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Frontiers in Research Review: Cutting-Edge Molecular Approaches to Therapeutics

POLYMERIC CORE-SHELL NANOPARTICLES FOR THERAPEUTICS

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SUMMARY

1. Nanobiotechnologies have recently attracted significant attention from chemists, biologists, engineers and pharmaceutical scientists. In particular, they have been widely applied to improve drug, protein/peptide and gene delivery.

2. This review presents recent advances in the field of drug, protein/peptide and gene delivery using natural and synthetic polymer nanoparticles and explains how polymeric nanoparticles are specifically designed to suit the needs for targeted delivery of small molecular drugs, proteins/peptides and genes. In addition, some of the challenges and prospects for these technologies are discussed.

Key words: cationic polymer nanoparticles, drug delivery, gene delivery, peptide/protein delivery, polymer core-shell nanoparticles, polymeric nanoparticles.

INTRODUCTION

According to the report by the Royal Society of London in July 2004, nanotechnlogies are the design, characterization, production and application of structures, devices and systems by controlling shape and size at the nanometre scale.[1] Nanotechnologies are not new and they have been studied for many decades. However, only in recent years have scientists gained an indepth understanding of nanostructured substances by using relatively new, sophisticated tools, such as the atomic force microscope. The recent advances in nanotechnologies, especially in nanoparticles, make them very promising in the delivery of therapeutics, drug discovery and diagnostics. Nanoparticles, with a hydrophilic surface, are especially desirable to transport the therapeutics to the target tissues or cells because they can escape the uptake of mononuclear phagocytes, macrophages and reticuloendothelial systems (RES) in the blood and organs. In addition, a biological signal can be chemically conjugated onto the surface to recognize specific tissues or cells. Although the nanoparticles as a drug carrier have their own disadvantages, such as low drug-loading capacity and wide size distribution, they have attracted increasing attention from chemists, biologists, engineers and pharmaceutical scientists because they provide the possibility of transporting bioactive compounds to specific tissues, cells and cell compartments. The nanoparticles can be made from inorganic and polymer materials. Polymeric nanoparticles are more desirable because they can be chemically designed to be biodegradable and biocompatible. In the present paper, nanoparticles, made from synthetic polymers or modified natural polymers, are reviewed for the delivery of small molecular drugs, proteins/peptides and genes. Figure 1 shows a schematic presentation of a polymer core-shell nanoparticle incorporating bioactive molecules.

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DELIVERY OF SMALL MOLECULAR DRUGS

Nanoparticles offer numerous advantages over conventional dosage forms, including the ability to protect drugs from biodegradation, target the drug to the site of action and reduce the side-effects of chemotherapy. They are made by forming drug–polymer complexes in which the drug is uniformly dispersed or by creating nanoscale vesicles (such as liposomes and micelles) to entrap drug molecules. The surface characteristics of nanoparticles are important in determining their susceptibility to uptake by RES. Modification of surface properties affects both the circulation time and ultimate fate of the nanoparticles. It has been reported that particles with more hydrophobic surfaces tend to be taken up rapidly by the liver, spleen and lungs,[2] whereas liposomes coated with hydrophilic polymers exhibit significantly prolonged circulation times *in vivo*.[3] It is believed that the dense surface concentration of hydrated polymer chains sterically hinders protein adsorption and opsonization of these liposomes.[4] The synthetic hydrophilic polymer polyethylene glycol (PEG) is the most common polymer used for nanoparticle coatings owing to its ability to avoid or retard nanoparticle recognition by the RES. Surface charge is another important parameter in determining how nanoparticles interact with cells, whose membranes are usually negatively charged.

The size of nanoparticles (typically smaller than 100 nm in diameter) allows drugs to accumulate in solid tumours, which are characterized by extensive angiogenesis, defective vascular architecture, impaired lymphatic drainage and an increased production of permeability factors.[5] This phenomenon is known as the enhanced permeability and retention (EPR) effect. It can be exploited to improve drug delivery to tumours and other sites, including inflammations and infarcts, which possess similar pathological characteristics. The EPR effect is considered a passive targeting mechanism, but more specific drug targeting can be achieved by binding targeting ligands to the surface of nanoparticles, such as monoclonal antibodies (mAbs), peptides and sugar moieties. Monoclonal antibodies were

