Molecular-Integrated Phospholipid Polymer Nanoparticles with Highly Biofunctionality

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Summary: Surface modifications of nanoparticles with phospholipid polymers composed of 2-methacryloyloxyethyl phosphorylcholine (MPC), are summarized. The MPC can be available for various polymerization methods such as conventional radical polymerization and living radical polymerization, and easily copolymerized with other vinyl compounds. The MPC polymers have been widely used as biocompatible coating and stabilizer for nanoparticles even when they are under biological environment. Additionally, for immobilization of biomolecules, such as antibody and enzyme, the MPC polymers having active ester group are applicable. These MPC polymers coated on the nanoparticles immobilize protein under mild condition and the protein maintained bioactivity well. Moreover, introduction of functional inorganic nanocrystals inside of the nanoparticles is effective to obtain good imaging tool for specific cells. The potential of molecular integration on nanoparticles based on MPC polymer chemistry will be expanded nanobiosensing, nanoimaging and nanodiagnostic system.

Keywords: 2-methacryloyloxyethyl phosphorylcholine polymer; bioactive molecules; immobilization; molecular integration; nanoparticles

Introduction

Colloidal nanoparticles have been accepted strong interest from viewpoint of life science, medical science and bioengineering. They have unique properties such as large surface area, electrostatic properties, and specific interaction by conjugation with biomolecules. Surface modification of the colloidal nanoparticles allows for the dispersion and stability of the nanoparticles in aqueous medium while they still maintain their original physical characteristics. One of the methods for surface modification of the nanoparticles is to use water-soluble polymers and block polymers as a coating material for nanoparticles surface. In the case of block copolymer, amphiphilic-like structure should be designed because it needs that one polymer segment interacts with surface on nanoparticles and the other segment interacts freely with outer aqueous environments. Recently, polymer brush surfaces prepared by living radical polymerization techniques such as atom transfer radical polymerization (ATRP) and reversible addition-fragmentation chain transfer (RAFT) polymerization have been interested.[1–3] These methods can be conducted polymer brushes with high density from initiator immobilized on nanoparticles surface and also control the molecular weight related to polymer length and polydispersivity by reaction time, temperature, etc. The state of polymer chains covered on the nanoparticles determines characteristics of the nanoparticles.

Surface modification needs to choose a kind of coating materials and methods

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according to environment. For biomedical application, the surface-coated nanoparticles have been widely applied as diagnostic reagents, bioimaging tool, drug delivery carriers and bioaffinity beads for separation.[4,5] However, in fact, a practicable surface coating polymer is limited under biological application. Multi-functionalities, for example, biocompatible, easy preparation, stable water-dispersibility, solubility in high salt concentration, etc. are required for providing stable dispersion.

Polymers composed of 2-methacryloyloxyethyl phosphorylcholine (MPC: Figure 1) are well-known hydrophilic and biomedical polymer.[6,7] The chemical structure of the MPC is as the same as a phosphatidylcholine with phosphorylcholine group, which is one of the phospholipids of cell membrane. The MPC polymers show an excellent biocompatibility based on inhibition of protein/cell adsorption and activation on the surface[8–12] and have been applied for surface coating of many medical devices including implantable artificial organs.[13–15] Thus, the MPC polymers have stealth property and also they fulfill required properties of easy preparation and water-solubility in high salt concentration.[16–18]

Here, as shown in Figure 2, it is indicated the advantages of MPC polymers for preparation of nanoparticles. Moreover, immobilization of biomolecules on the MPC polymer-coated nanoparticles to obtain bioaffinity. The nanoparticles demonstrate efficient separation and capturing of target molecules by specific bioaffinity of immobilized biomolecules. And finally, it is demonstrated functionalization of core part of the nanoparticles with semiconductor nanocrystals to obtain fluorescence probe for bioimaging.

