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FOCUS REVIEW

Surface engineering of nanoparticles for therapeutic applications

Kenya Kobayashi[1], Jinjian Wei[2], Ryo Iida[2], Kuniharu Ijiro[3] and Kenichi Niikura[3]

Nanoparticles with a diameter of <100 nm are regarded as potential medical materials, as this size allows nanoparticles to circulate *in vivo* and possibly reach targeted tumors. Inorganic nanoparticles in particular are able to interact with light and/or magnetic fields, thus extending their potential applications to such fields as fluorescence labeling, magnetic resonance imaging and stimulus-responsive drug delivery that are essential to the diagnosis and treatment of disease. To facilitate their use in such applications, the appropriate design of surface ligands on these nanoparticles is necessary. The surface ligands determine the physicochemical properties of the surface, such as hydrophilicity/hydrophobicity and zeta potential as well as dispersibility in solution. These properties have an especially important role in determining nanoparticle–cell associations, such as cellular membrane permeability, immune responses and localization *in vivo*. This review focuses on recent advances in the surface engineering of nanoparticles for therapeutic applications.
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INTRODUCTION

Recently, therapeutic applications of polymer particles with diameters ranging from several nanometers to one micron have become an extremely important research topic.[1,2] These nanoparticles are referred to as 'therapeutic nanoparticles' and commonly function as drug molecule carriers. For example, drug-loaded polymeric micelles with diameters of 30 nm can reach poorly permeable pancreatic tumors to deliver an antitumor drug load.[3] Furthermore, 'smart' drug-delivery systems (DDSs) capable of controlled release have also been reported.[4] In addition to their use as delivery carriers, polymer nanoparticles are feasible as vaccine adjuvants that enhance immune responses. Biodegradable poly(γ-glutamic acid) nanoparticles were shown to work as adjuvants with a level of activity comparable to that of conventional aluminum adjuvants, supporting the notion that polymer-based particles show promise as effective vaccines.[5,6]

Recently, inorganic and semiconductor nanoparticles have been widely used as therapeutic tools owing to the ability of inorganic nanoparticles to interact with light or magnetic fields, affording a useful nanomaterial for application to fields including fluorescence or magnetic resonance imaging,[7] X-ray imaging,[8] Raman imaging[9] and DDS with an external stimulus-triggered release function.[10] Inorganic metal nanoparticles with controlled diameters ranging from ~ 2 to no more than 100 nm can be readily prepared. This feature itself can be an advantage in therapeutic applications. With regard to biomedical applications, for example, gold nanoparticles (AuNPs) have some advantages over other inorganic nanoparticles. Simple synthetic protocols for the preparation of AuNPs with controlled sizes and shapes have been generally established,[11,12] and these AuNPs are biocompatible owing to their inertness, allowing wide biomedical applications. The cellular uptake of AuNPs into mammalian cells has been recently studied.[13,14] The surface modification of AuNPs with target molecules is easily achieved via strong thiol–gold interactions, providing various types of platforms on which to attach drugs including monolayers,[15–17] layer by layer,[18] silica gel coatings[19] and liposomes[20] (Figure 1). The encapsulated drugs can be released by light irradiation, as AuNPs show high efficacy of light-to-heat conversion.[21,22] Poly(ethylene glycol) (PEG)-coated AuNPs have been used as a representative carrier in which drugs are encapsulated in either a covalent[23] or a noncovalent[24] manner. Light scattering from AuNPs at a plasmon wavelength[25] or efficient surface-enhanced Raman spectroscopy (SERS) signals from attached dyes[26] also make AuNPs available for use as imaging tools. The use of AuNPs has not been limited to monodispersed forms. Self-assembled nanoparticle vesicles (NVs) have emerged as a potential new carrier in which drugs can be encapsulated and released by light irradiation.[27]

In this review, we describe recent advances in the surface engineering of AuNPs, with a particular focus on the following three topics: (1) surface design for enhanced cellular uptake, (2) capsule-like assembly of AuNPs for drug delivery and (3) engineering of AuNPs for use as vaccine adjuvants. We also discuss the importance of surface ligands in regulating and enhancing cell–nanoparticle interactions toward the creation of smart therapeutic nanomaterials.

[1]Nano Medical Engineering Laboratory, RIKEN, Saitama, Japan; [2]Graduate School of Chemical Sciences and Engineering, Hokkaido University, Sapporo, Japan and [3]Research Institute for Electronic Science (RIES), Hokkaido University, Sapporo, Japan
Correspondence: Dr K Niikura, Research Institute for Electronic Science (RIES), Hokkaido University, Kita 21, Nishi 10, Kita-Ku, Sapporo, Hokkaido 001-0021, Japan.
E-mail: kniikura@poly.es.hokudai.ac.jp
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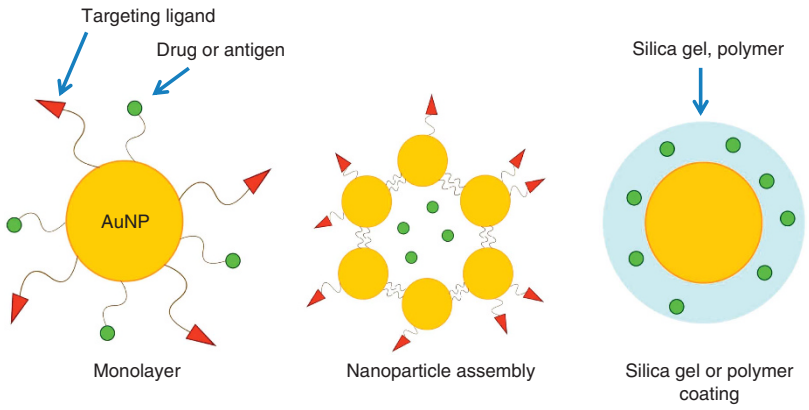

Figure 1 The surface modification of gold nanoparticles (AuNPs) for use as a drug-delivery system (DDS) carrier.

