho et al. 2008). Nanoparticles, for example, have both ideal size and surface characteristics, which aid in a drug's biodistribution and circulation (Cho et al. 2008).They also have been designed to bring drugs to cancer cells, penetrate them, and remain in the tumor microenvironment (Cho et al. 2008). In addition, nanoparticles have been designed to actively target tumors using ligands or antibodies, which further increase the therapeutic specificity (Cho et al. 2008). Finally, nanoparticles may also be able to minimize drug resistance because they can collect in cells without being recognized by P-glycoprotein, a key mediator of multidrug resistance (Cho et al. 2008). Cancer treatments may also become more customized to individuals through the development of multifunctional and multiplex nanoparticles, which are currently underway (Cho et al. 2008). Currently used in clinical trials, new molecularly targeted anti-cancer agents such as imatinib mesylate (Gleevec®), gefitinib (Iressa®), trastuzumab (Herceptin®), and cetuximab (C225, Erbitux®) can be made more effective by using nanomedicines, including nanoparticles, liposomes, dendrimers, polymeric nano-drug-conjugates and micelles which are being studied in preclinical and clinical studies (Danhier et al. 2010). For detailed information the reader is referred to a recent review (Danhier et al. 2010).

Core-shell magnetic NPs are also used in biomedicine (Arruebo et al. 2007). These NPs have a metal or metallic oxide core, which is surrounded by a synthetic or polymeric shell that helps to make the NPs biocompatible and stable, and provides support for biomolecules (Arruebo et al. 2007). The metallic characteristics allow the NPs to be used in many ways, including as:

i. Magnetic contrast agents in magnetic resonance imaging (MPI) (Cunningham et al. 2005)
ii. Hyperthermia agents, where the magnetic particles are heated selectively by applying a high frequency magnetic field (Johannsen et al. 2005)
iii. Magnetic vectors that are directed using a magnetic field gradient towards a certain location, such as in the case of the targeted drug delivery (Jurgons et al. 2006).

Magnetic NPs may result in medical discoveries relating to cancer diagnosis and treatment and drug delivery (Arruebo et al. 2007).

Hydrogel nanoparticles, or nanogels, also show great potential as a drug delivery system because they combine both properties of hydrogels (e.g. hydrophilicity and high water content) with the small size of nanoparticles (Sasaki and Akiyoshi 2010; Hamidi et al. 2008). Both natural and synthetic polymeric hydrogel nanoparticle systems have been studied and different benefits and disadvantages have been noted (Hamidi et al. 2008). Most research on natural polymeric hydrogel nanoparticles has included chitosan and alginate while the most researched synthetic polymeric hydrogel nanoparticle systems have included poly(vinyl alcohol), poly(ethylene oxide), poly(ethyleneimine), poly(pyrrolidone), and poly-N-isopropyl acrylamide (Sasaki and Akiyoshi 2010; Hamidi et al. 2008). These natural and synthetic polymeric hydrogel nanoparticles have different drug delivery properties (Sasaki and Akiyoshi 2010; Hamidi et al. 2008). Despite the type of polymeric hydrogel nanoparticle used, the release mechanism of the loaded agent from the hydrogel nanoparticles is complex because it occurs via drug diffusion, hydrogel swelling, and chemical reactivity of the drug/matrix (Sasaki and Akiyoshi 2010; Hamidi et al. 2008). Crosslinking has also been used to create hydrogel matrices, which are grouped into chemically and physically induced crosslinking (Sasaki and Akiyoshi 2010; Hamidi et al. 2008). Unlike polymeric nanoparticles, which have a densely packed polymer inside the core structure, nanogels are able to stably retain bioactive compounds such as drugs, proteins, and DNA/RNA inside their nanospace within crosslinked polymer networks (Sasaki and Akiyoshi 2010). Moreover, nanogels show a rapid response to micro environmental factors such as temperature and pH because of their nano-scaled dimension. These properties are useful for the controlled release of bioactive compounds (Sasaki and Akiyoshi 2010). Kabanov et al. reported the first chemically cross-linked nanogels made from poly(ethylene glycol) (PEG) and polyethylenimine (PEI) for the delivery of antisense oligonucleotides (Vinogradov et al. 2004). Akiyoshi et al. reported the first physically cross-linked nanogels using self-assembly of cholesterol-bearing polysaccharides in water through the study of self-organization of amphiphilic polymers (Akiyoshi

