Nanoscale

Cite this: *Nanoscale*, 2012, **4**, 360

www.rsc.org/nanoscale

FEATURE ARTICLE

Strategies in biomimetic surface engineering of nanoparticles for biomedical applications

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Received 14th September 2011, Accepted 20th October 2011
DOI: 10.1039/c1nr11297j

Engineered nanoparticles (NPs) play an increasingly important role in biomedical sciences and in nanomedicine. Yet, in spite of significant advances, it remains difficult to construct drug-loaded NPs with precisely defined therapeutic effects, in terms of release time and spatial targeting. The body is a highly complex system that imposes multiple physiological and cellular barriers to foreign objects. Upon injection in the blood stream or following oral administation, NPs have to bypass numerous barriers prior to reaching their intended target. A particularly successful design strategy consists in masking the NP to the biological environment by covering it with an outer surface mimicking the composition and functionality of the cell's external membrane. This review describes this biomimetic approach. First, we outline key features of the composition and function of the cell membrane. Then, we present recent developments in the fabrication of molecules that mimic biomolecules present on the cell membrane, such as proteins, peptides, and carbohydrates. We present effective strategies to link such bioactive molecules to the NPs surface and we highlight the power of this approach by presenting some exciting examples of biomimetically engineered NPs useful for multimodal diagnostics and for target-specific drug/gene delivery applications. Finally, critical directions for future research and applications of biomimetic NPs are suggested to the readers.

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1. Introduction

Nanometre-sized particles, such as proteins or viruses, take an active part in diverse cellular activities. Much attention has been focused on understanding the mechanisms of interaction between natural or synthetic NPs and the cell membrane, as a means to use them to control NP import/export in and out of cells. Synthetic NPs can transport drugs, proteins, or other molecules of interest to the interior of the cell, evading the protection mechanisms built into the cell membrane. A number of pertinent reviews have been published recently on the preparation and use of NPs designed for the diagnosis and therapy of specific diseases in targeted organs.[1–5] One needs to remember, however, that nanoparticles may have deleterious effects *in vivo*. Examples abound of promising NPs that turned out to be cytotoxic *in vivo* or *in vitro*.[6,7]

An inherent feature of NPs is that they are rapidly recognized as foreign substance *in vivo* unless their external surface is endowed with stealth properties.[8–11] Most NPs are rapidly eliminated from the bloodstream immediately upon intravenous injection. They accumulate in tissues of the mononuclear phagocyte system (MPS), mainly Kupffer cells in the liver and macrophages in the spleen.[12,13] For example, standard poly(lactide), poly(lactide-coglycolide) or starch-coated iron oxide nanoparticles are cleared from the blood within a few minutes post injection.[14,15] Unless this defense mechanism is evaded, there can be no use for NPs in controlled drug targeting and delivery to tissues other than the MPS. Mechanistic studies have indicated that NP elimination from the blood is initiated by the adsorption of plasma proteins (opsonins) onto the surface of the NPs, followed by phagocytic recognition.[8,16] Therefore, one prerequisite for engineering long-circulating NPs is to design for them a coat acting as a shield or "stealth" against opsonic adsorption and subsequent removal from the blood by phagocytic cells.[17] Several strategies have been developed to achieve this goal. The predominant one consists in adsorbing or grafting on the NP surface a hydrophilic polymeric shell having no affinity towards opsonins. Polyethylene glycol (PEG) has proven to be particularly effective in this function.[14,15] The stealth properties of PEG chains have been attributed to a combination of structural features, including charge neutrality, hydrophilicity, chain flexibility, and capacity for hydration. PEG-modified ("PEGylated") particles are camouflaged, or invisible, to phagocytic cells. The blood circulation time of PEGylated NPs varies from several minutes to several days, depending on the length and surface density of the PEG chains. The PEG chains can also be used to achieve site-specific release of therapeutic agents entrapped in the NPs, since their chain end can be linked to targeting groups.[18–20] There are limitations to the use of PEGylated NPs. Several groups have reported that the stealth properties of PEG chains are transient, so that eventually opsonization and macrophage clearance still occur.[21,22]

Surface modification of NPs following principles derived from studies of the composition, physicochemical properties, and biological functions of the cell membrane creates new opportunities for developing nanoparticles for biomedical applications. Cell membrane mimetic strategies derived from these studies are the main focus of this review. The methods of biomimetic NP surface engineering include surface coating with a phospholipid bilayer or monolayer, surface decoration with proteins, carbohydrates, peptides or antibodies.