DESIGN OF SURFACE LIGANDS TO ENHANCE CELLULAR UPTAKE

Surface ligands for water-dispersible nanoparticles with high colloidal stability

The biological use of nanoparticles requires both stability and dispersibility in water. The surface modification of ligands has an important role in endowing these properties. Both strong anchoring of the surface ligands to the nanoparticle surface and the display of hydrophilic moieties are required to increase colloidal stability in water. For example, thiol and disulfide derivatives are frequently employed for the modification of AuNPs and semiconductor quantum dots (QDs) owing to their strong interaction with inorganic surfaces. Zubarev and coworkers[28] demonstrated that the alkyl-chain packing of ligand molecules is a key factor for colloidal stability. Gold nanorods (AuNRs) are typically synthesized in the presence of excess cationic surfactant bearing C16 alkyl chains (cetyltrimethylammonium bromide (CTAB)) that noncovalently covers the AuNR surface. The replacement of CTAB with peptides is hindered by the remaining CTAB;[29] however, modification with C16 thiol molecules (16-mercaptohexadecyltrimethylammonium bromide) can result in the stable dispersion of 16-mercaptohexadecyltrimethylammonium bromide/AuNRs in water.[28]

The water dispersibility of nanoparticles can be provided by the attachment of hydrophilic functional groups to the ligands such as PEG, carboxylic acids, sulfonic acids, ammonium salts or zwitterions. Charged nanoparticles show higher water dispersibility than do noncharged nanoparticles. Grzybowski and coworkers[30] synthesized AuNPs coated with two thiol ligands: 11-mercaptoundecaoic acid as an anionic ligand and 11-mercaptohexadecyltrimethylammonium chloride as a cationic ligand. They demonstrated fine tuning of nanoparticle charge by a co-display of cationic and anionic ligands, and the AuNPs were stable under both high and low pH conditions. The cellular uptake of AuNPs can be influenced by surface charge.[31] In general, positively charged nanoparticles can be readily taken up into cells owing to their high affinity for the cell membrane, whereas negatively charged nanoparticles show longer circulation in the blood stream. These two factors are important to the therapeutic use of nanoparticles and should be carefully balanced. As the net charge of nanoparticles is adjustable by the co-display of anionic and cationic ligands, this approach is expected to be applicable to pH-responsive cellular uptake.

AuNPs must also be robust against biological component-induced precipitation. For example, the exposure of nanoparticles to media containing electrolytes like sodium chloride cause aggregation as the salt neutralizes the electronic repulsion among nanoparticles. In addition, biomolecules of thiol derivatives, such as glutathione or cysteine, also induce aggregation via ligand exchange. Susumu, Mattoussi, Medintzz and coworkers[32,33] mentioned that multidentate ligand anchorage to the surface of nanoparticles is required for stabilization of nanoparticles in water. Nanoparticles coated with dithiol ligands, such as dihydrolipoic acid-terminated PEG derivative ligands, are more stable than those coated with monothiol ligands. The authors synthesized multidendate poly(ethylene glycol) ligands that were constructed from two dihydrolipoic acid anchor groups to provide higher stability to the nanoparticles via strong multi-thiol bonds with the nanoparticle surface. In fact, QDs and AuNPs coated with the multidendate ligands remained in a dispersed state for several months in media that were both highly acidic and basic and that contained a high concentration of NaCl and dithiothreitol as a mimic of a thiol-containing biomolecule.[33] Vachet and coworkers[34] also reported the difficulty of replacing bisthiol ligands attached to the AuNPs with thiol-containing biomolecules within the cell, whereas monothiol ligands are easily released. The advantage of increasing the number of anchors for colloidal stabilization is not limited to the case of AuNPs or QDs. Iron oxide nanoparticles, frequently coated with catechol anchor ligands, can be further stabilized by increasing the number of catechol anchors.[35–37] For example, Mefford and coworkers[35] reported that ligands bearing three catechol moieties afford magnetite nanoparticles higher durability against aggregation in phosphate-buffered saline (PBS) than do monocatechol ligands.

The density of the surface ligand also affects the dispersity of NPs. Textor and coworkers[38] compared the stability of Fe_3O_4 NPs modified with linear and dendritic PEG. Because it covered the surface at a higher density, dendritic PEG better stabilized the NPs than did linear PEG.

Polymers are also employed for the modification of nanoparticles.[39–41] Although modification with polymers can provide stability or biocompatibility to nanoparticles similar to that realized by the use of various ligands, the thickness of the surface coating and polymer architecture can be controlled by changing the polymerization conditions. While addition of thiol-terminated polymers to the nanoparticles is one approach for modification, atom transfer radical polymerization[42,43] and reversible addition-fragmentation chain transfer[44] polymerization on the surface of nanoparticles are also promising protocols for controlled surface modification. In contrast, Lin and coworkers[45] demonstrated that star-like polymers branched from a β-cyclodextrin core were excellent templates for nanoparticle production (Figure 2). Near-monodispersion of AuNPs

