Bioinspired phospholipid polymer biomaterials for making high performance artificial organs

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Abstract

Novel polymer biomaterials, which can be used in contact with blood, are prepared with strong inspiration from the surface structure of biomembrane. That is, the polymers with a phospholipid polar group in the side chain, 2-methacryloyloxyethyl phosphorylcholine (MPC) polymers were synthesized. The MPC polymers can inhibit surface-induced clot formation effectively, when they are in contact with blood even in the absence of an anticoagulant. This phenomenon was due to the reduction of plasma protein and suppression of denaturation of adsorbed proteins, that is the MPC polymers interact with blood components very mildly. As the molecular structure of the MPC polymer was easily designed by changing the monomer units and their composition, it could be applied to surface modification of artificial organs and biomedical devices for improving blood and tissue compatibility. Thus, the MPC polymers are useful polymer biomaterials for manufacturing high performance artificial organs and biomedical devices to provide safe medical treatments.

Keywords: Biomaterials; Phospholipid polymer; Artificial organs; Biocompatibility; Blood compatibility

1. Introduction

Various polymeric materials have been used as raw materials for manufacturing biomedical devices including artificial organs which could be used in contact with blood [1]. However, the only polymers presently used are conventional materials, such as poly(vinyl chloride), polyethylene, poly[methyl methacrylate (MMA)], segmented polyetherurethane (SPU), poly(dimethylsiloxane), poly(tetrafluoroethylene) (PTFE), cellulose, and polysulfone (PSf). These materials do not have enough blood compatibility and biocompatibility; therefore, infusion of an anticoagulant is required during clinical treatments using these biomedical devices to avoid clot formation.

The development of new biomaterials was proposed based on the mimicking of a simple component present on the extracellular surfaces of the lipid bilayer that forms the matrix of the plasma membranes of cells, namely, the phosphorylcholine group of phosphatidylcholine and sphingomyelin. Phosphorylcholine, an electrically neutral zwitterionic head group, which represents the dominant property of the phospholipid head groups present on the external surface of cells, is inert in coagulation assays.

Some researchers confirmed the blood compatibility of a polymer surface coated with synthetic phospholipids [2,3]. They arranged the polymerizable phosphatidylcholines onto a support and polymerized them. The clotting time of blood was extended on the phospholipid-treated surface compared with that on the non-treated substrate. From the results of these studies, it can be said that these polymerizable phospholipid derivatives are useful for making polymer biomaterials.

2. Synthesis of 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer

A new concept was proposed for making blood-compatible polymer materials that have good stability, processability and applicability using a methacrylate monomer with a phosphorylcholine group, 2-methacryloyloxyethyl phosphorylcholine (MPC) [4]. The synthesis of the MPC was difficult; however, in 1987, the synthetic route and purification method of MPC was finally developed and a sufficient amount of MPC with excellent purity could be obtained [4]. Thus, it became possible to prepare the MPC polymer with various other alkyl methacrylates or styrene, and their blood compatibility was carefully evaluated [5]. In Fig. 1, the chemical structures of typical MPC polymers are shown.
The solubility of the MPC polymer strongly depends on its molecular structure, the composition of MPC and other monomer units, and its molecular weight [6]. The homopolymer of the MPC easily dissolves in water and alcohol, but is insoluble in acetone, acetonitrile, tetrahydrofuran and a specific composition of an aqueous solution of ethanol (60–92 vol% ethanol). The introduction of other monomer units could change the solubility. This means, it is very easy to design the MPC polymer structure to adapt to the substrate that is modified with the MPC polymer. The radical copolymerization of MPC (M1) and styrene (St) (M2) in ethanol at 50°C resulted in the following copolymerization parameters similar to those of the copolymerization of typical methacrylate, MMA and St, that is, \( r_1 = 0.39, r_2 = 0.46, Q_1 = 0.76 \), and \( e_1 = +0.51 \) [7]. These polymerization characteristics are very important for obtaining a suitable MPC polymer for use as a biomaterial. Controlling the monomer ratio determines the MPC composition in the polymer. The MPC polymer was also prepared with other polymer architectures such as block-type copolymers and graft-type copolymers by a conventional radical polymerization technique [8,9]. Telomerization also proceeded during polymerization of the MPC using chain transfer reagent such as mercapt compounds and a reactive functional group could be introduced at one terminal of the MPC polymer chain. The functional MPC polymer is useful for surface modification and can convert block-type and graft-type copolymer.