2. The cell membrane in relation to biomimetic surface engineering

2.1 Composition and functions of the cell membrane

The cell membrane can be viewed as an integral phospholipid bilayer with embedded proteins and tethered carbohydrates (as shown in Fig. 1). It separates the interior of a cell from the exterior environment and serves as a permeable barrier by controlling the trafficking of large molecules and ions in and out of the cell. Thus, the integrity of the lipid bilayer is essential for the function of the cell. Phospholipids consist of a hydrophilic head containing a phosphate group linked to a fatty acid acting as a hydrophobic tail. Phospholipids self-assemble into a bilayer with the hydrophilic regions facing towards the outside of the cell and the cytoplasmic (interior) face of the cell. The hydrophobic (hydrocarbon-rich) regions of each layer face each other and point away from the membrane/water interfaces. The phospholipid bilayer forms the basic structure of the cell membrane. Phosphorylcholine (PC) is the headgroup of most phospholipids, especially those in the outer membrane layer. The zwitterionic nature of PC confers excellent biocompatibility to the cell. The phospholipid bilayer is fluid and allows molecules to move laterally along the plane of the membrane. The stability of the membrane can be increased by incorporation of longer fatty acid tails and insertion of cholesterol and proteins.

Membrane proteins are asymmetrically distributed in the lipid bilayer of all biological membranes. Transmembrane proteins have a specific orientation. One side of the protein might be shaped such that it can act as a receptor for a signaling molecule, while the other side changes shape in response to the binding signal. Some cells are specialized to be anchored to the extra cellular matrix. In these cells, the externally exposed portion of the protein locks onto extracellular polymers, while the internal protein region might tie to the cytoskeleton. Some eukaryotic cells have carbohydrate moieties on their external surface. Most of the carbohydrates of the membrane are covalently bound to proteins forming the class of proteins known as glycoproteins. Other carbohydrate moities are bound to the head group of lipids

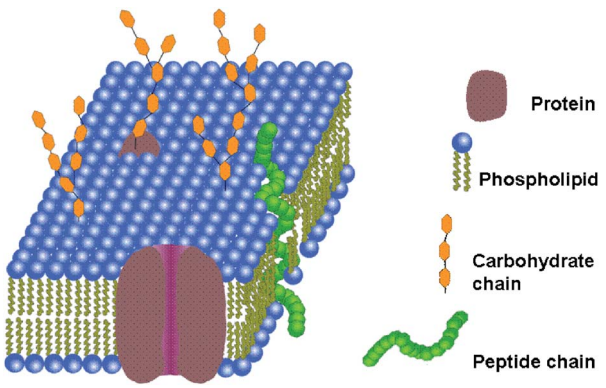

Fig. 1 Schematic representation of the cell membrane.

forming glycolipids. Membrane carbohydrates are recognition sites, enabling cells to be recognized by proteins of adjacent cells.

The composition, structure and function of the cell membrane have inspired numerous studies in the biomedical field aimed at the engineering of NP surfaces to promote long-circulation and specific targeting. Coating nanoparticles with lipid bilayers that mimic cellular envelopes provides several advantages,[23–25] such as enhancement of circulation time and accumulation in tumor cells, as demonstrated for example in the case of the liposome-based delivery of doxorubicin,[26] better biocompatibility, decreased toxicity and immunogenicity,[23,27–30] and enhanced structural stability, compared to liposomes.[23,31]

2.2 Strategies of biomimetic surface engineering

Biomimetic nanoparticles composed of a particle core coated with phospholipids possess the advantages inherent to both nanoparticles and phospholipids. The solid core confers mechanical stability to the phospholipid layer while the phospholipid envelope endows biocompatibility to the nanoparticles. Strategies of biomimetic surface engineering of nanoparticles with phospholipids are depicted in Fig. 2 and Fig. 3.