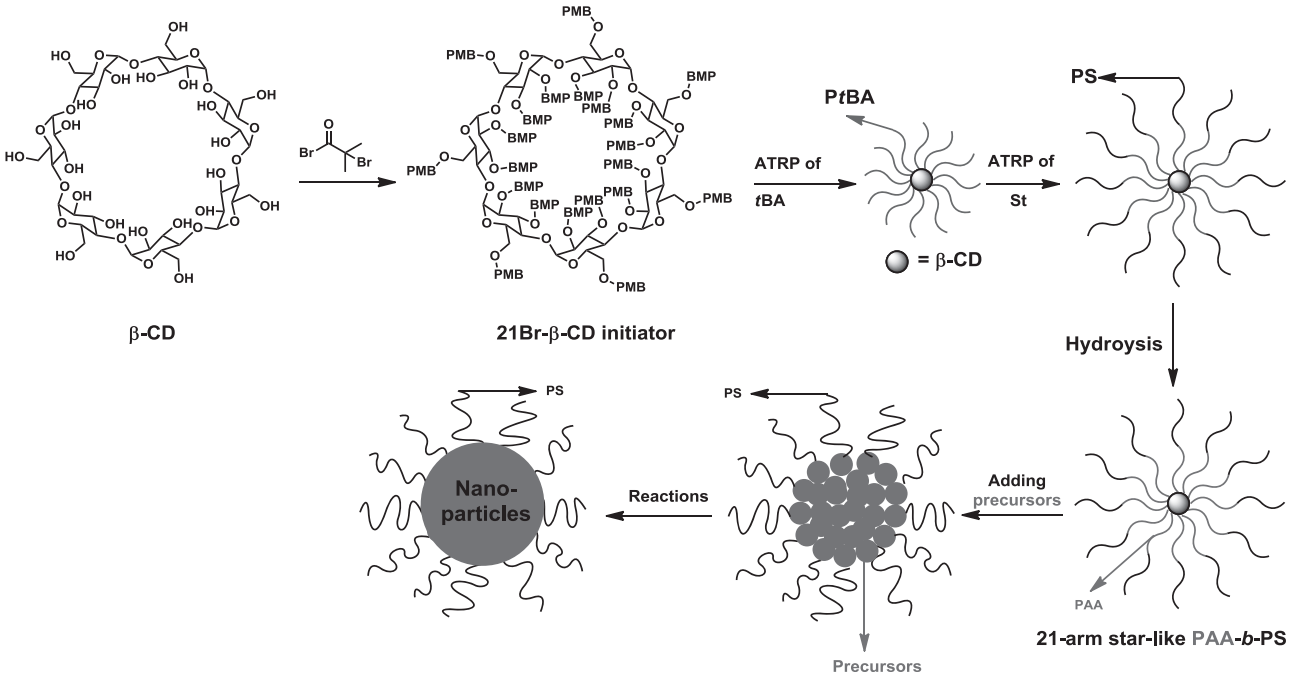

Figure 2 A star-like-polymer template strategy for the synthesis of monodispersed colloidal nanoparticles. Reproduced from Pang *et al*.[45] with permission from the Nature Publishing Group. A full color version of this figure is available at the *Polymer Journal* online.

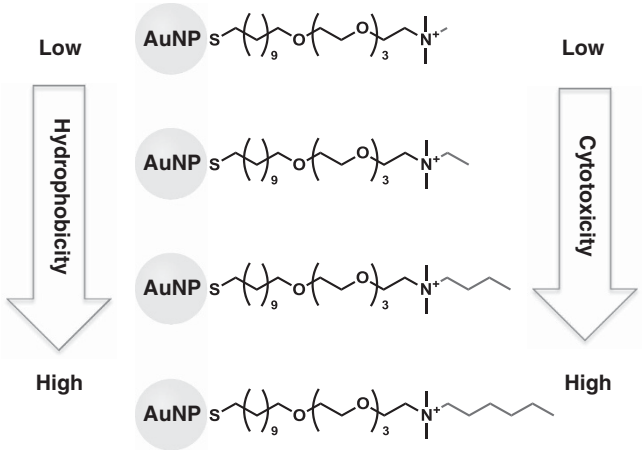

Figure 3 Relationship between hydrophobicity of ligands and cytotoxicity (see Kim *et al*.[46]). A full color version of this figure is available at the *Polymer Journal* online.

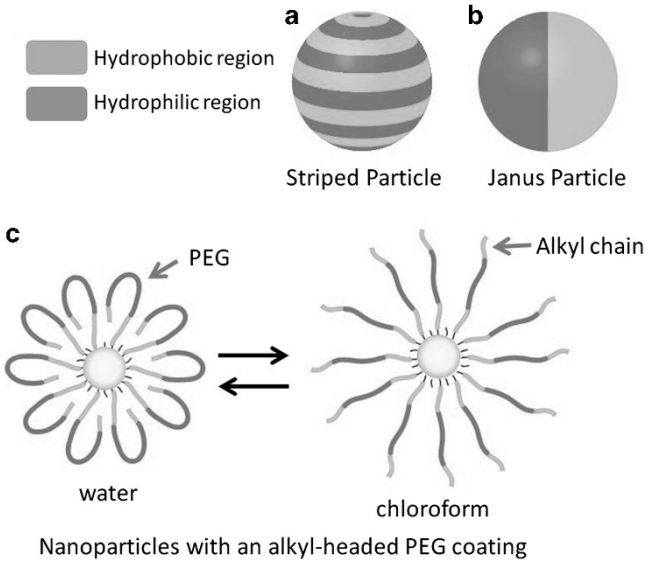

Figure 4 Surface engineering of nanoparticles for cell membrane permeation. (**a**) Striped particles. (**b**) Janus particles. (**c**) Alkyl-headed poly(ethylene glycol) (PEG) nanoparticles. A full color version of this figure is available at the *Polymer Journal* online.

bearing various functionalities on the surface was achieved by templating various polymers.

Surface ligands for cell membrane-permeable nanoparticles

As mentioned in the above section, nanoparticles require hydrophilicity for good dispersion in water or serum to prevent aggregation. However, hydrophobicity is also required to enhance the interaction of nanoparticles with the cellular membrane to encourage uptake into cells. Cationic ligands, such as amines or ammonium salts, achieve high affinity with the cellular membrane, allowing a high level of endocytic cellular uptake.[46,47] However, rather than being an advantage, cationic nanoparticles disrupt the cellular membrane, resulting in cytotoxicity by changing the cell membrane potential and intracellular concentration of calcium ions.[46–49] This trend is particularly true for cationic nanoparticles with higher levels of surface hydrophobicity.[46] Indeed, increasing the length of the alkyl chain on the quaternary ammonium cation of surface ligands increased their cytotoxicity (Figure 3).