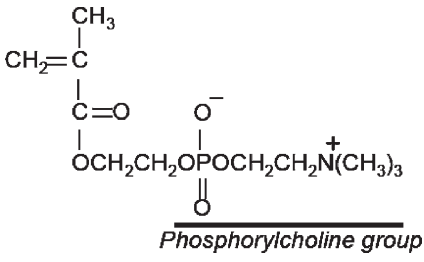

Figure 1.
Chemical structure of 2-methacryloyloxyethyl phosphorylcholine (MPC).

Nanoparticles with MPC Polymers

Table 1 summarizes nanoparticles prepared with the amphiphilic MPC polymer and surface modification of inorganic particles with the MPC polymers. Despite zwitterionic monomer, MPC can polymerize from the surface of nanoparticles by a conventional radical polymerization (RP)[19,20] and ATRP[22,24] easily and formed poly(MPC) chains as brush-like form. Grafting of poly(MPC) on inorganic nanoparticles was carried out by the living radical polymerization. They showed improved dispersity in aqueous medium due to brush shaped poly(MPC) chain. And also reduced protein adsorption of proteins was observed. For example, Jiang *et al.* demonstrated the silica nanoparticles grafted with poly(MPC) were available for peptide separation as column packing. The column has good separation efficiency without nonspecific adsorption.[20] Matsuno *et al.* prepared magnetic beads grafted with the poly(MPC) for magnetic separation of biomolecules.[23] Dispersion ability of the magnetic nanoparticles was significantly increased compared with non-treated particles.

Very recently, Matsuno *et al.* also developed smart technologies for graft polymerization of MPC by RAFT using surfactant-type initiator.[25] The grafting from surfactant-stabilized QD progressed RAFT manner and controlled polymer chain length.

Armes *et al.* synthesized several block-type polymers composed of poly(MPC) segment and polymer segment with amino groups in the side chain.[22,24,28,29] They tried to prepare nanoaggregate with DNA molecules through ionic interactions. In general, when the ionic interaction between cationic polymer and DNA was generated, the polymer complex may be precipitated due to neutralization of cationic part and anionic groups in the DNA. Although the cationic segment could bind DNA, the

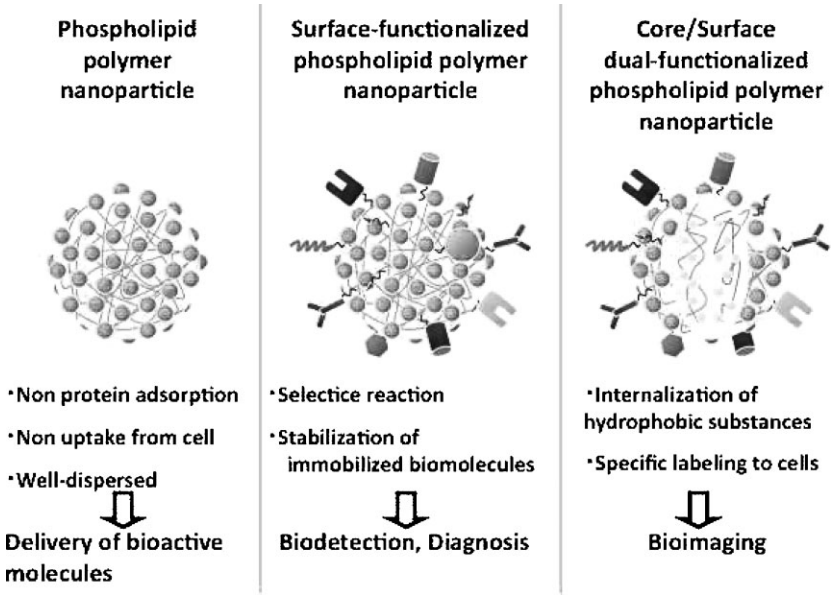

Figure 2.
Preparation and surface modification of nanoparticles with the phospholipid polymer, MPC polymers. Left: complexation with biomolecules or surface modification by MPC polymer, Center: surface modification with the MPC polymer and immobilization of biomolecules to obtain bioaffinity, Right: functionalization of both surface and core of the nanoparticles.

poly(MPC) segment in the block-type polymer did not affect for making the complex and maintained highly water-solubility, the complex did not precipitate. This is advantageous points for utilizing the MPC segment. As DNA vector, one of the random polymers, poly(MPC-*random*-2-aminoethyl methacrylate (AEMA)) delivered DNA into the cells and protein formation was observed inside cells.[27]

Table 1.
Preparetion and surface modification of nanoparticles with MPC polymers.