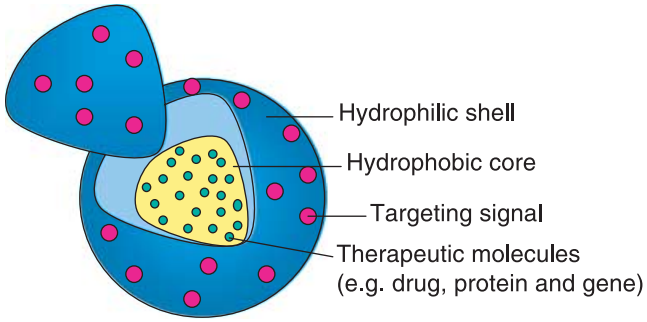

Fig. 1 A schematic presentation of a polymer core-shell nanoparticle.

first shown to bind to specific tumour antigens in 1975,[6] but it is only in the past decade that antibody targeted treatments for specific cancers have become available.[7] Torchilin *et al*. have reported a relatively simple procedure for the attachment of specific ligands to distal ends of liposome-grafted PEG chains.[8] The method allows the single-step binding of proteins and small molecules that contain primary amino groups. Peptides have also been considered as pilot molecules for drug delivery vehicles because they offer excellent tissue penetration and can be easily synthesised and conjugated. Shadidi and Sioud identified a novel cell-targeting peptide for the SKBR3 breast cancer cell line.[9] A modified luteinizing hormone-releasing hormone (LHRH) was conjugated to PEG–camptothecin (an anticancer drug) for targeting camptothecin to human ovarian tumour xenografts[10] and arginine–glycine–aspartic acid (RGD)-4C peptide was conjugated to doxorubicin, an anticancer drug, for drug targeting to tumour vasculature.[11] In addition, sugar moieties can be used for site-specific drug delivery,[12] although most reports have so far been limited to delivery to liver tissue. Moreover, biotin[13] and small molecular compounds, such as folic acid,[14] can be used for anticancer drug targeting. The use of targeting signals improves the drug concentration in tumours.[13,14] Monoclonal antibodies provide many advantages, including great targeting specificity and stability in the blood circulation, over other targeting signals. Monoclonal antibodies have been widely used in basic and clinical research. A number of mAbs, which include murine, chimeric and humanized antibodies, have been approved by the US Food and Drug Administration (FDA) for the treatment of cancer.[15] Cytotoxic drugs, such as doxorubicin, DM1, CC-1065, second-generation taxanes, monomethyl auristatin E and geldanamycin, have been conjugated onto the antibodies to achieve direct killing of tumour cells and, thus, minimize toxicities of cytotoxic drugs against normal tissues. The drawbacks of using mAbs for targeting cytotoxic drugs include immunogenicity and difficulty of penetration into solid tumours. The replacement of murine and chimeric antibodies with human ones may overcome the immunogenicity related problem. Conversely, there are a number of barriers, including the vascular endothelium, stromal and epithelial barriers, as well as high interstitial pressure, for therapeutic agents to overcome before entering solid tumours.[16] Therefore, it is difficult for the therapeutic agents, especially large molecules, to be transported into the tumour. It was reported that single-chain antibodies penetrated into the tumour with higher efficacy than the parental antibodies.[17] However, the single-chain antibodies are not stable in the blood circulation and have short half-lives. Targeting the endothelium of tumour blood vessels may be a better approach because of its accessibility.[18]

Drug targeting can also be achieved by introducing stimuli-sensitive materials into nanoparticles.[19] Temperature-[20,21] and pH-sensitive[13,22] polymers have been used in drug targeting. Temperature- and pH-sensitive polymers have been investigated extensively because many pathological processes are known to be accompanied by local temperature increases and/or acidosis. Poly(*N*-isopropylacrylamide) (PNIPAAm) and its copolymers are the most extensively studied thermosensitive polymers. One of the drawbacks of using thermosensitive nanoparticles is that heating is inaccessible to deep tissues or organs. We have recently developed polymer core-shell nanoparticles that were both pH and temperature sensitive. The temperature sensitivity was induced by pH changes.[23] These nanocarriers were stable in the normal physiological environment, but deformed and precipitated in acidic environments, such as the tumour interstitium and intracellular compartments. These nanoparticles are superior to carriers that are only thermosensitive and pH sensitive because they can target deep tissues where it may not be possible to trigger drug release by hyperthermia and their phase alternation triggered by external pH changes is much more abrupt than that of pH-sensitive nanoparticles.