et al. 1993). Recently, they have also applied the physically cross-linked nanogels for use as nanocarriers in development of DDS (Morimoto and Akiyoshi 2010). Nanogels can be prepared using a basic scheme depicted in Fig. 2.3.

Bae et al. fabricated biosynthetic nano-delivery platforms (∼size ranging 50–200 nm) for drug delivery applications for hyperthermic combination chemotherapy and thermal drug targeting. Geldanamycin (GA), a heat shock protein 90 inhibitor, was conjugated to novel thermosensitive poly (K) (8)-poly (VPGXG) (60) block copolymers [K (8)-ELP (1-60)] with guest residues as valine, alanine and glycine in a 5:2:3 ratio at the 'X' position. The conjugates were completely soluble in PBS and showed a characteristic thermosensitive inverse phase transition. Relevant to systemic drug delivery in vivo, these nanomaterials stably dispersed in aqueous solution (Bae et al. 2007).

A layer-by-layer (LbL) deposition strategy for preparing protein nanotubes have been attempted by many for drug loading and targeted delivery. Hou et al. showed that glucose oxidase nanotubes prepared in this way catalyzed glucose oxidation and that hemoglobin nanotubes retained their heme electro activity (Hou et al. 2005). Furthermore, for the glucose oxidase nanotubes, the enzymatic activity increased with the nanotube wall thickness (Hou et al. 2005). Nair et al. formed protein nanotubes by layer-by-layer assembly which could penetrate cells and act as nanopores for direct transmembrane delivery of chemical compounds (Nair et al. 2014). This LbL strategy is discussed in detail later elsewhere in this brief in Chap. 4.

Nanoparticles have the ability and chemical properties to be conjugated with various other peptides or moieties for better functionalization and uptake (Nair et al.

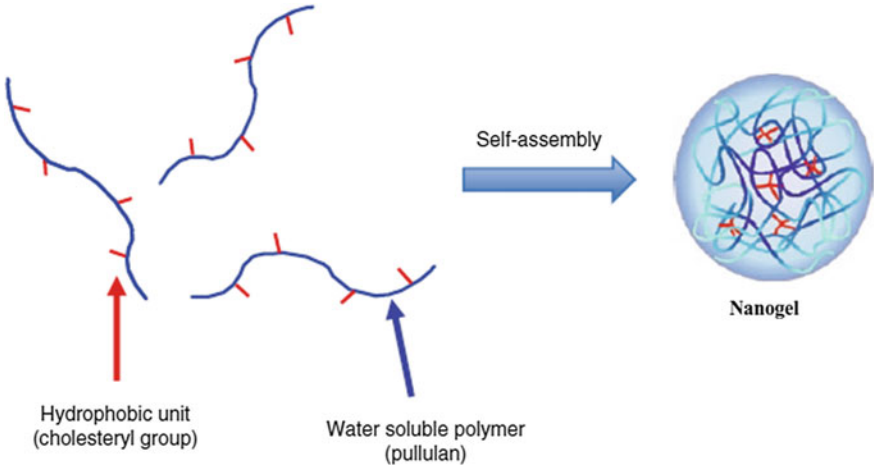

Fig. 2.3 Schematic for preparing a nanogel utilizing 2 basic moieties: hydrophobic and hydrophilic (Morimoto and Akiyoshi 2010)