In this way, noncationic ligands enabling higher levels of cellular uptake of nanoparticles are in high demand for safety reasons. In addition, nanoparticles with cationic surfaces tend to be taken up by cells via endocytosis, and this is undesirable for the delivery of many drugs, such as small interfering RNA and antibodies.

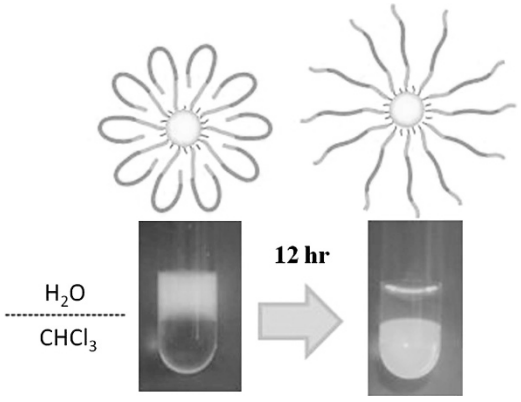

Figure 5 Phase transfer of C4-headed ligand-displaying CdTe quantum dots (QDs); (Niikura, K. & Ijiro, K., unpublished data). A full color version of this figure is available at the *Polymer Journal* online.

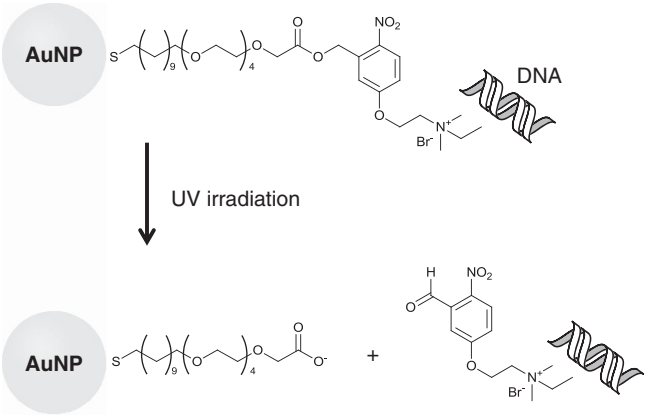

Figure 6 Charge reversal surface ligands upon ultraviolet (UV) irradiation and concomitant release of DNA in cells (see Han *et al.*[59]). A full color version of this figure is available at the *Polymer Journal* online.

To overcome the issues of toxicity and other limitations associated with the use of cations, there has been much emphasis on the development of noncationic, cellular membrane-permeable nanoparticles and ligands. Stellacci and coworkers[50,51] reported striped gold nanoparticles, the surface of which was coated with *n*-octanethiol and sodium 11-mercapto-1-undecanesulfonate in a striped pattern that could penetrate the cell membrane of mammalian cells (Figure 4a). Van Lehn and Alexander-Katz[52] simulated Janus particles with one hydrophilic and one hydrophobic hemisphere that could favorably interact with the cellular membrane (Figure 4b). Bishop and coworkers[53] demonstrated that Janus AuNPs with similar hydrophobic/hydrophilic faces could penetrate the lipid bilayer of liposomes, implying that Janus AuNPs are applicable as DDS carriers. Eychmüller and coworkers[54] reported that quantum dots coated with methyl-terminated PEG ligands could penetrate the cell membrane.

In the process of cell membrane penetration, nanoparticles must cross from the hydrophilic serum to the hydrophobic cell membrane, necessitating an amphiphilic nanoparticle surface for this type of transfer. Ijiro and coworkers[55] developed alkyl(C2–C8 in alkyl length)-head PEG ligands that can impart amphiphilicity to gold nanoparticles (Figure 4c). The ligand is constructed from a hydrophobic alkyl moiety at the head of the ligand, a hydrophilic PEG moiety in the middle and a thiol anchor for attachment to the AuNP surface. AuNPs coated with C8-head ligand can cross from water to chloroform phases. Similar results were obtained for CdTe QDs. Figure 5 indicated the phase transfer of C4-headed CdTe nanoparticles from water to chloroform. In the aqueous phase, PEG moieties on ligands were exposed to the medium whereas the alkyl heads were hidden inside the ligand layer (Figure 4c). However, the alkyl heads were exposed in chloroform because of the stretched form of the ligands. The switchability of the ligand conformation based on the surrounding solvent is a key to endowing amphiphilicity; however, a driving force to power passage through the membrane is still required.

Stimulus-responsive nanoparticles

Nanoparticles require a balance between hydrophilicity and hydrophobicity to maintain dispersibility in serum and allow facile cellular uptake. However, when hydrophobic nanoparticles within the cell membrane penetrate into the cytosol, the surface of the nanoparticle should become hydrophilic to allow facile permeation. If the hydrophobicity/hydrophilicity of the nanoparticles can be changed by stimuli, cell permeation will be accelerated. Typically, several stimuli such as light[56–60] and temperature[61,62] have been employed as stimuli to change the surface properties of nanoparticles. Hydrophilic/hydrophobic photoswitching on spiropyrane-immobilized nanoparticle surfaces has been accomplished by several groups.[57,58] Rotello and coworkers[59] demonstrated the charge reversal of nanoparticles with the aim of gene release within cells upon ultraviolet irradiation. They developed a PEG-derivative thiol ligand possessing *o*-nitrobenzaldehyde with a cationic moiety at the terminus. AuNPs coated with the ligands are initially cationic; however, *o*-nitrobenzaldehyde can be cleaved upon ultraviolet irradiation and converted to carboxylic acid. Thus, the gene, which is attached by electrostatic interactions, can be released upon ultraviolet irradiation (Figure 6). By coating the nanoparticle surface with thermo-responsive polymers, the aggregational state can be controlled by temperature.[61,62]

These methods provide dynamic changes to the surface properties; however, the stimuli used to change the particle properties should be both biocompatible and applied internally rather than externally to allow *in vivo* use. Ester capping of a carboxylic acid moiety is a promising concept observed in prodrug technology to promote cellular uptake.