Polymers used for the fabrication of nanoparticles include polylactide (PLA),[24] poly(lactide-coglydcolide) (PLGA),[25] PLA/PLGA-*b*-PEG,[26] poly(β-amino ester),[22] poly(L-histidine)-*b*-PEG,[13] PNIPAAm-based copolymers,[20,21,23,27] PEG–poly(β-benzyl-L-aspartate) block copolymer,[28] PEG–poly(ϵ-caprolactone)–PEG[29] and poly(trimethylene carbonate)–PEG–poly(trimethylene carbonate)[30] triblock copolymers. A drug can be loaded into the nanoparticles by various methods, such as an oil-in-water emulsion method if the drug is water insoluble[22] or a water-in-oil-in-water double-emulsion process if the drug is water soluble.[24] Membrane dialysis is a widely used process for the fabrication of drug-loaded micellar nanoparticles.[13,14,20,21,23,26–30] The drug-loading capacity of the nanoparticles depends on the properties of the drug, fabrication parameters and the structural compatibility between the drug and the hydrophobic segments of the amphiphilic polymers.[31] It has always been a challenge to incorporate highly water-soluble drugs into polymeric nanoparticles. Recently, ionic complexation was used to load drugs such as doxorubicin and verapamil into dextran sulphate, an anionic polymer with relatively high loading levels.[32]

In summary, polymeric nanoparticles provide a promising carrier for the delivery of poorly water-soluble drugs. They not only improve the water solubility and bioavailability of the drugs, but also allow for drug targeting by conjugating biological signals on the surface of the nanoparticles or using stimuli-sensitive polymers. Although the introduction of targeting signals to the nanoparticles leads to a higher concentration of the drug at target sites, such as tumours, more drug molecules are still trapped in the liver,[14] possibly due to the leakage of the drug molecules from the nanoparticles or the sophisticated liver structure rich in RES. Therefore, future research efforts in the field of drug delivery may be focused on enhancing drug stability in the nanoparticles before reaching the target site and drug accumulation at the target sites by using stable polymer core-shell nanoparticles and specific targeting signals.

DELIVERY OF PROTEINS/PEPTIDES

Peptide- and protein-based therapeutics have recently received increasing attention because of their high biological activity and specificity.[33,34] However, despite the advantages offered by these

drugs, their application may suffer because of the high molecular weight, hydrophilicity and low stability, which are reflected in poor biopharmaceutical properties.[35,36] In particular, peptides and proteins undergo rapid clearance from the body, which takes place by a combination of events, including proteolysis, renal ultrafiltration, liver clearance and starvation by the immune system. Interaction and accumulation within tissues can represent an additional pathway for removal of peptides and proteins from the blood.[36]

Many approaches to enhancing protein/peptide delivery have been proposed. Cyclosporine A (CsA), an immunosuppressive agent, is very hydrophobic and water insoluble. The water solubility and bioavailability of CsA can be significantly improved when it is loaded into polymeric nanoparticles, such as stearic acid,[37] chitosan,[38] and poly(methacrylic acid-*co*-methacrylate)[39] nanoparticles. In addition, polyoxyethylene cetyl ether-grafted dextran and hydroxypropylcellulose have been developed to incorporate CsA into micellar nanoparticles.[40] The apical to basal permeability of CsA across Caco-2 cells was higher when loaded in the micellar nanoparticles compared with free CsA. Recently, methoxy poly(ethylene oxide)-*b*-poly(epsilon-caprolactone) micellar nanoparticles were also used to deliver CsA.[41] The release of CsA was well sustained compared with the commercial formulation containing Cremophor EL (BASF Aktiengesellschaft, Ludwigshafen, Germany), a low molecular weight surfactant. The blood circulation was significantly prolonged using the polymeric nanoparticle formulation.

Insulin is another peptide of great interest in pharmaceutical applications. Protection of insulin against self-aggregation and enzymatic degradation is an important issue for its *in vivo* delivery. Insulin was encapsulated into poly(isobutylcyanoacrylate) nanocapsules. Although the nanocapsules increased the survival time of insulin in the intestines, they did not give significant enhancement of insulin adsorption.[42] Insulin was also loaded into PLGA nanoparticles.[43] The nanoparticles with fumaric anhydride oligimer and iron oxide additives were able to control plasma glucose levels after oral administration. In addition, water-soluble, amphiphilic polyesters, poly[(vinyl-3-(diethylamino)-propylcarbamate-*co*(vinyl acetate)-*co*(vinyl alcohol)]-*graft*-PLA, containing a positively charged backbone, were synthesised to carry insulin by a self-assembly process. A high loading level of insulin was achieved. These self-assembled nanocomplexes may offer the potential for mucosal insulin delivery.[44] More recently, chitosan–insulin nanoparticles were prepared by ionotropic gelation and administered orally to streptozotocin (STZ)-induced diabetic rats. The nanoparticles sustained the serum glucose at prediabetic levels for at least 11 h owing to the strong interaction between the rat intestinal epithelium and the chitosan nanoparticles.[45] Insulin-loaded poly(isobutylcyanoacrylate) nanoparticles were also fabricated and administered subcutaneously or orally to STZ-diabetic rats. Both formulations significantly prolonged the duration of the hypoglycemic effect of insulin compared with free insulin.[46]