2010, 2012). Internalization of nanoparticles conjugated with cell penetrating peptides is a promising approach to various drug delivery applications (Nair et al. 2012). Cell penetrating peptides such as transactivating transcriptional activator (TAT) peptides derived from HIV-1 proteins are effective intracellular delivery vectors for a wide range of nanoparticles and pharmaceutical agents, due to their intrinsic ability to enter cells and render minimum cytotoxicity (Nair et al. 2012). Nair et al. (Nair et al. 2012) studied the intracellular localization and trafficking of TAT peptide conjugated superparamagnetic iron oxide nanoparticles (TAT-SPIONs) using 3-D electron tomography (Nair et al. 2012). 3-D tomograms clearly showed the location of TAT-SPIONs in-vitro and their sustained release from the endocytic vesicles into the cytoplasm (Nair et al. 2012). Thus the results clearly demonstrated the applicability of this technique for the development of nano-drug delivery systems (Nair et al. 2012).

Induced pluripotent stem cells (iPSCs) have been generated from fibroblasts using a non-viral magnetic nanoparticle-based transfection method that employed biodegradable cationic polymer PEI-coated super paramagnetic nanoparticles (Lee et al. 2011). Nanoparticle synthesis, development and application will be really helpful for treatment of Parkinson's (PD) and Alzheimer's (AD) diseases. Nitrosative stress resulting from elevated levels of Nitric Oxide (NO) is known to modulate the development of PD and AD as well (Pal et al. 2011). Specifically, elevated levels of NO disrupt the redox activity of protein-disulfide isomerase (PDI), a key endoplasmic reticulum-resident chaperone by S-nitroso modification of its redox-active cysteines (Pal et al. 2011). This leads to accumulation of misfolded AD- and PD-specific protein debris leading to disease progression in PD and AD. Pal et al. demonstrated in vitro that polyphenolic phytochemicals, curcumin and masoprocol, can rescue S-nitroso-PDI formation by scavenging NOx (Pal et al. 2011). In this study, using dopaminergic SHSY-5Y cells, they monitored the aggregation of green-fluorescent protein (GFP)-tagged synphilin-1 (a known constituent of PD Lewy neurites) as a function of rotenone-induced nitrosative stress (Pal et al. 2011). Importantly, it was demonstrated that a marked decrease in synphilin-1 aggregation when the cell line was previously incubated with 3,5-bis (2-flurobenzylidene) piperidin-4-one (EF-24), a curcumin analogue, prior to rotenone insult (Pal et al. 2011). Furthermore, their data also revealed that rotenone attenuated PDI expression in the same cell line, a phenomenon that can be mitigated through EF-24 intervention (Pal et al. 2011). Together, these results suggested that EF-24 could exert neuroprotective effects by ameliorating nitrosative stress-linked damage to PDI and the associated onset of PD and AD (Pal et al. 2011). Essentially, EF-24 can serve as a scaffold for the design and development of PD and AD specific prophylactics (Pal et al. 2011). However the EF-24 is tedious to synthesize and usually 10–200 μM solution is needed for such studies (Pal et al. 2011). So by synthesizing EF-24 nanoparticles the efficiency of this compound towards mitigating endpoints of PD and AZ can be achieved.