3. Bioresponse at the MPC polymer surface

Excellent antithrombogenic properties were observed when the MPC polymers came in contact with human whole blood even in the absence of an anticoagulant [10]. In Table 1, the total blood coagulation time of human whole blood on the polymer measured by the Lee–White method is summarized. The blood coagulated on glass within 8.4 ± 0.46 min with no significant difference observed in coagulation time when compared with that on poly(n-butyl methacrylate (BMA)). With a coating of poly(2-hydroxyethyl methacrylate (HEMA)) and MPC polymers (PMM and PMB, see Fig. 1), the coagulation time was significantly increased compared with that on glass and a poly(BMA) coating case \( P < 0.01 \). On the surface of PMB with 30 mol% of MPC unit, the maximum time for coagulation, 28 ± 2.6 min, was observed.

Fig. 2 shows the SEM pictures of the polymer surfaces after contact with human whole blood. On the surface of poly(BMA), a fibrin net completely covered the bead surfaces and many blood cells were adhered. On the other

![Fig. 1. Chemical structure of various MPC polymers.](image1)

![Fig. 2. Scanning electron microscopic picture of polymer membrane after contact with human whole blood in the absence of an anticoagulant for 15 min. (A) poly(BMA); (B) PMB (30 mol% of MPC unit).](image2)
hand, no fibrin deposition and cell adhesion could be found on PMB. These results clearly show that MPC polymers have excellent antithrombogenic properties and MPC moieties in the polymer are an important element in the antithrombogenicity of the polymers.

Protein adsorption is one of the most important phenomena in determining the biocompatibility of materials [11]. In general, proteins adsorb on a surface within a few minutes after the material comes in contact with body fluids such as blood, plasma or tears. Protein adsorption on the MPC polymers from human plasma determined by radioimmunoassay and an immunogold-colloid labeling technique showed that the amount of adsorbed protein was quite small and decreased with an increase in the MPC moiety [12]. In Fig. 3, proteins existed at plasma-contacting surface after a 60 min contact with poly(BMA) and PMB were determined by radioimmunoassay. Not only major components of plasma proteins such as albumin (Alb), fibrinogen (Fib), and γ-globulin (IgG) but also minor components were observed on the surface of every material. Protein adsorption was reduced with an increase in MPC unit composition. In the case of the PMB with 30 mol% of MPC unit, every adsorbed protein was reduced drastically compared with that on poly(BMA). During the last six years, many research groups in the world reconfirmed the antithrombogenicity and protein adsorption resistance observed at the MPC polymer surfaces [13–17].

4. Mechanism of protein adsorption resistance on the MPC polymers

The characteristics of water in the material or on the surface of the material are important to recognize the interactions between proteins and polymeric materials. Particularly, the structure of water surrounding the proteins and the polymer surfaces is considered to influence the protein adsorption.

The equilibrium amount of proteins, bovine serum albumin (BSA) and bovine plasma fibrinogen (BPF), adsorbed on the polymer surface is measured and represented with free water fraction in the hydrated polymers as shown in Fig. 4 [18,19]. The amounts of both proteins adsorbed on poly(HEMA), poly(acryl amide(AAm)-co-BMA) and poly(N-vinylpirrolidone(VPy)-co-BMA) were larger than those on the MPC polymers. The increase in the MPC mole fraction was effective in reducing the amount of protein adsorption. It was reported that the theoretical amounts of BSA and BPF adsorbed on the surface in a monolayer state are 0.9 and 1.7 μg/cm², respectively. On the surface of the MPC polymers (PMB and PMD), the amount of adsorbed proteins was less than these theoretical values. This means that the proteins attached to the surface could be very easily detached by rinsing. Thus, it
is considered that the phosphorylcholine group can reduce protein adsorption effectively.