The delivery of other peptides/proteins has also been explored using polymeric nanoparticles. For instance, CGP 57813, a peptidomimetic inhibitor of human immunodeficiency virus type 1 (HIV-1) protease, was loaded into pH-sensitive methacrylic acid copolymer nanoparticles.[47] The nanoparticles provided sufficient plasma levels of CGP 57813 following oral administration. Positively charged salmon calcitonin was ion paired with sodium oleate to form salmon calcitonin–oleate complexes, which were further formulated into PLGA nanoparticles with a high loading efficiency. A greater amount of salmon calcitonin-loaded nanoparticles was delivered into Caco-2 cells compared with free salmon calcitonin and was readily taken up *in vivo* following oral and intravenous administration.[48] Surface chemistry dominantly affects the permeability of the nanoparticles through Caco-2 monolayers.[49] Chitosan-coated salmon calcitonin-loaded tripalmitin nanoparticles induced a significant and prolonged reduction in the serum calcium levels compared with free salmon calcitonin solution and PEG-coated nanoparticles loaded with salmon calcitonin.

In summary, polymeric nanoparticles hold promise for peptide and protein delivery. A few key factors need to be taken into consideration in the design of polymeric nanoparticles for successful *in vivo* delivery of a specific peptide, including the surface chemistry of the nanoparticles, the compatibility of the peptide or protein with the polymer, the fabrication process of the nanoparticles and the bioactivity of the peptide or protein after being formulated into the nanoparticles. The surface chemistry should be designed to overcome various biological barriers for peptide or protein uptake and to prolong its blood circulation. The choice of polymer and fabrication process of the nanoparticles must be based on the nature of the peptide or protein so that a high loading level can be achieved and the bioactivity of the peptide or protein remains intact during the fabrication processes.

DELIVERY OF NUCLEIC ACIDS

Gene therapy refers to the transmission of DNA encoding a therapeutic gene of interest into the targeted cells or organs with consequent temporary or 'permanent' expression of the transgene. The purpose of delivering transgene into the targeted cells or organs is to treat the disease caused by genetic disorders, mutation or genetic defects, such as leukaemia and tumours. Although gene therapy has been extensively studied, the US FDA has not yet approved any human gene therapy product for clinical application. Current gene therapy is experimental and has not proven very successful in clinical trials. Little progress has been made since the first gene therapy clinical trial began in 1990. This is because there are limited safe and efficient gene carriers available. Basically, there are two types of gene delivery vectors, viral and non-viral vectors. Non-viral vectors have recently received increasing attention because they are easier to produce, transport and store and induce less of an immune response. Many natural and synthetic materials have been explored as non-viral gene vectors, including cationic liposomes,[50,51] cationic polymer nanoparticles[52] and inorganic nanoparticles (e.g. silica nanoparticles,[53] carbon nanotubes[54] and metal nanorods[55]). Of these non-viral vectors, cationic polymer nanoparticles are the most attractive because they can be easily tailored and synthesised to suit the special requirements encountered by gene delivery. The cationic polymers also include natural polymers carrying positive charges. The cationic polymers most commonly used as gene vectors include branched and linear polyethylenimine (PEI), copolymers of PEI,[56,57] poly(L-lysine) and its copolymers,[58,59] chitosan[60] and dendrimers.[61] Nanoscaled complexes of cationic polymer and DNA can be fabricated by simply mixing these cationic polymer solutions with DNA. Of these cationic polymers, PEI polymers are the most popular gene vectors. In particular, branched PEI with a molecular weight of 25 kDa is often used as a control to evaluate the gene expression efficiency of other non-viral vectors because it provides high gene transfection efficiency in various cell lines. The PEI

polymers can effectively condense DNA molecules to form homogeneous spherical particles with a size of approximately 100 nm, enabling efficient *in vitro* gene transfection.[62,63] Conversely, PEI offers significantly more efficient protection against nuclease degradation than other cationic polymers, such as poly(L-lysine), probably because of its high charge density and efficient complexation with DNA. The amino groups of PEI may also provide significant buffering capacity to the polymer over a wide range of pH values.[64] This property, known as 'proton sponge', is essential for escape of the DNA complexes from the endosome.[65]