2.4.2 For Incorporation in Medical Implants

The assembling of high-surface zone, unagglomerated nano-sized (1–100 nm) bioceramic particles are of enthusiasm for some applications including injectable/controlled setting bone concretes, high quality permeable/non-permeable engineered bone unions, and the strengthening stage in nano-composites that endeavor to imitate the mind boggling structure and unrivaled mechanical properties of bone. In a study by Philips et al. (2003) the assembling of nano-molecule hydroxyapatite powders by a few wet concoction techniques, which join a stop drying step was reported. Specifically, it was found that the emulsion-based combinations yielded powders with high surface territories and little essential molecule sizes. Stop drying as opposed to broiler drying of powders arranged by customary wet substance amalgamation yielded a nano-sized powder with a similarly higher surface territory of 113 m^2/g. All powders were calcined in air in a heater at 900 °C to research the impacts of combination technique on stage virtue and surface range. The materials were described by a scope of explanatory systems including Fourier-change infrared spectroscopy utilizing the photograph acoustic (PAS-FTIR) testing procedure, BET surface region investigation, X-beam powder diffraction (XRD), and the particles were inspected utilizing a transmission electron magnifying lens (TEM). The outcomes acquired in this study uncovered that a noteworthy maintenance of surface range could be accomplished by stop drying of the wet hydroxyapatite channel cakes after ("bulk") co-precipitation contrasted with the routine broiler drying system. Without a doubt, the utilization of stove drying of wet channel cakes resulted in critical agglomeration of particles and decrease in the total surface area (as much as 30%). Free streaming powders containing nano-sized particles of hydroxyapatite with moderately extensive surface territories (>200 m^2/g) can be incorporated in the centers of water in oil emulsions that contained as much as 40% water content. The blended HA powder properties were not essentially diverse between emulsions containing either 10 or 40% volumes of water in oil stage. Particles arranged by such a course demonstrated astounding wettability when calcined and thus would be required to show predominant sinterability for the arrangement of thick, abandon free sintered earthenware plates, giving expanded mechanical properties. These earthenware production would be suitable for orthopedic applications, for example, bone plates, acetabular compartments and coatings (Phillips et al. 2003). Nano-structured implants and tissue engineering scaffolds are explained in detailed in another section of this brief as well in Chap. 4.

2.4.3 Photothermal Nanotherapeutics and Nanodiagnostics

Nanoparticles have been used to identify and kill pathogenic bacteria. Zharov et al. (2006) described a new method for selective laser killing of bacteria targeted with light-absorbing gold nanoparticles conjugated with specific antibodies. The

multifunctional photothermal (PT) microscope/spectrometer provides a real-time assessment of this new therapeutic intervention. In this integrated system, strong laser-induced overheating effects accompanied by the bubble-formation phenomena around clustered gold nanoparticles are the main cause of bacterial damage. PT imaging and time-resolved monitoring of the integrated PT responses assessed these effects. Specifically, this technology was used for selective killing of the Gram-positive bacterium Staphylococcus aureus by targeting the bacterial surface using 10-, 20-, and 40-nm gold particles conjugated with anti-protein A antibodies. Labeled bacteria were irradiated with focused laser pulses (420–570 nm, 12 ns, 0.1–5 J/cm^2, 100 pulses), and laser-induced bacterial damage observed at different laser fluences and nanoparticle sizes was verified by optical transmission, electron microscopy, and conventional viability testing.

In another study by Norman et al., they used gold nanorods that were covalently bonded to primary antibodies to kill Pseudomonas aeruginosa, a gram-negative bacterium (Norman et al. 2008). The authors found that the bacteria's cell viability was greatly diminished after they attached gold nanorods to the bacteria's cell surface and exposed it to near-infrared (NIR) radiation (Norman et al. 2008). These studies assert that this kind of nanotechnology may provide a potential solution to the growing problem of antibiotic resistant bacteria. Huang et al., also, used gold nanoparticles as a photothermal medium to kill pathogenic bacteria (Huang et al. 2007). The authors developed polygon-shaped gold nanoparticles that could absorb NIR light via a photochemical reaction (Huang et al. 2007). They then immobilized Vancomycin onto the surface of the gold nanoparticles, and used them as the photothermal medium to irradiate bacterial growth under an NIR light (808 nm) (Huang et al. 2007). The vancomycin-bound gold nanoparticles selectively fastened themselves onto the pathogenic bacteria's cell walls (Huang et al. 2007). Within 5 min, more than 99% of the bacteria was killed under the NIR light because of the heat (Huang et al. 2007). Other studies have shown that gold nanospheres, nanoshells, nanorods, and nanoclusters can be used in photothermal (PT) therapy to treat cancer cells, bacteria, viruses, and DNA (Khlebtsov et al. 2006).