Core	Polymer	Polymer synthesis	Application	Ref.
SiO_2	Poly(MPC)	Grafting-from by RP	Nonthrombogenic materials	[19]
SiO_2	Poly(MPC)	Grafting-from by RP	Column packing	[20]
SiO_2	Poly(MPC)	Grafting-from by ATRP	Analytical system	[21]
Au	Poly(MPC-*block*-DMA)	ATRP	Detection system	[22]
Fe_3O_4	Poly(MPC)	Grafting-from by ATRP	Magnetic separation	[23]
Fe_3O_4	Poly(MPC-*block*-DEA)	ATRP	Imaging agent	[24]
Quantum dots	Poly(MPC)	Grafting-from by RAFT	Cell imaging tool	[25]
Carbon nanotube	Poly(MPC)	Grafting-from by ATRP	Analytical system	[26]
DNA	Poly(MPC-*random*-AEMA)	RP	DNA vector	[27]
DNA	Poly(MPC-*block*-DEA)	ATRP	DNA vector	[28]
DNA	Poly(MPC-*block*-DPA)	ATRP	DNA vector	[29]
Cancer drug	Poly(MPC-*randam*-BMA)	RP	Drug carrier	[30]
PLA	Poly(MPC-*randam*-BMA)	RP	Drug carrier	[31]
Phospholipid liposome	Poly(MPC), Poly(MPC-*random*-BMA)	RP	Drug carrier	[32]

DMA: 2-(N, N-dimethylamino)ethyl methacrylate, MEONP: *p*-nitrophenyloxycarbonyl poly(ethylene glycol) methacrylate, DEA: 2-(N,N-diethylamino)ethyl methacrylate, AEMA: 2-aminoethyl methacrylate, DPA: 2-(N, N-diisopropylamino)ethyl methacrylate, BMA: *n*-butyl methacrylate.

Water-soluble amphiphilic MPC polymers form polymer aggregate over critical aggregation concentration in aqueous medium. The representative polymer was poly(MPC-*random*-*n*-butyl methacrylate (BMA)) (PMB).[30,31] Even the MPC unit mole fraction is only 0.30, it can dissolve in water by controlling the molecular weight below 10^5. The BMA units are very hydrophobic, so the polymer chains gather by hydrophobic interaction and provide hydrophobic domain. The diameter of the PMB aggregate was about 20 nm when the concentration of the polymer was 1.0 wt%. In the MPC polymer aggregate, hydrophobic bioactive agents could be solubilized.[30] One of the cancer drugs, paclitaxel (PTX) is very poorly solubility (< 0.3 μg/mL) in aqueous medium and it is difficult to administrate to patients by an injection. By using aqueous solution containing PMB aggregate, the PTX could be dissolved more than 2 mg/mL. This PTX formulation is biocompatible and effective to treat cancer, which was revealed by in vivo animal test.[33]

Polymeric nanoparticles covered with the MPC polymer were prepared by a simple solvent evaporation method using the PMB as the surface modifier and emulsionaizer.[31] To PMB aqueous solution the dichloromethane solution containing hydrophobic polymer, such as, poly(L-lactic acid) (PLA) and polystyrene (PSt), was added. And then sonication was applied to make a suspension. The dichloromethane was evaporated under reduced pressure. The polymer nanoparticles suspended in the aqueous medium ware obtained. The diameter of the nanoparticles was depended to the concentration of the MPC polymers, but that was in the range between 200 nm and 350 nm. Surface analysis of the nanoparticles with X-ray photoelectron spectroscopy indicated that the surface was completely covered with the MPC unit and zeta-potential of the nanoparticles was almost zero (−0.4 mV). Low-molecular weight hydrophobic molecules could adsorb at the surface of core polymer through the MPC polymer layer, however, high-molecular weight proteins did not adsorb due to the characteristics of the MPC polymer. That is, the polymer nanoparticles showing biostealth characteristics are obtained.