In addition to these commonly used cationic polymers, a variety of other cationic polymers, such as polybrene,[66] gelatin,[67] tetraminofullerene,[68] polyphosphoramidate (PPA) with a spermidine side chain,[69] polysaccharide–oliguamine-based conjugates[70] and linear β-cyclodextrin-based polycations,[71] have been studied as vectors for efficient gene delivery. Although many of these cationic polymers provide high *in vitro* gene transfection levels, the stability of the cationic polymer/DNA complexes, the ability of targeting to diseased tissues or cells and sufficient *in vivo* gene transfection efficiency remain a challenge. Hydrophilic polymers such as PEG have been grafted onto various cationic polymers to improve the stability of the polymer/DNA complexes in the blood.[72] However, the introduction of PEG causes a decrease in gene transfection efficiency, possibly due to the lower endolysosomal escaping capacity and weaker DNA-binding ability caused by the shielding effect of PEG.[72,73] Conversely, although cationic polymers have many advantages over viral vectors, their gene transfection efficiency is much lower than that induced by viral vectors, limiting their clinical application. Therefore, a fusogenic peptide has been conjugated to PEG, which has been further coated onto PEI/DNA complexes to improve gene transfection efficiency.[73] A nuclear localization signal has also been introduced to DNA to enhance its transport from the cytoplasm to the nuclei of target cells.[74] Targeting signals, such as transferrin, epidermal growth factor,[75] galactose[76] and folate,[77] have been chemically linked to PEG-PEI for targeted gene delivery.

Small interfering RNA (siRNA) can be used as a method to silence target genes, which has recently become a powerful tool in drug development. However, *in vivo* siRNA delivery is typically challenging and delivery methods effective for other nucleic acids may not be suitable for siRNA. The commonly used method to deliver siRNA *in vivo* is to incorporate siRNA into a viral vector.[78,79] Recently, scientists have also attempted to deliver siRNA using non-viral polymer-based nanoparticles. The siRNA with the target sequence 5′-AATGACATGCCGATCTACATG-3′ for downregulating EphA2 was loaded into liposomes composed of neutral lipid 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine.[80] The successful delivery of siRNA decreased protein expression in the tumours established in female athymic nude mice by i.p. injection of ovarian cancer cells and significantly suppressed tumour growth when combined with the anticancer drug paclitaxel. In addition, vascular endothelial growth factor receptor (VEGFR) 2-targeted siRNA was designed and complexed with PEGylated-PEI with an RGD peptide ligand attached at the distal end of PEG, a signal to target tumour neovasculature expressing integrins.[81] The intravenous injection of RGD-PEG-PEI/siRNA complexes into female nude mice bearing N2A tumours induced sequence-specific inhibition of the target gene, reduction in angiogenesis and inhibition of tumour growth. Block amphiphilic copolymer-coated calcium phosphate/siRNA nanoparticles were also reported to deliver siRNA.[82] The siRNA targeting the GL3 luciferase gene was mixed with calcium and phosphate solutions and the resulting complexes were then stabilized by PEG-*b*-poly(aspartic acid) to form organic–inorganic hybrid nanoparticles with a core-shell structure. The core was composed of calcium, phosphate and siRNA, surrounded by a hydrophilic tethered layer of PEG. The siRNA-incorporated nanoparticles successfully silenced GL3 luciferase gene expression in HeLa cells.[82] In addition, GL3 or lamin A/C-targeted siRNA was delivered into HuH-7 cells by PEG-*b*-poly(3-[(3-aminopropyl)amino]propylaspartamide) copolymer to knockdown GL3 luciferase gene expression and lamin A/C mRNA expression, respectively.[83]

In summary, cationic polymer nanoparticles provide a strong gene-binding ability, high gene transfection efficiency in various cell lines and relatively low toxicity. Successful *in vivo* gene transfection has also been achieved, which may have potential for clinical immunization.[84] However, for some diseases, such as cancers, gene transfection induced by cationic polymer nanoparticles is still not efficient enough. Targeting signals may be conjugated to the nanoparticles or DNA to improve cell uptake and nucleus targeting.

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