Ester-hydrolysis-triggered cellular uptake

Drug molecules in which a carboxylic acid moiety is capped by an ester react with esterase to produce the original form of the drug.[63–65] The esterase-triggered release of coumarin dye, which was conjugated to the AuNRs, was demonstrated by Ito and coworkers.[66] Ijiro and coworkers[67] adapted this idea to the enhanced internalization of AuNPs by coating them with ester-terminal ligands. In this approach, the hydrophobic terminus of alkyl-headed ligands,[55] in which alkyl moieties were connected by an ether bond, was replaced by an ester bond (Figure 7). The hydrolysis of the ester groups is expected to provide a driving force for the cellular uptake of AuNPs.

To examine the above hypothesis, four types of PEG-derived thiol ligands bearing ethyl ester (C2-Ester), *n*-butyl ester (C4-Ester), ethyl ether (C2-Ether) or *n*-butyl ether (C4-Ether) at their termini were synthesized and then conjugated to AuNPs with diameters of 10 nm (Figure 7). The dispersion of AuNPs (C2-Ester- and C2-Ether-AuNPs) in PBS was mounted on dichloromethane to form a heterogeneous system. Both AuNPs were transferred from the PBS layer to the dichloromethane layer. Successive addition of sodium hydroxide to the PBS phase resulted in phase transfer of the C2-Ester-AuNPs to the PBS phase from the dichloromethane phase. However, the C2-Ether-

AuNPs remained in the dichloromethane phase (Figure 8a). This phase transfer triggered by ester hydrolysis appears to support the hypothesis that esterase promotes the penetration of the cellular membrane by AuNPs and their localization in the cytosol. Ester-headed AuNPs were applied to HeLa cells, and the level of cellular uptake was measured by inductively coupled plasma atomic emission spectroscopy. A small number of C2-Ether-AuNPs were internalized to the HeLa cells after incubation for 3 h, whereas a larger number of C2-Ester-AuNPs were taken up by the HeLa cells. Extension of the incubation time from 3 to 24 h resulted in greater cellular uptake of both AuNPs, although the C2-Ester-AuNPs were still taken up in larger numbers (Figure 8b).

Transmission electron microscopy imaging reveals that internalized C2-Ester-AuNPs were mainly localized in the multivesicular bodies, although some AuNPs were found within the cytosol (Figure 8c), implying that these AuNPs were internalized via cell membrane penetration. These data suggest that intracellular stimuli-triggered cellular uptake is a promising concept to increase the level of nanoparticle uptake.

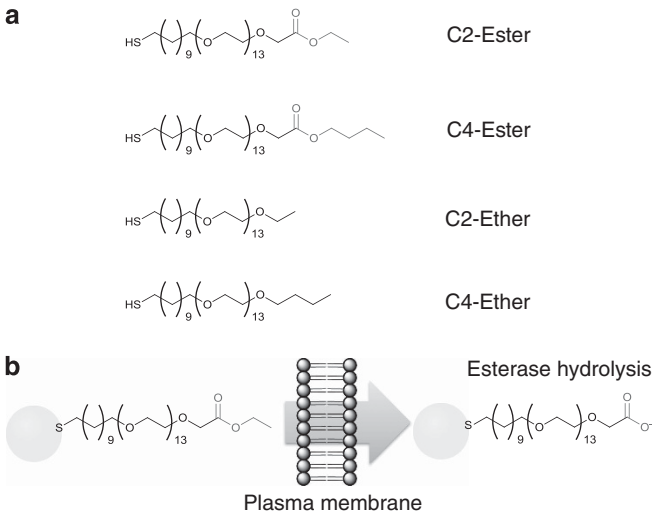

Figure 7 (**a**) Chemical structures of alkyl(ester and ether)-headed poly(ethylene glycol) (PEG) ligands. (**b**) Speculated mechanism for the cellular uptake of ester-headed nanoparticles. A full color version of this figure is available at the *Polymer Journal* online.

Specific localization of nanoparticles in cells

In nanomaterial-based therapy, the tumor cell-specific delivery of nanoparticles is a crucial issue. It is generally accepted that tumor cells tend to uptake larger numbers of molecules and retain the internalized molecules longer than do normal cells owing to enhanced permeation and retention effects. However, for the effective use of drugs and the minimization of side effects as well as dosage, the accumulation of drugs or nanoparticles into the target organ should be controlled and cell specific. Thus, studies on the relationships among the nanoparticle surface, localization of accumulated nanoparticles with the organ and cell specificity are required. Rotello and coworkers[46] reported the relationship between the surface functionality of AuNPs and their accumulation in organs. For example, charged nanoparticles (both cationic and anionic) can be cleared faster than AuNPs bearing