2.4.4 Lipid-Based Nanotherapeutics for Nucleic Acid Delivery

Cationic lipids were introduced as carriers for DNA and RNA over 2 decades ago. Cationic lipids interact with negatively charged nucleic acids through electrostatic interactions forming complexes called lipoplexes. The proposed mechanism of formation of lipoplexes involves negatively charged nucleic acids binding to positively charged lipid vesicles. Additional positively charged vesicles adsorb to the solvent-exposed nucleic acids. This process causes formation of a multilamellar structure of positively charged lipid bilayers $\sim$3.7 nm thick, spaced $\sim$2 nm apart from each other by negatively charged nucleic acids (Schroeder et al. 2010). One of

the first cationic lipids to be used for DNA delivery was cationic phospholipid DOTMA (1, 2-di-O-octadecenyl-3-trimethylammonium propane). Upon hydration, DOTMA formed liposomes either alone, or in presence of other lipids. These liposomes could be downsized into small unilamellar vesicles (SUVs) <100 nm in diameter. Cholesterol plays a role in many cellular membrane-related events such as membrane fusion, macropinocytosis and caveolin and lipid-raft-mediated endocytosis (Lu et al. 2009). Introducing cholesterol as a component of certain DNA/RNA carriers has been reported to improve transfection in vivo in comparison with carriers not containing cholesterol (Liu et al. 1995). When formulated in delivery vehicles at more than 25 mol%, cholesterol can decrease carrier permeability, increase carrier circulation time (Senior and Gregoriadis 1982) and increase structural rigidity and stability of the carrier (Mouritsen 2005). Furthermore, cholesterol is reported to protect nucleic acids from extra-liposomal degradative entities such as RNases (Liu et al. 1997). The importance of cholesterol for internalization of siRNA into cells has been exemplified by extracting cholesterol from cell membranes, and then exposing the cells to siRNA lipoplexes. In the cholesterol-depleted cells siRNA uptake and transfection were totally abolished (Lu et al. 2009).

Conjugating cholesterol to siRNA improves cellular uptake and transfection, and decreases siRNA degradation in serum (Rossi 2004; Whitehead et al. 2009). Wolfrum et al. (2007) showed that introducing cholesterol-conjugated siRNA into plasma resulted in association of these particles with either high-density lipoproteins (HDLs), which in vivo targets the liver, gut, kidney and steroidogenic organs, or with low-density lipoproteins (LDLs), which targeted the liver primarily (Wolfrum et al. 2007). siRNA conjugated to other hydrophobic molecules, with more than 22 carbons, also showed HDL and LDL association (Wolfrum et al. 2007). The association of the conjugated siRNA with HDL/LDL may protect siRNA from being degraded by plasma components. Cholesterol may play a dual role in the delivery of siRNA. When incorporated in the carrier, cholesterol may help facilitate cell fusion or endosomal internalization of the carrier. When conjugated to siRNA, cholesterol seems to act as a targeting entity (Schroeder et al. 2010). Derivatives of cholesterol have also been shown to improve the performance of cationic liposomes. Han et al. (Han et al. 2008), showed that cationic liposomes enriched with an amine-based cholesterol derivative, cholesteryloxypropan-1-amine, increased delivery efficiency of siRNA in serum, in comparison with ordinary cholesterol.

2.4.5 Nanotherapeutics for Chemotherapy

Cancer nanotherapeutics are rapidly progressing and are being implemented to solve several limitations of conventional drug delivery systems such as nonspecific biodistribution and targeting, lack of water solubility, poor oral bioavailability, and low therapeutic indices (Cho et al. 2008). To improve the bio-distribution of cancer