Polymer Nanoparticles Covered with the MPC Polymers and Immobilization of Biomolecules

To provide much more functionality on nanoparticles surface, immobilization of biomolecules, such as antibody, enzyme, and DNA, should be conducted. At that time, the surface requires to maintain the structure and activity of these biomolecules. Also, to enhance bioaffinity of the nanoparticles to the target molecules, unfavorable non-specific binding must be inhibited. The MPC polymer surfaces are suitable for this purpose, so functional groups for immobilizing the biomolecules under mild physical condition are introduced. A new MPC polymer having active ester groups has been prepared by radical polymerization among MPC, BMA and *p*-nitrophenyloxycarbonyl poly(ethylene glychol) methacrylate (MEONP).[34–36] By controlling the composition of MPC unit, it was obtained water-soluble and amphiphilic MPC polymer (PMBN). Using the same procedure for preparation of polymer nanoparticles using PMB, the polymer nanoparticles covered with the PMBN were prepared.[37–42] The MPC and MEONP units were located at the surface of the nanoparticles and proteins can be immobilized by just mixing with the nanoparticles and stored at 4 °C for 24 h. The activity of antibody immobilized on the MPC polymer nanoparticles was 100 times higher than that on PSt nanoparticles.[42] Amount of protein adsorbed on the nanoparticles covered with MPC polymer was smaller about 300 times than that on PSt nanoparticles. That is, non-specific adsorption of biomolecules was almost inhibited. Affinity separation of albumin (BSA), one of the plasma proteins, from protein mixture using anti-BSA antibody immobilized nanoparticles could be realized with highly efficiency.

Table 2.
Functions of nanoparticles coverd with PMBN.

Core	Immobilized biomolecules	Application	Ref.
PLA	Anti-CRP monoclonal antibody	CRP detection system	[37]
PLA	Acetylcholine esterase and choline oxidase	Microdialysis biosensor	[38]
PLA	Luciferase	Microdialysis biosensor	[39]
PSt	Alexa Fuor 488-labeled anti-CRP IgG and Alexa Fuor 555-labeled anti-CRP IgG	Biomolecular recognition system using FRET	[40]
PLA	Anti-IgG antibody, Alkaline phosphatase	Medical treatment agent	[41]
PLA	Anti-albumin antibody	High affinity separation system for proteins	[42]

CRP: C-reactive protein, IgG: Immunogloburin G, PTX: Paclitaxel.

These properties are due to biocompatible platform based on the MPC polymer.

Table 2 summarizes characteristics and functions of polymer nanoparticles coated with PMBN and immobilized biomolecules. The nanoparticles have potential for use in highly selective separation, a promising tool for a diagnostic system, and a screening device for bioactive reagents. For example, the detection limit of serum-free C-reactive protein (CRP), which is one of the marker proteins for immunoreaction, was extended from 0.01 to 10 mg/dL when anti-CRP antibody was immobilized on the nanoparticles.[37] Denaturation of anti-CRP antibody immobilized on the nanoparticles hardly occurred despite increasing the temperature.

To have instantaneous determination for bimolecular recognition it was installed fluorescence resonance energy transfer (FRET) system in polymer nanoparticles covered with PMBN.[40] The favorable characteristics are suppression of non-specific protein adsorption and simple bioassay protocol relative to conventional enzyme-linked immunosorbent assay (ELISA). In the case of immunoassay, non-specific interaction and complicate protocol are known to dominant problems. To improve these issues, it was designed FRET-installed polymer nanoparticles. Agglutination of the nanoparticles is fundamental immunoassay; however, it is not quantitative. If it can be evaluated the degree of agglutination by fluorescence intensity, the resulting information is capable of being diagnosis. Therefore, the FRET system was installed at the surface of nanoparticles. The CRP and osteopontin (OPN) were used as target biomarkers for the instantaneous determination, and the resulting fluorescence intensity was well correlated with change in concentration of the target molecules. The immunoassay protocol is quite simple, only mixing of FRET-installed nanoparticles and target molecules such as CRP and OPN antigens. It was successfully evaluated the concentration of target biomarkers, even if human serum albumin was contained as an interference molecule.