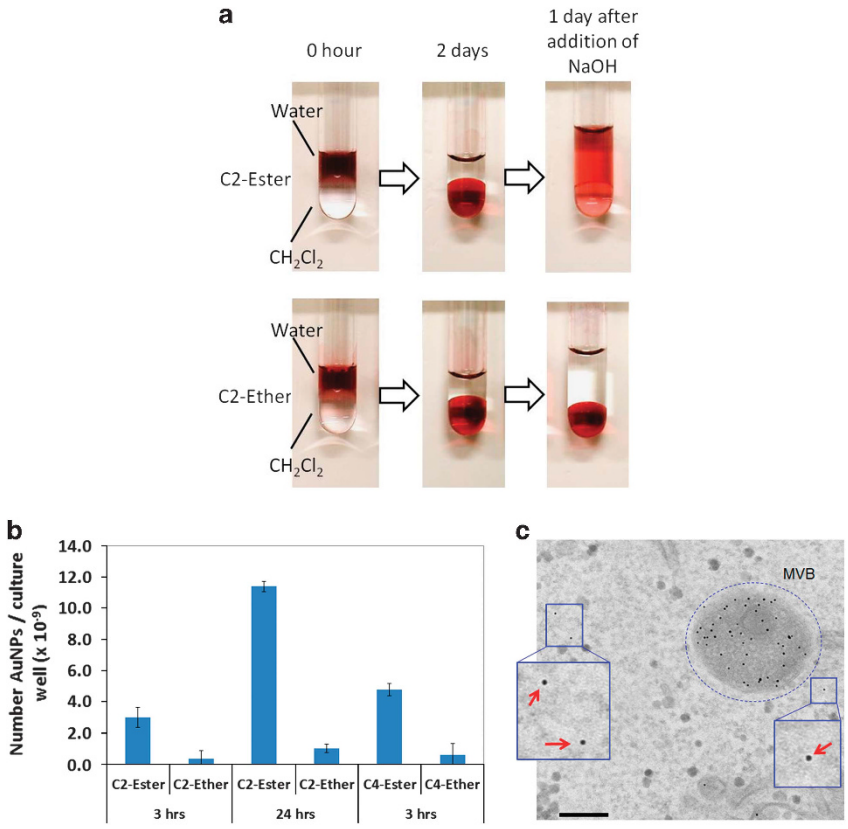

Figure 8 (**a**) Phase transfer of gold nanoparticles (AuNPs; top: C2-Ester-AuNPs, bottom: C2-Ether-AuNPs). (**b**) Number of AuNPs internalized into HeLa cells per well (1.0×10^5 cells per well). (**c**) Ultra-thin section image of HeLa cells observed by transmission electron microscopy (TEM). Red arrows indicate nanoparticles within the cytosol. Scale bar: 200 nm. Reproduced from Kobayashi *et al.*[67] Copyright 2014 Royal Society of Chemistry.

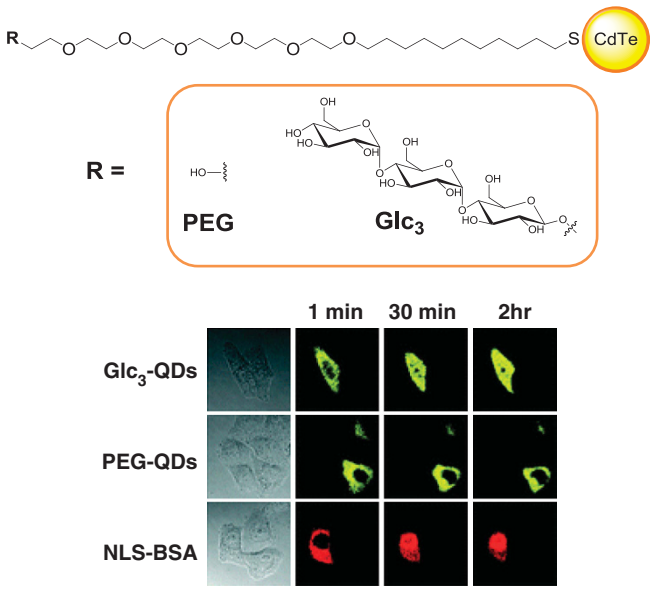

Figure 9 Chemical structures of maltotriose ligand and maltooligosaccharide derivatives immobilized on quantum dots (QDs). Pictures show confocal microscopy of differential interference contrast and fluorescence images of living HeLa cells after microinjection of Glc3-QDs, poly(ethylene glycol) (PEG)-QDs and nuclear localization signal (NLS)-attached bovine serum albumin (BSA). Reproduced from Sekiguchi *et al.*[72] Copyright 2012 Royal Society of Chemistry.

zwitterions or a neutral surface when administered via intravenous injection.[46] Cell-specific delivery with the aid of targeting agents is also a promising method for the effective use of drugs. A characteristic feature of tumor cells is the overexpression of certain receptors or molecules relative to normal cells. For example, SK-BR-3 cells, a breast cancer cell line, overexpresses the Herceptin receptor HER-2. Thus, nanoparticles conjugated with Herceptin as a targeting agent are selectively internalized in SK-BR-3 cells.[68,69] In addition to HER-2 overexpression, tumor cells overexpress certain sugar receptors. For example, nanoparticles bearing galactose selectively enter HepG2 cells rather than HeLa cells owing to receptor-mediated endocytosis.[70,71] The localization of nanoparticles within the cell after cellular uptake can be controlled by surface modification. Ijiro and coworkers[72] demonstrated the nuclear import of a maltotriose-QD conjugate that is enhanced by the high affinity between the nuclear pore and the sugar pendant on the surface of the QDs. PEG-modified and mono- and disaccharide-QDs were not transportable into the nuclei, whereas trisaccharide-QDs could enter the nuclei owing to the high affinity of trisaccharide ligands for the nuclear pore (Figure 9). Recently, Galan and coworkers[73] applied QDs functionalized with different types of sugar to intercellular localization. They demonstrated that the type of sugar on QDs could affect cellular uptake and lactose-QDs could serve as a 'Trojan horse' to assist the internalization of QDs with other noninternalizable sugar moieties into the cell. Furthermore, sialic acid-QD conjugates were shown to have high retention in the bloodstream.[74]

CELLULAR INTERNALIZATION OF NANOPARTICLE ASSEMBLIES

Encapsulation strategies associated with the controlled delivery of active pharmaceutical ingredients to the disease site for biomedical applications are in high demand. Fabrication of micro- or nanocapsules, such as liposomes, polysomes and micelles, by self-assembly strategies, has been successfully and extensively investigated.[75–78]

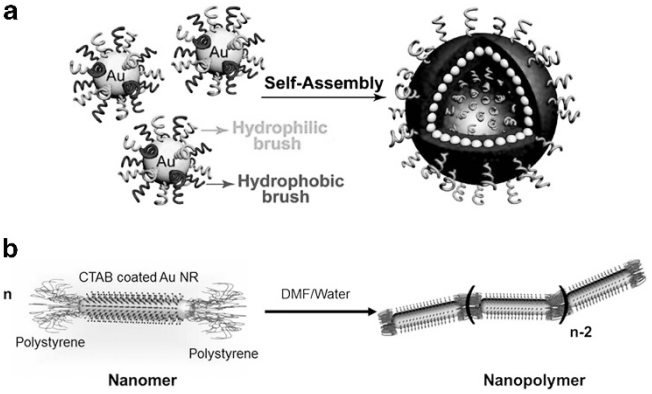

Figure 10 Self-assembly of polymer-coated gold nanoparticles (AuNPs) and gold nanorods (AuNRs). (**a**) Formation of nanoparticle vesicles by displaying two polymers (poly(ethylene glycol) (PEG) and poly(methyl methacrylate)) on AuNPs. Reproduced from Song *et al.*[27] with permission from the American Chemical Society. (**b**) Self-assembly of AuNRs to construct a gold nanopolymer. Reproduced from Liu *et al.*[81] with permission from the American Chemical Society. A full color version of this figure is available at the *Polymer Journal* online.