2.2.1 Coating of nanoparticles with phospholipid bilayers

Electrostatically-driven physical adsorption. The incorporation of anionic or cationic lipids in a phospholipid bilayer yields charged vesicles that can be adsorbed onto oppositely charged polymeric or inorganic nanoparticles *via* electrostatic attraction. Successful adsorption of a lipid monolayer on a nanoparticle results in a *ca.* 10-nm increase of the mean average diameter of the particle, as observed in the case of mixed vesicles/polystyrene latex dispersions.[32] Cationic lipid bilayers were also deposited on functionalized silica particles. The supported membrane acts as a barrier preventing the escape of encapsulated dye molecules.[33,34] Optimal cationic lipidic bilayer deposition on particles was achieved by coalescence under careful adjustment of the parameters, such as the pH and ionic strength of the dispersion medium and the concentrations of the interacting components (nanoparticles and lipid vesicles).[35]

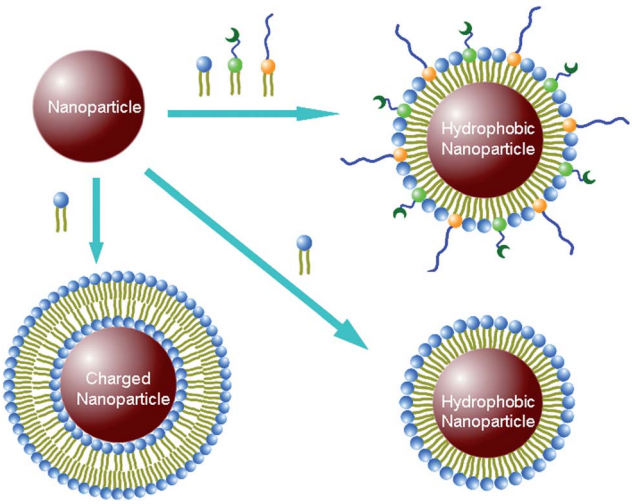

Fig. 2 Schematic illustration of the biomimetic surface engineering of nanoparticles with phospholipids. Various nanoparticles loaded with diagnostic and therapeutic agents can be encapsulated by phospholipid monolayers or multilayers to protect their cargo from enzymatic degradation and to reduce toxicity and immunogenicity. Targeting ligands such as antibodies, aptamers or small molecules can be bound to phospholipids to help the particles to lock onto target cells. Hydrophilic polymers such as PEG, carbohydrates are also incorporated in the lipids to extend the particle circulation half-time in blood.

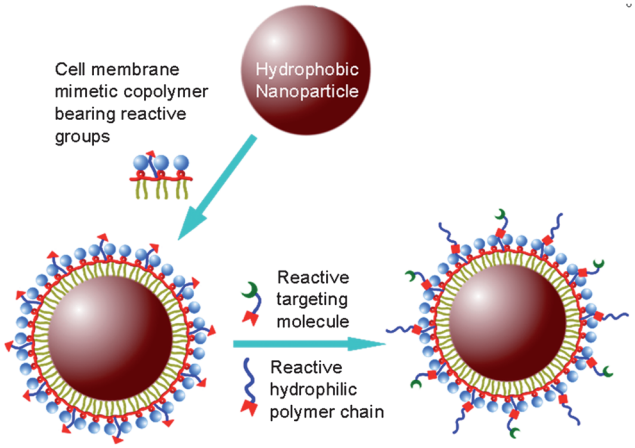

Fig. 3 Schematic illustration of biomimetic surface engineering of nanoparticles with phospholipid copolymers bearing reactive groups. The copolymer contains hydrophilic phosphorylcholine groups and hydrophobic alkyl groups as side chains. A reactive group such as an active ester or an aldehyde, can be incorporated on the polymer chain. After coating the copolymer onto the nanoparticles, the reactive groups of the polymer can be linked readily to amino groups of proteins, peptides, or other functional molecules dissolved in the aqueous dispersion medium. The main advantage of this strategy is that it permits one to introduce simultaneously different functionalities onto the surface.

Hydrophobically-driven physical adsorption. Neutral phospholipids, such as phosphatidylcholine and dipalmitoylphosphatidylcholine (DPPC), adsorb and self-assemble onto hydrophobic polymer nanoparticles through hydrophobic interactions in order to reduce the free energy of the system. The hydrophobic tails of lipids adsorb onto the hydrophobic NP surface, while the hydrophilic head groups of the lipids extend into the external aqueous environment, forming a lipid monolayer coated polymeric nanoparticle, and imparting to the NPs their stability in water. As more and more lipids are added to the NP dispersion, vesicles form in addition to lipid monolayer coated NPs. The latter can interact with the vesicles *via* van der Waals interaction, resulting in further deposition of lipid bilayers on the NPs, such that an increasingly larger (odd) number of monolayers will be deposited on polymeric NPs. Several such lipid–polymer hybrid NPs have been reported and used in pharmaceutical applications, including lipid-supporting particles based on poly (lactic-*co*-glycolic acid) and poly-L-lactide.[35–42]