Park et al. reported one possible mechanism for understanding protein adsorption on the polymer surface [20]. When a protein adsorbs on a polymer surface, water molecules between the protein and the polymer need to be displaced. That is, removal of water molecules induces direct contact between the amino acid residues and the polymer surface (Fig. 5). A repulsive solvation interaction — a hydration interaction — arises whenever water molecules are associated with surfaces containing hydrophilic groups, and its strength depends on the energy necessary to disrupt the ordered water structure and to ultimately dehydrate the surface. When water molecules are associated with hydrophobic surfaces, an attractive solvation interaction (hydrophobic interaction) occurs and its strength depends on the hydrophobicity of the surface or surface groups. The protein adsorbed on the surface loses bound water at the surface-contacting portion. This phenomenon induces the conformational change in the proteins; that is the hydrophobic part of the protein is exposed and comes in direct contact with the polymer surface. If the water state at the surface is similar to that in the aqueous solution, the protein need not release the bound water molecules even when the protein molecules contact the surface. This means that the hydrophobic interaction does not occur between the proteins and the polymer surface. Moreover, the conformational change during the protein adsorption on contact with the surface was also suppressed.

Tsuruta also considered the importance of water structure on biomedical polymers [21]. He has described in his review article that the polymers having a hydroxyl group such as poly(HEMA) could incorporate water molecules at the surface and form a network structure of water molecules. The protein adsorption starts with protein-trapping by the network structure of water molecules on the surface. The longer the contact of a protein on the surface, the greater is the chance of the protein interacting with the surface, undergoing a conformational change, and inducing irreversible adsorption. This is a very acceptable consideration to explain the difference in protein adsorption behavior between the MPC polymers and other amphiphilic polymers including poly(HEMA). Most of the polymeric materials have a negative surface charge even if they do not have any negatively charged groups. In fact, surface ζ-potentials of poly(ethylene terephthalate) (PET) and poly(BMA) are $-41$ and $-36$ mV, respectively. The hydrated polymer, poly(HEMA) also has a negative ζ-potential, of $-16$ mV. On the other hand, when the PET surface was covered with the PMB, the ζ-potential became zero, $-0.4$ mV. This characteristic is important for understanding the surface water structure of these polymers. On the PET, poly(BMA) and poly(HEMA), water molecules bind to the surface through electrostatic interactions (dipole–dipole interactions). The PMB effectively prevents the interaction between the surface and water molecules. This also reduces the binding of water at the surface of MPC polymers.

The circular dichroism (CD) spectrum of a protein adsorbed on a polymer surface gives us useful information about the conformational change during adsorption. From the CD spectra of proteins adsorbed on the polymer surfaces, the secondary structure of the adsorbed proteins was determined to calculate the α-helix content. Fig. 6 indicates the α-helix content of a protein adsorbed on the polymer surface [18]. The α-helix contents of BSA and BPF in PBS, which are assumed to be the ‘Native’ secondary structure, were 54 and 19%, respectively. When these proteins adsorbed on the polymer surface, changes in the α-helix content were observed. On the surface of the MPC polymers, the α-helix content of both adsorbed BSA and BPF was almost at the same level as that of native proteins. The proteins adsorbed on the MPC polymers could maintain their original higher α-helix level compared with that on poly(HEMA).

It is concluded that when the free water fraction on the polymer surface is kept at a higher level, the proteins can contact the surface reversibly without significant conformational change. The free water fraction must be one of the important factors to be considered for the blood compatibility of polymeric materials. Thus, the phospholipid polymer having a phosphorylcholine group, such as the MPC polymers, is one of the effective biomedical materials, and it could provide materials to develop new blood-contacting artificial organs.
5. Small diameter vascular prosthesis covered with the MPC polymer

The surface modification of SPU to improve the blood compatibility using the MPC polymer was performed. For in vivo evaluation, the artificial heart made by SPU which was coated with the MPC polymer was implanted in sheep. There was no clot formation and calcification on the surface even after one month of the implantation.