Inorganic nanoparticles are particularly intriguing candidates as building blocks for self-assembly in the fabrication of various two- and three-dimensional hierarchical superstructures for application to material, analytical and medicinal sciences.[79] In particular, the vesicular assembly of AuNPs serving as a DDS carrier has attracted much attention in recent years owing to their collective plasmonic properties that differ from those of single NPs.[80–86] To date, the most popular method for the synthesis of AuNVs is based on the use of polymer brushes to functionalize the AuNP surface, followed by self-assembly in selective solvents (Figure 10a).[27] The main driving force in the nanoparticle self-assembly process can be attributed to hydrophobic interactions. Kumacheva and coworkers[81] successfully fabricated AuNR vesicles and chain structures using hydrophilic CTAB-coated gold nanorods, with termini modified with hydrophobic polystyrene by changing the solvent quality of the building blocks (Figure 10b).

Recently, Duan and coworkers[82] developed photo-responsive plasmonic vesicles with AuNPs embedded in a hydrophobic shell of photo-responsive hydrophobic poly(2-nitrobenzyl arylate). They demonstrated that plasmonic vesicles could not only serve as drug delivery carriers but also offer real-time feedback using SERS to detect the drug release process. This type of vesicle can act as a new class of theranostic platforms for simultaneous SERS detection and chemo-photothermal therapy against specific cancer cells. The biodegradable AuNVs, with a strong localized surface plasmon resonance peak in the near-infrared region, showed high photothermal conversion for photoacoustic imaging and enhanced photothermal therapy.[83,84]

Another promising approach to the preparation of AuNVs is based on the modification of AuNPs with small surface ligands instead of complex polymers. Small surface molecules, such as ligands, significantly decrease nanogaps, resulting in enhanced plasmon coupling and thereby expanding the plasmonic applications. Ijiro and coworkers[87] reported the formation of three-dimensional superlattices using fluorinated tetraethylene glycol-stabilized AuNPs via a fast drying process. After modification with the fluorinated ligand using oligo(ethylene glycol), the AuNPs coated with semi-fluorinated ligands provided sub-100-nm hollow nanostructures in a selective solvent with high efficiency, and the nanoparticle vesicles were SERS active in solution (Figure 11a).[85] Therefore, the solvophobic effect

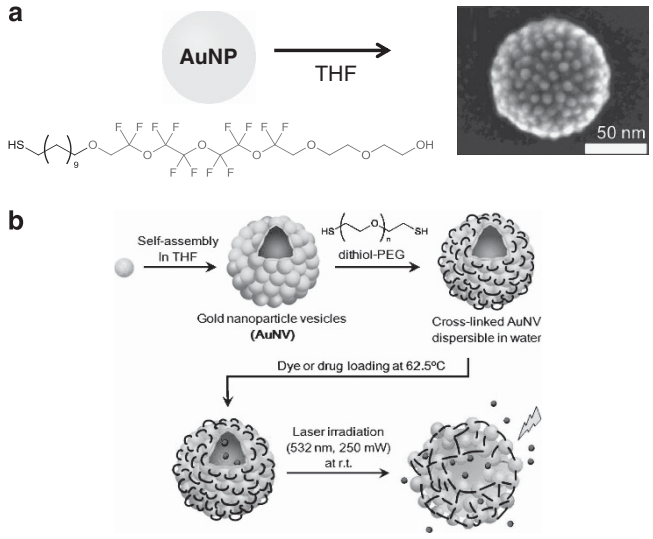

Figure 11 (**a**) Chemical structure of fluorinated poly(ethylene glycol) (PEG) ligand and transmission electron microscopy (TEM) image of gold nanoparticle vesicles. Reproduced from Niikura *et al.*[85] with permission from the American Chemical Society. (**b**) Schematic diagram showing water-dispersible nanoparticle vesicle (AuNV) formation by linking PEG and irradiation-triggered release of encapsulated drug molecules. Reproduced from Niikura *et al.*[86] with permission from the American Chemical Society. A full color version of this figure is available at the *Polymer Journal* online.

derived from the small fluorinated ligands can induce AuNP self-assembly. Another advantage of using fluorinated ligands on nanoparticles is the possibility of 19F imaging.[88] Furthermore, fluorinated dendrimers were shown to express excellent gene transfection in several cell lines.[89] Thus, nanoparticle vesicles induced by fluorinated ligands would be a strong candidate to simultaneously allow imaging and efficient drug delivery.

After crosslinking each nanoparticle using thiol-terminated PEG, the vesicle structure, which was maintained in water, could work as a DDS carrier to deliver doxorubicin into cells (Figure 11b).[86] Upon irradiation, 'closed' AuNVs were transformed to an 'open' status, thus enabling the rapid release of the encapsulated drug. This remote-controlled drug-release function introduces the possibility of nanoparticle vesicles as a carrier in DDS for localized therapy.