Drug loaded nanoparticles enveloped by phospholipids were also designed specifically to improve their biocompatibility. An inherent drawback of such lipid-coated NPs lies in their short circulation time, as a consequence of the progressive loss of the physically adsorbed lipidic layer. Recent progress in lipid-based nanoparticles as pharmaceutical drug carriers has been reviewed in several excellent articles.[43,44]

2.2.2 Coating of nanoparticles with phosphorylcholine polymers. To overcome the limitations imposed by the reversible adsorption of phospholipids onto NPs, several groups have explored the use of amphiphilic copolymers bearing along their main chain hydrophilic phosphorylcholine (PC) groups and hydrophobic moieties.[45–51] Since phosphorylcholine is the major hydrophilic group of the cell outer membrane, amphiphilic copolymers bearing hydrophilic phosphorylcholine groups are called "cell membrane mimetic polymers". Gong *et al.* have depicted a scheme for cell membrane mimicking by self-assembly of an amphiphilic copolymer (Fig. 4).

Three methods were devised to anchor phosphorylcholine groups onto NP surfaces by the cell membrane mimetic polymer strategy. The simplest one consists of coating nanoparticles with an amphiphilic copolymer bearing hydrophilic phosphorylcholine groups. In an aqueous medium, the hydrophobic chains/blocks of the amphiphilic copolymer adsorb onto the hydrophobic surface of the nanoparticles, and the hydrophilic phosphorylcholine groups orient themselves toward the aqueous phase, thus forming, with the copolymer, a coating of structure analogous to the cell outer membrane.[53–56] The second method relies on the surface-initiated grafting of a PC-containing polymer onto the particle surface. For example, nonthrombogenic NPs were obtained by grafting silica nanoparticles with poly-(2-methacryloyloxyethyl phosphorylcholine) (poly-MPC) *via* radical graft polymerization of MPC.[57] In the third strategy, an amphiphilic PC-containing copolymer is used alone. The composition of the copolymer is such that nanoparticles form upon dispersion of the copolymer in water by self-assembly driven by hydrophobic interactions between the hydrophobic groups, which form the core of the NPs while the hydrophilic phosphorylcholine groups distribute themselves on the water/micelle interface.[58–62]

Nanoparticles covered by phosphorylcholine groups have been applied extensively in the biomedical field. The covering of phosphorylcholine groups enables the nanoparticles to maintain the function of the colloid core within a biological environment. The cell membrane mimetic coating enhances the dispersion and stability of the nanoparticles in aqueous solution due to the prevention of biomolecule adsorption and stealth in biological environment. Interestingly, a special class of amphiphilic PC-copolymers, known as "amphipols", has been described by Diab *et al.* PC-containing amphipols have the unique capacity to protect transmembrane proteins against aggregation in water. The protection mechanism involves: (1) hydrophobic interactions between the hydrophobes on the polymers and the hydrophobic surface of the proteins; and (2) stabilization in water *via* the PC groups present at the interface between water and the protein/amphipol NP.[63–65]

2.2.3 Stabilization of phospholipid coatings. High-performance applications require phospholipid coatings to remain stable under harsh denaturing conditions. Accordingly, synthetic strategies are often proposed to increase the chemical and mechanical stability of phospholipid assemblies. The polymerization of self-assembled phospholipid structures is a frequently used strategy that results in robust biocompatible architectures. Polymerization can be designed in either the hydrophobic region[66] or the hydrophilic region[67,68] of the phospholipid. Recently, biomimetic designs and performances of polymerizable lipids have been reviewed in detail.[69]