drugs, nanoparticles have been designed for optimal size and surface characteristics to increase their circulation time in the bloodstream. They are also able to carry their loaded active drugs to cancer cells by selectively using the unique pathophysiology of tumors, such as their enhanced permeability and retention effect and the tumor microenvironment. In addition to this passive targeting mechanism, active targeting strategies using ligands or antibodies directed against selected tumor targets amplify the specificity of these therapeutic nanoparticles (Cho et al. 2008). Drug resistance, another obstacle that impedes the efficacy of both molecularly targeted and conventional chemotherapeutic agents, might also be overcome, or at least reduced, using nanoparticles. Nanoparticles have the ability to accumulate in cells without being recognized by P-glycoprotein, one of the main mediators of multidrug resistance, resulting in the increased intracellular concentration of drugs. Multifunctional and multiplex nanoparticles are now being actively investigated and are on the horizon as the next generation of nanoparticles, facilitating personalized and tailored cancer treatment. Drug resistance has emerged as a major obstacle limiting the therapeutic efficacy of chemotherapeutic agents. Among several mechanisms of drug resistance, P-glycoprotein is the best known and most extensively investigated (Gottesman et al. 2002). It has been suggested that nanoparticles may be able to circumvent P-glycoprotein–mediated resistance. One possible mechanism is that nanoparticles may avoid recognition by the P-glycoprotein efflux pump by means of being enveloped in an endosome when entering the cell, leading to high intracellular drug concentrations. Ligand-targeted strategies, especially those using receptor-targeting ligands, may have particular potential for overcoming drug resistance because these ligands are usually internalized via receptor-mediated endocytosis. Indeed, a folate receptor–targeted, pH-sensitive polymeric micelle containing doxorubicin (Lee et al. 2005) and transferrin-conjugated paclitaxel-loaded nanoparticles (Sahoo and Labhasetwar 2005) exhibited greater inhibitory activity against drug-resistant MCF-7 cells and/or xenografts than their non-targeted free drug counterparts.

In a novel study by Nair et al. magnetic nanoparticles were developed and for the selective removal of target cancer cells using aptamer conjugated magnetic nanoparticles controlled by an externally applied three-dimensional rotational magnetic field (Nair et al. 2010). Additionally, this system can be tuned towards the selective removal of complex cancers from diverse tissues by incorporating various target specific ligands on these magnetic nanoparticles (Nair et al. 2010).

Others have shown that cancer cells can be selectively targeted with light-absorbing microparticles and nanoparticles (Pitsillides et al. 2003; Zharov et al. 2003, 2005). Pitsillides et al. used short laser pulses to heat light-absorbing microparticles and nanoparticles to target and damage selected cells (Pitsillides et al. 2003). The authors assert that this method is like chromophore-assisted laser inactivation and photodynamic therapy, but works exclusively via light absorption (Pitsillides et al. 2003). To study how light-absorbing microparticles killed cells, Pitsillides et al. used peripheral blood lymphocytes (Pitsillides et al. 2003). These cells were incubated with 0.83-μm oxide-doped latex microspheres covered with anti-CD8 IgG, and double-labeled with anti-CD8 IgG conjugated to

R-phycoerythrin (Pitsillides et al. 2003). Bright field microscopy showed the microparticles attached to the surface of CD8+ cells (Pitsillides et al. 2003). When the cells were irradiated with short (20-ns) laser pulses at 565 nm, the particles were quickly heated and the fluid layer around each particle was vaporized (Pitsillides et al. 2003). The authors equate this reaction to a microscopic underwater explosion and cavitation bubble formation (Pitsillides et al. 2003).

Letfullin et al. (2006) assert that gold nanoparticles (GNs) show the most potential as photothermal sensitizers because they absorb well, are photostable, are nontoxic, can be conjugated to antibodies and proteins, and have adjustable optical properties (West and Halas 2003; Hirsch et al. 2003; O'Neal et al. 2004). However, researchers (Zharov et al. 2003, 2004) also found that GN clusters (GNC) on cell membranes greatly increased bubble formation efficiency, led to greater cancer cell damage at low laser fluences (60–80 mJ/cm^2), and were safer for normal tissue (Zharov et al. 2005b). Previous research on GNC bubble formation, further, suggests that effective bubble formation in GNCs corresponds with optical (Khlebtsov et al. 2006) and thermal (Zharov et al. 2005b) amplification effects, including overlapping nanobubbles from a single GN as separate nucleation centers or the creation of one large bubble around a GNC as a single nucleation center (Zharov et al. 2005a, b). Researchers have also used different methods to develop GNCs (Zharov et al. 2005c) such as targeting natural clustered cancer markers (Zharov et al. 2005b), clustering secondary monoclonal antibodies (Zharov et al. 2005a, b, 2006), conducting special engineering, such as identifying a human breast carcinoma MDA-MB-231 marker (Zharov et al. 2005a, b), clustering viruses with an incorporated GN into cancer cells (Zharov et al. 2005c), concentrating GNs within vesicles during endocytosis internalization (Zharov et al. 2003, 2005a, b), using laser-induced GN aggregation (Zharov et al. 2005b), using silver and gold kits to enhance GNCs (Zharov et al. 2005b). Letfullin et al. (2006) also noted that nanoparticle explosions may be effective in killing cancer cells because they can co-occur with optical plasma, shock waves with supersonic explosions, and particle fragmentation, which are also effective in killing cancer cells.