INFLUENCE OF THE SURFACE, SIZE AND SHAPE OF NANOPARTICLES ON IMMUNOLOGICAL RESPONSES

In addition to their use as drug carriers, the potential application of AuNPs as vaccine platforms is also attractive. Recent efforts to produce effective and safe vaccines have focused on subunit vaccines in which an antigen alone is linked to a strong immunogen, such as proteins or virus-like capsules. However, the antigen–protein conjugate induces antibodies specific for both the antigens and protein carriers, making multiple vaccinations inefficient owing to exclusion by carrier-specific antibodies. To prevent antibody production against the carrier itself, synthetic carriers are promising candidates.[90] AuNPs have been used as antigen carriers for subunit vaccines without the production of anti-AuNP antibodies. The effectiveness of AuNPs as an antigen carrier has been demonstrated for various viruses.[91,92] In these studies, conventional adjuvants such as complete Freund's adjuvant or alum, which are used to increase the induction of immunity, were coadministered with the nanoparticle carrier.

There have been a few recent reports that AuNPs themselves can induce immunological responses, such as antibody and cytokine

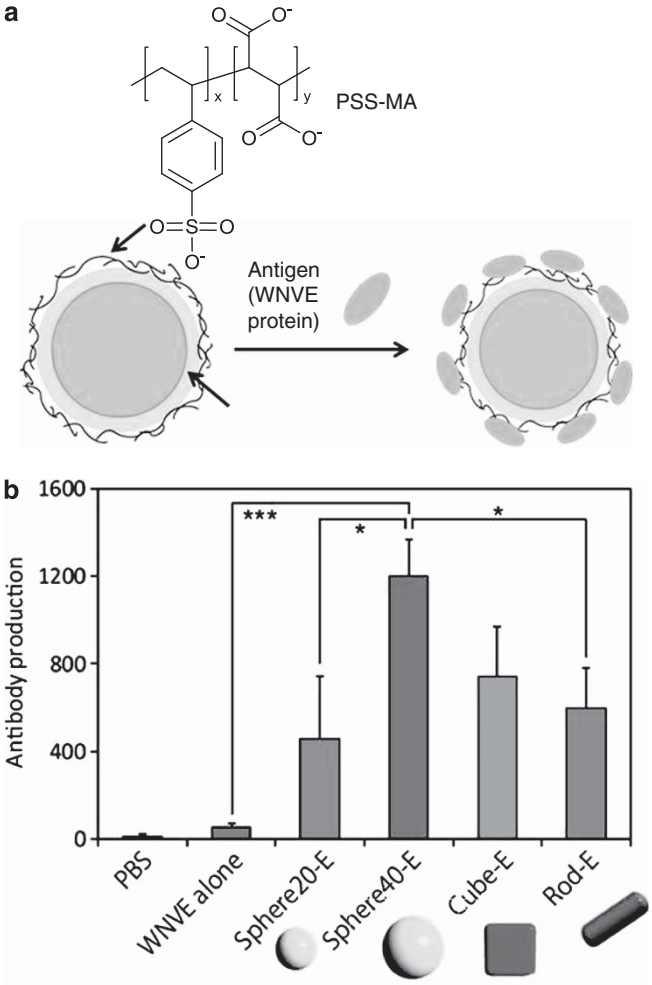

Figure 12 (**a**) Conjugation of West Nile virus envelope (WNVE) virus protein to gold nanoparticles (AuNPs) through poly(4-stylenesulfonic acid-co-maleic acid (PSS-MA). (**b**) WNVE-antibody production in mice. Reproduced from Niikura *et al.*[97] with permission from the American Chemical Society. Significant diffrences: $*P<0.05$; $***P<0.001$ (means (s.e.m., $n=10$). A full color version of this figure is available at the *Polymer Journal* online.

secretion.[93,94] Hence, an understanding of the effects of nanoparticles on cytokine release is important for their further application to vaccine adjuvants. Maysinger and coworkers[95] reported the AuNP shape dependence of the inflammatory response in microglial cells. Rotello and coworkers[96] reported that nanoparticle surface hydrophobicity is correlated with immune response.

Niikura *et al.*[97] prepared CTAB-coated spherical (20 and 40 nm in diameter), rod (40×10 nm) and cubic ($40\times40\times40$ nm) nanoparticles and compared their adjuvant activities. The nanoparticles were coated with poly(4-stylenesulfonic acid-co-maleic acid) according to the method of Gole and Murphy[98] followed by West Nile virus envelope (WNVE) protein via electrostatic interactions (Figure 12). The authors found that large spherical nanoparticles (40 nm) were more effective as a platform for antibody production than other shapes (cube and rod) or smaller spheres (20 nm). These data indicate that, in addition to surface properties, the size and shape of AuNPs should be considered in the development of effective nanoparticle-based vaccines. Furthermore, the effects of antigen immobilization chemistry on immune responses should be clarified in additional studies on the use of AuNPs as adjuvants.

CONCLUSION

Various types of inorganic nanoparticles have been developed, some of which are expected to have applications as therapeutic nanomaterials. In this review, we have focused on the importance of the surface engineering of these nanoparticles for therapeutic applications. In particular, the following three topics related to the biomedical application of AuNPs were discussed: (1) cellular membrane-permeable nanoparticles, (2) self-assembled nanoparticles and (3) nanoparticle-based vaccines.

Colloid and surface chemistry is a research field with a long history. Nevertheless, various new nanoparticle functions derived from surface ligands have been found, such as high cellular membrane permeability and strong immune responses. The accumulation of this knowledge regarding these functions will lead to new insights into and concepts for the design of surface-engineered nanoparticles applicable to medical uses. Furthermore, when considering the practical use of nanoparticles *in vivo*, the influence of serum proteins cannot be ignored,[99–101] as nanoparticles are exposed to high concentrations of proteins in the blood. We must consider different factors than those associated with cell-based *in vitro* experiments when dealing with nanoparticles under physiological conditions. Therefore, to control protein adsorption *in vivo*, functional polymer ligands, which can display various functionalities on the surface of nanoparticles, would offer much promise toward the development of the next generation of therapeutic nanoparticles.

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