Some researches about multiple-immobilization of enzymes on the nanoparticles coated with PMBN have been reported.[38–41]

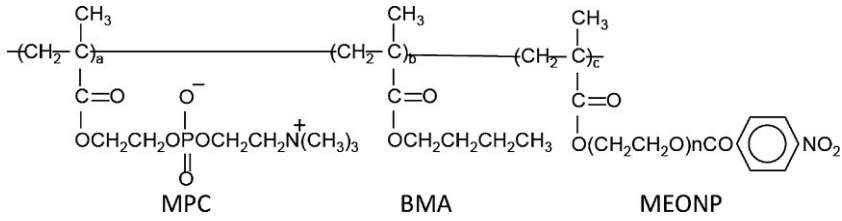

Figure 3.
Chemical structure of MPC polymer for immobilization of biomolecules (PMBN).

In these systems, continuous enzymatic reactions were observed. That is, a product of an enzymatic reaction became a substrate of the next enzymatic reaction. These reactions were quick compared with that in homogeneous solution containing enzymes due to shortened diffusion path between enzymes. When acetylcholine oxidese and peroxydase were immobilized on the nanoparticles, concentration of the acetylcholine was determined with the concentration of hydrogen peroxide generated by the enzymatic reactions. By combination with microdialysis sensing system with the nanoparticles, the concentration of the acetylcholine was determined easily in electrochemical sensor.[37] When enzyme luciferase was immobilized on the nanoparticles additionally, the hydrogen peroxide generated with the enzymatic reaction. It reacted with adenosine triphosphate, luciferin and oxygen and photoluminescence was observed and detected by photosensing system in microdialysis system.[38]

Quantum Dot Embedding Polymer Nanoparticles Covered with the MPC Polymer for Bioimaging

Functionalization inside of core part of polymer nanoparticles with semiconductor nanocrystal or magnet nanoparticles is promising for developing new tool for bioanalysis. To prepare stable and highly-sensitive bioimaging fluorescence probe, polymer nanoparticles embedding quantum dots (QDs) covered with PMBN were designed by making assemble of phosphorylcholine groups as platform and oligopeptide as bioaffinity moiety immobilized on the surface of nanoparticles.[43] They were prepared by a simple solvent evaporation technique using an amphiphilic water-soluble PMBN and PLA with QDs. The PLA nanoparticles embedding QDs with 20 nm in diameter were covered with PMBN and dispersed well in an aqueous medium. The polymer matrix did not affect the optical and fluorescence properties of QDs. The nanoparticles after immobilization of a simple amino acid, glycine, showed resistance to cellular uptake from HeLa cells owing to nature of the MPC polymers. When the arginine octapeptide (R8) was immobilized at the surface of nanoparticles, they could penetrate into cell membrane of HeLa cells. The R8 is well known as cell membrane penetrating peptide, so its specific functions are observed with the polymer nanoparticles. Cytotoxicty of the nanoparticles was not observed even after immobilization of oligopeptide. Thus, it could be obtained stable fluorescent polymer nanoparticles covered with MPC polymer as an excellent bioimaging probe and a novel evaluation tool of oligopeptide functions to the target cells.

Conclusions

The molecular integration on the polymer nanoparticles with phospholipid polymer platform was summarized. The nanoparticles modified with the MPC polymers to provide stability in an aqueous medium under biological condition. They showed excellent biofunctionality based on immobilization of biomolecules have strong advantages for applying in bioscience and bioengineering fields. Also, introduction of functional particles and nanocrystals could be embedding inside core of the nanoparticles. These nanoparticles will be applied in the field of cell/tissue engineering and biomedical engineering as an excellent bioimaging tool.

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