2.4.6 Quantum Dots for Traceable Therapeutic Delivery

Photoluminescent semiconductor nanocrystals, or quantum dots (QDs), hold promise as contrast agents for the diagnosis and characterization of disease using optical imaging (Walkey and Chan 2014). Yet, the diagnostic capabilities of a QD can be extended by coupling therapeutic molecules to its surface to form a theranostic QD (Walkey and Chan 2014). Theranostic QDs act as nanoscale therapeutic delivery vehicles (NDVs), transporting therapeutic molecules selectively to their site of action, while avoiding their interaction with sensitive healthy tissues and their degradation or modification within a biological environment (Walkey and Chan 2014). The optical contrast of the nanocrystal core allows the trafficking and localization of the construct, as well as the dynamics of therapeutic release, to be

monitored in a biological environment (Walkey and Chan 2014). By monitoring the therapeutic delivery process, barriers preventing effective therapeutic delivery can be identified and overcome (Walkey and Chan 2014). While theranostic QDs have demonstrated potential in proof-of-concept applications using cultured cells and small animal models, concern surrounding the toxicity of the semiconductor core has prevented their clinical translation (Walkey and Chan 2014). Engineering total body clearance or replacing the semiconductor core with a biocompatible and/or biodegradable nanomaterial prior to clinical translation may facilitate the eventual application of theranostic QDs in human patients (Walkey and Chan 2014). Even if theranostic QDs are never applied clinically, they will still be useful for elucidating the fundamental mechanisms by which NDVs interact with biological systems and in establishing structure-activity relationships to guide NDV design (Walkey and Chan 2014).

Nanoparticle-based drug delivery (NDD) has the potential to increase drug effectiveness. In studies, NDD has decreased drug toxicity, increased bio-availability and circulation time, and provided better controlled drug release and identification. While different kinds of nanoparticles have been used, quantum dots (Qdots) have become important as both a potential medium for NDD and as a means to study NDD processes. As just one example, Qdots have been used to observe intracellular uptake and trafficking. Traditionally, this process has been difficult to observe because NPs cannot easily travel through cell membranes. However, microinjection or electroporation processes were found to enable cytosolic NP delivery (Chen and Gerion 2004; Derfus et al. 2004; Yum et al. 2009). Using such processes, Qdots have been distributed into cell cytoplasm to study how surface ligands affected intercellular NP trafficking and how peptides targeted organelles. Yum et al. noted that by injecting Qdots via nanotubes into cell nuclei and cytoplasm, researchers were able to observe NP dispersal for 30 min (Yum et al. 2009). Additionally, Derfus et al. found that 25-nm Qdots enabled researchers to see NP movement through the nuclear core complex and into the mitochondria (Derfus et al. 2004).

Qdots have also been used to trace nanocarrier biodistribution because of their unique ability to serve as imaging agents—especially when used with whole-body fluorescence imaging, intravital microscopy, (Gao et al. 2004; Cai et al. 2006; Zhelev et al. 2011) or with other tracers such as positron emission tomography and scintigraphic imaging (Jung et al. 2011; Ducongé et al. 2008). This imaging capability has helped distinguish between the NP and its environment, which is important since NPs are often identified as foreign objects and amass in the mononuclear phagocyte system (MPS) organs (Li and Huang 2008).