To protect fluorescent tags from unfavorable protein adsorption, Saavedra and researchers[70] coated the probes with a cross-linked polylipid coating. Rhodamine–protamine fluorescent molecules were encapsulated in silica nanoparticles (Si NPs), and the luminescent Si NPs themselves were coated with a cross-linkable phospholipid. The fluorescently tagged Si NPs were used as fluorescent probes to label HeLa cells. The phospholipid coating on the Si NPs was photopolymerized to produce a chemically cross-linked polylipid coating that effectively protected the luminescent Si NPs from undesirable association/aggregation. Before and after cross-linking, lipid-coated NPs had no interaction with HeLa cells; however, in the presence of a denaturing surfactant, the non-crosslinked coating readily desorbed from the particle surface, yielding bare Si NPs, which adsorbed to the cells' surface. The cross-linked phospholipid coating prevented the adsorption of Si NPs to HeLa cells in the presence of surfactants, proving the stability of the polylipid coating. Furthermore, the phospholipid bioactivity was demonstrated when biotin was bonded to the cross-linked phospholipid on the Si NPs, and biotin-functionalized NPs successfully targeted the conjugation to protein receptors.[69]

Other methods of fabricating stable phospholipid coatings include chemical bonding and crosslinking. For example, phospholipids bearing thiols can chemisorb onto gold and silver nanoparticles to form a stable self-assembled monolayer.[71–73] Reactive groups, such as trimethoxysilyl group[52,74] and active esters[75,76] were incorporated in a phosphorylcholine copolymer

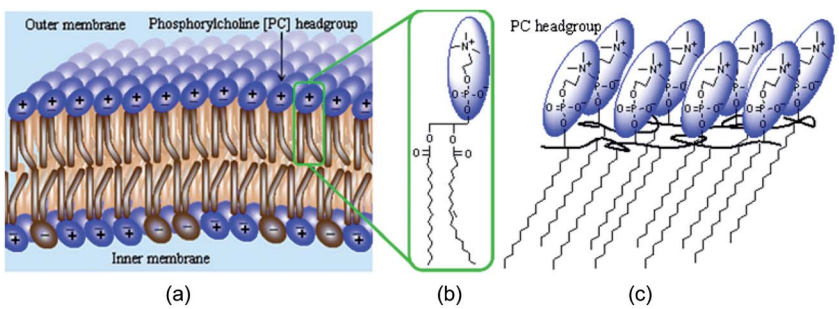

Fig. 4 Schematic structures of cell membrane and the outer membrane mimicking polymeric assembly: (a) lipid bilayer of cell membrane; (b) phosphatidylcholine; (c) cell membrane mimetic polymer assembly. Reproduced with permission from ref. 52. Copyright 2008 Elsevier B.V.

that forms a cell membrane mimetic coating on nanoparticles. Reactions between the reactive groups or with a crosslink agent immobilize the polymer chain and, accordingly, increase the stability of the coating.

2.2.4 Coating with other cell membrane molecules. Membrane proteins are found inserted in the lipid bilayer. They perform diverse functions in cells, such as transport of specific solutes into or out of cells; signal transduction (relaying hormonal messages to the cell); cell–cell recognition (allowing other proteins to attach two adjacent cells together); intercellular joining of adjacent cells with gap or tight junctions. Inspired by the functions of membrane proteins and peptides, scientists have coupled NP surfaces with specific antibodies and peptides, to interact selectively with cells bearing the corresponding antigens or receptors. For example, the amyloid protein antibody was coated onto nanoparticles functionalized with a phospholipid monolayer to develop novel targeting approaches for detecting and treating cerebrovascular amyloid deposits.[77] Similarly, gold nanoparticles were coated with a short peptide to promote intracellular delivery of membrane-impermeable proteins.[78] Interestingly, not only the particle size, but also the targeted organs, can be tuned by variations in nanoparticle peptide coating.[79]

As described in the introduction, carbohydrates present on the cell membrane are usually branched oligosaccharides covalently linked to lipids, forming glycolipids, or more commonly to proteins, forming glycoproteins. The oligosaccharides vary from species-to-species, from individual to individual, and even from cell type to cell type within the same individual. This variation distinguishes each cell type, such as A, B, AB, and O groups of human red blood cells. The carbohydrates attached on cell surface may play a critical role for stabilization by both hydrophilicity and steric repulsion. Similar effects were used to stabilize nanoparticles.[80–82] A new MRI platform was developed by coupling *N*-acetyl-D-glucosamine and D-mannose onto magnetic nanoparticles *via* amidation of the amine groups in the outer shell of apoferritin containing magnetic nanoparticles.[83] Metal nanoparticle-based biomimetic strategies using conjugated carbohydrates have been reviewed as potential replacements for native biomolecules in immunoassays and catalysis applications.[84]

It is expected that by mimicking the function of cell membrane proteins and carbohydrates, it will be possible to obtain long circulation nanoparticles, as well as specific targeting, highly efficient therapeutic and diagnostic nanoparticles.

3. Biomimetic surface engineering for long circulation

NPs are generally removed from the blood stream by macrophages of the reticuloendothelial system (RES), resulting in NP accumulation in both the liver and the spleen and subsequent clearance from the circulation.[85] In order to overcome the elimination of nanoparticles by the RES, a number of effective methods have been developed to render nanoparticles 'invisible' to the RES, resulting in long-circulating nanoparticles (NPs), which are also referred to as stealth NPs. For example, NPs coated with poly(ethylene glycol) (PEG) exhibit decreased levels of uptake by the MPS and an increased circulation time in the blood.[86] The surface engineering of NPs with PEG moieties has emerged as a platform for the incorporation of active-targeting molecules, thereby providing the drug carriers with specific tumor-targeting properties.[87] Recently, the surface PEGylation and application in cancer targeting of NPs including liposomes, micelles, polymer NPs and lipid NPs, have been well reviewed.[88] In this section, our discussions will be limited to the recent progress on surface engineering of NPs with biomolecules, mainly with the cell membrane mimetic composition and structure.

3.1 Steric repulsion effect of carbohydrates

Shen *et al.* have reported that the blood circulation half-life of a PEGylated PLA NPs is lower than that of PLA NPs coated with a water soluble chitosan (WSC). More interestingly, the NP circulation half-life was enhanced from several minutes to 63 h by coating the NP with both PEG and WSC.[17] The synergistic action of the WSC and PEG was ascribed to the neutralization of the surface charge and the dense brush-like repulsive conformation of the hydrophilic polymers. A recent report describes a preparation of long-circulating PEG-NPs suitable for magnetic tumor targeting starting with starch-coated NPs that were crosslinked, aminated, and modified with PEG using *N*-hydroxysuccinimide functional groups as intermediates. The modified NPs showed 61–98 fold longer plasma half-life in rats, compared to the unmodified parent starch NPs.[14] Ohyanagi *et al.* reported that quantum dots (QDs) coated with a phosphorylcholine self-assembled monolayer showed much better long-term stability than PEG-coated QDs.[71] More importantly, live animal NIR fluorescence imaging indicated that the *in vivo* circulation half-life of phosphorylcholine coated QDs increased by a factor of 10, or more, after conjugation with α-sialic acid residues.

3.2 Steric repulsion effect of PEG/Lipid monolayer

Micelle-like nanoparticles were prepared by condensing plasmid DNA with a chemical conjugate of phospholipid with polyethylenimine, followed by coating the complexes with a lipid monolayer envelope. The addition of PEG–phosphatidylethanolamine led to spherical hard-core nanoparticles loaded with DNA. The coating allowed for complete protection of the loaded DNA from enzymic degradation, resistance to salt-induced aggregation, and reduced cytotoxicity. The NP also demonstrated prolonged blood circulation and low RES accumulation.[18,20] The PEGylated phospholipid coating was also employed to expand the circulation half-life of magnetic nanoparticles.[89]

4. Biomimetic surface engineering for specific targeting

Nanoparticle targeting can be achieved by mimicking naturally occurring targeting mechanisms, such as by covalently attaching tissue-specific antibodies,[90–92] peptides,[93–96] folate, saccharides[97] and hormones onto the particle surfaces.[4]

4.1 Antibody targeting

Antibodies can be immobilized chemically onto nanoparticles *via* two routes. One approach is to modify the nanoparticles with

reactive groups, as a first step. Subsequently, the reactive NPs are mixed and coupled with purified antibodies. In the other method, the antibody is activated first. Then, this activated antibody is coupled to nanoparticles. For example, antibodies against biomarkers of interest were modified with transcyclooctene and used as scaffolds to couple tetrazine-modified nanoparticles onto live cells.[98] This technique is fast, chemoselective, adaptable to metal nanomaterials, and scalable for biomedical use. In addition to chemical modifications, carbon nanovectors can also be linked to antibody *via* noncovalent interactions, such as hydrophobic interactions. This targeted drug delivery assembly could enable high-throughput screening of drug/antibody combinations.[99]