To treat complex diseases (e.g. cancer) with NPs, researchers must understand how NP properties affect diseases in the body. They also need a high-resolution, high-sensitivity, and cost effective model system that can be used to study NDD stages (Nie 2010). Qdots may be one such model system. In one study, for example, Zhang et al. covalently conjugated Qdots with a type 1 insulin-like growth factor receptor (IGF1R) antibody, AVE-1642, to identify and measure IGF1R levels in breast cancer cells (Zhang et al. 2009). Zhang et al. found that AVE-1642 Qdots

only attached to IGF1R-indicated cells and measured IGF1R fluorescence emission levels at 655 nm (Zhang et al. 2009). After attaching to IGF1R-indicated cells, AVE-1642 Qdots experienced receptor mediated endocytosis, were confined to the endosome, and then later moved into the nucleus (Zhang et al. 2009). Furthermore, when AVE-1642 Qdots were used to treat MCF-7 cells, IGF1R levels were downregulated and cell growth was partly impeded (Zhang et al. 2009). These results indicate that researchers may be able to use AVE-1642 Qdots to identify IGF1R expression and measure cell surface receptor levels.

2.4.7 Strategies to Improve Implant Tolerance

The induction of donor-specific tolerance to transplanted cells and organs, while preserving immune function as a whole, remains a highly sought after and elusive strategy for overcoming transplant rejection (Hlavaty et al. 2015). Tolerance necessitates modulating a diverse array of cell types that recognize and respond to allo-antigens, including antigen presenting cells and T lymphocytes. Nanotherapeutic strategies that employ cellular and biomaterial engineering represent an emerging technology geared towards the goal of inducing transplant tolerance. Nanocarriers offer a platform for delivering antigens of interest to specific cell types in order to achieve tolerogenic antigen presentation. Furthermore, the technologies also provide an opportunity for local immunomodulation at the graft site. Nanocarriers delivering a combination of antigens and immunomodulating agents, such as rapamycin, provide a unique technology platform with the potential to enhance outcomes for the induction of transplant tolerance. Nanocarriers have been fabricated from a range of materials including polymers (e.g. PLGA, polystyrene), lipids (e.g. micelles, liposomes), gold, and carbon-based materials. These carriers can be loaded with antigen or drug by attachment to the surface (chemical conjugation or adsorption) or encapsulation within the carrier. Additionally, for promoting an immune response, these carriers typically have an adjuvant, such as aluminum salts or monophospholipid A (MPLA). Generating a specific, desired immune response using a nanocarrier is dependent on a number of factors, such as delivery route, size, shape/conformation, charge, incorporation of immune mediators, and presence/type of an adjuvant (McCarthy et al. 2014). Relative to traditional vaccines, nanocarriers offer the advantage of enhanced antigen stability, improved immunity, targeted delivery, and additional delivery routes.

Antigen presenting cells (APCs), which internalize and present allo-antigens via the indirect pathway, are an attractive target for nanotherapies to generate a donor-specific tolerant state in immune cells. Nanocarriers containing antigens or immunomodulatory factors can be directed to specific receptors of tolerogenic APCs for uptake and re-presentation to CD4+ and CD8+ T lymphocytes. The APCs mediating alloantigen presentation include circulating or tissue-specific subsets of dendritic cells and macrophages. Of note, APCs within the liver, the spleen, and lymphoid tissue—liver sinusoidal endothelial cells (LSECs), Kupffer cells (KCs),

splenic marginal zone macrophages, lymphatic endothelial cells (LECs), and plasmacytoid DCs—have been documented in mediating tolerance (Fehres et al. 2014; Tel et al. 2013; Reynoso and Turley 2009; Ebrahimkhani et al. 2011; Getts et al. 2013; Hirosue et al. 2014; Reddy et al. 2006).

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