Progress in surface coating design of iron oxide nanoparticles for targeted cancer therapy have been discussed systematically in a recent account.[100] Recent achievements include the active tumor-targeting of single chain prostate stem cell antigen antibody by conjugation on the surface of docetaxel/superparamagnetic iron oxide loaded nanoparticles using a functional poly(ethylene glycol).[101] Selective targeting of antibody conjugated multifunctional nanoclusters to epidermal growth factor receptors in cancer cells was also achieved. Iron oxide nanocluster cores were coated with gold in order to promote near-IR absorbance, while the absorbance of AlexaFluor 488 labels conjugated to iron oxide/gold nanoclusters was used to correlate the NIR signal to the number of adsorbed NPs.[102] These stable and tumor-targeting polymer NPs could be promising multifunctional vehicles for simultaneous targeting imaging, drug delivery and real time monitoring of the therapeutic effect.

In another study, nanoparticles bearing functionalized phospholipid monolayers were coated with a monoclonal antibody against fibrillar human amyloid-42. The targeting ability of these nanoparticles to cerebrovascular amyloid provides opportunities for the prevention and treatment of cerebral amyloid angiopathy.[77] For a more complete description of various lipid-coated and antibody-conjugated nanoparticles for drug delivery and monitoring the reader is referred to a review by Namiki *et al.*[44] It is believed that the continuing development of lipid-based nanomedicine will lead to fast progress towards highly sensitive and minimally invasive cancer treatments

4.2 Peptide targeting

To achieve stable and highly sensitive bioimaging with fluorescence probe on target cells, polymer nanoparticles with embedded quantum dots were covered with an artificial cell membrane. These nanoparticles were designed by assembling phospholipid polar groups as a platform onto which oligopeptides were immobilized as bioaffinity moieties. The polymer nanoparticles were resistant against HeLa cell uptake due to the intrinsic properties of the phosphorylcholine groups. An arginine octapeptide was immobilized on the surface of the nanoparticles by reaction with active ester groups present on the artificial cell membrane coating. The resulting NPs were able to penetrate the membrane of HeLa cells effectively without any cytotoxic effect.[103]

Micelle-like nanoparticles based on covalent conjugation of a phospholipid and polyethylenimine core enveloped by a phospholipid/PEG layer showed a good capacity to complex and protect siRNA from serum degradation. The effectiveness of the NPs was similar to that of polyethyleneimine, but the NPs exhibited improved biocompatibility properties, compared to polyethyleneimine, including neutral surface charge and absence of *in vitro* cytotoxicity. The phospholipid cell outer membrane surface decorated with a few PEG chains makes the nanoparticles long-circulating and an efficient delivery system to B16F10 cells.[18] Also, the combination of PEI complexes with several liposomal formulations led to nontoxic and efficient *in vitro* delivery of siRNA.[104] Similarly, PEI/siRNA complexes[105] encapsulated in PEG-stabilized liposomes were successfully tested for targeted delivery by coupling an antibody to the surface of the liposomes.

4.3 Folate targeting

Folate (vitamin B_9) is a vital nutrient required by all living cells for nucleotide biosynthesis and for the proper metabolic maintenance of 1-carbon pathways. More interestingly, folate displays high affinity for the folate receptor, a glycosylphosphatidyinositol-linked protein overexpressed in most cancers acting as a recognized tumor antigen/biomarker. Therefore, folate targeting methods have been exploited extensively for cancer diagnosis[106–110] and therapy.[111–113]

In order to develop a novel tumor-targeting and site-specific labeling NP, the tumor-targeting folic acid group was conjugated to the surface of QDs *via* a cell-penetrating γ-cyclodextrin coating. Results of flow cytometry and confocal laser scanning microscopy revealed that the folate-receptor targeted QDs could more effectively recognize cancer cells, known to over-express the folate receptor, compared to non-targeted QDs.[106] Biocompatible, hydrophilic, magneto-fluorescent bimodal imaging nanoparticles with surface-pendant amine, carboxyl, and aldehyde groups were designed using *O*-carboxymethyl chitosan. After conjugation of folic acid and its aminated derivatives, these magneto-fluorescent nanoparticles were internalized at much higher level by HeLa cells, compared to normal L929 fibroblast cells.[103] Folate receptor-targeted NPs were also prepared for cancer cell imaging using two-photon microscopy.[108,109] More impressively, a novel multifunctional nanoparticle for simultaneous magnetic resonance (MR) and fluorescence imaging, cell targeting and photosensitization treatment was prepared by a three step process. Superparamagnetic magnetite nanoparticles and fluorescent dyes were co-encapsulated inside nonporous silica nanoparticles as the core to provide dual-imaging capabilities. Then, tetra-substituted carboxyl aluminum phthalocyanine was covalently linked to the mesoporous silica shell as the photosensitizer. Finally, the surface of the nanoparticle was modified with folic acid to enhance the delivery of the particle to the targeting cancer cells that over express the folate receptor.[110]

Folate-decorated chitosan nanoparticles loaded with doxorubicin were used successfully for targeted delivery to retinoblastoma.[111] Similarly designed NPs loaded with DNA were used in a nonviral gene therapeutic approach. It was shown that hydrodynamic delivery helped to enhancing the transfection efficiency of folate-chitosan-DNA nanoparticles *in vitro* and *in vivo*. Moreover treatment with these folate-chitosan-DNA NPs resulted in a significant decrease in inflammation in arthritic rats.[112] Interestingly, a folate-functionalized degradable

amphiphilic dendrimer-like star polymer (FA-DLSP) with a well-defined poly(L-lactide) star polymer core and six hydrophilic polyester dendrons based on 2,2-bis(hydroxymethyl) propionic acid was successfully synthesized.[113] This unimolecular micelle could be a promising nanosize anticancer drug carrier with excellent targeting property.

4.4 Carbohydrate targeting

Carbohydrates on cell surfaces contribute to a variety of communications between the cell and its environment, and are believed to act as markers for cellular recognition. Coating a nanoparticle with carbohydrate has a series of advantages, including dispersibility in aqueous media, biocompatibility, stability, and targeting properties. The selection of suitable carbohydrates for specific targeting biomarkers opens up the possibility to employ more NPs in diagnostics and/or therapy.[114] *N*-Acetylglucosamine conjugated polyethylenimine was used to target various vimentin-expressing cells, such as fibroblasts and tumor cells.[115] A carbohydrate recognition-based approach for efficiently targeting B cells *in vivo* can offer improved treatment options for patients with B-cell malignancies.[116] 2-Methacryloyloxyethyl phosphorylcholine polymer nanoparticles bearing hydrazide groups can react with unnatural carbohydrates with ketone groups, which are present on human cervical carcinoma cell (HeLa) surfaces, leading Iwasaki *et al.* to suggest cellular-specific drug delivery by means of such novel nanoparticles.[117] Water-dispersible rhamnose-coated iron oxide nanoparticles were shown to confer targeting properties to nanosystems, since this sugar is a substrate of lectins of the skin.[118] Hyaluronic acid grafted with hydrophobic moieties forms stable and long-circulating drug delivery nanoparticles as a potential cancer-targeted drug delivery system.[119]

5. Conclusion and future perspective

Biomimetic surface engineering of nanoparticles offers numerous advantages as a biomedical application platform, including wide range of flexible strategies, ease of surface functionalization, prolonged circulation half-time, more specific cancer targeting, reduced toxicity, and increased stability of particles. All of these features make the cell membrane mimetic nanoparticles an ideal cargo delivery platform for cancer diagnosis and therapy. Although great progress has been made in the area of synthesis, characterization and applications of the cell membrane mimetic nanoparticles, a number of key challenges remain unmet and need to be addressed, in order to promote this new nanoparticle platform as a robust cargo delivery system for medical applications.

First, highly selective targeting is critical for decreasing the toxicity against normal cells in cancer therapy. To improve the selectivity in recognition and targeting, two or more different targeting molecules can be coupled on the particle surface and a synergistic effect may be expected. Second, optimizing the targeting ligand density on the nanoparticle surface is crucial to achieve optimal therapeutic efficacy. As many kinds of targeting ligands have been conjugated to improve the accumulation of the modified nanoparticles to their specific sites of action, it would be desirable to screen and optimize the ligand density through *in vivo* experiments. Lastly, precise control of the surface structure and composition of the coated layer on the nanoparticles remains challenging.

Fortunately, amphiphilic copolymers bearing phosphorylcholine and other functionalizable groups, can be designed to serve as multifunctional platforms for surface engineering. The multifunctional coating can provide biocompatibility from the cell membrane mimetic structure, as well as permit the conjugation of desirable amounts of different ligands.

Acknowledgements

This work is supported by the National Natural Science Foundation of China (No. 20774073, 20974087), and Beijing National Laboratory for Molecular Sciences (BNLMS).

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