To obtain a more reliable modification of SPU with the MPC polymer, the blending of the MPC polymer as a polymeric additive was considered [22]. Polyester and PTFE are representative materials for artificial vessels. To use these materials for artificial vessels in the body, a living surface has to be formed on their surfaces. These vessels can be used only for arteries with an internal diameter greater than 6 mm. However, the small vessels with an internal diameter less than 4 mm are needed for the repair of coronary artery, etc. As shown in Fig. 7, the SPU (Tecoflex®) or SPU/PMEH (See Fig. 1) blend was coated on a polyester prosthesis with a 2 mm internal diameter and grafted to a rabbit carotid artery [23]. The coating process was simple using solvent evaporation method. Even the composition of the PMEH was 10 wt% against that of SPU, the MPC units were located near the surface, which was revealed by an X-ray photoelectron spectroscopy (XPS). The mechanical properties of the SPU did not change after blending with the PMEH. The prosthesis coated with SPU occurred due to the adherent and activated blood cells within 90 min after implantation. On the other hand, the prosthesis coated with SPU/PMEH functioned for more than eight months. The surface of the prosthesis covered with the SPU/MPC polymer blend membrane was clean and no protein deposition and clot formation was observed. These results suggested that the stability of the MPC polymer was sufficient even when the MPC polymer was in contact with blood continuously for eight months.

6. The MPC polymer membrane for artificial kidney

Hemodialysis using a cellulose membrane is one of the most useful methods to purify blood. Although the cellulose membrane has both good permeability and mechanical strength, its compatibility with blood must be improved. Therefore, if the MPC polymer chains are immobilized on the surface of the cellulose membrane, the blood compatibility can be improved [24]. For achievement of efficient modification of the cellulose membrane, a water-soluble cellulose grafted with poly(MPC) (MGC) was synthesized [25]. Coating the cellulose membrane with the MGC made it blood-compatible, such as preventing both protein
adsorption and clot formation even when the membrane was in contact with blood without an anticoagulant.

Recently, the MPC polymer (PMBU, See Fig. 1) was blended with PSf for making asymmetric porous membrane and hollow fiber membrane [26–28]. As shown in Fig. 8, these membranes could be processed by the wet-membrane processing method. The permeability of these porous membranes was of the same level as that of PSf membrane, which is commercially available. Protein adsorption and platelet adhesion was suppressed by blending with the PMBU. Moreover, permeation of solute after contact with protein solution did not change significantly in the case of PSf/PMBU membrane but it decreased dramatically in the case of PSf membrane. That is, the combination of PSf with the MPC polymer could provide high performance hemodialysis membranes.

7. Glucose sensor of artificial pancreas covered with the MPC polymer membrane

The wearable artificial endocrine pancreas was then applied to ambulatory diabetic patients and they first demonstrated that perfect glycemic control for a long period could be obtained with the system. However, for the long-term clinical use of this wearable artificial endocrine pancreas, there are several problems to be solved, especially regarding the glucose sensor. It was considered that the major reason for sensor stability when in vivo continuous monitoring is undertaken might be due to protein adsorption on the sensor surface. To prepare a stable, long-life glucose sensor, the sensor surface is covered with a PMB membrane [29]. As shown in Fig. 9, the glucose sensor showed excellent stability compared with the polystyrene (PVA) membrane case, that is, the output current after seven days was 94% of the initial value and 74% even after a fourteen day continuous insertion subcutaneously. The sensor was applied to human volunteers and it exhibited a satisfactory performance during continuous monitoring over fourteen days.

When the glucose sensor covered with the PMB membrane was combined with the insulin infusion pump system, control of the glucose level using the system was achieved for more than eight days continuously. Thus, the MPC polymer is useful for prolonging the life of the biosensors, particularly, implantable sensors.

8. The MPC polymer nanoparticles for drug delivery systems

The biodegradable poly(l-lactic acid) (PLA) nanoparticles covered with the MPC polymer were prepared by the solvent evaporation technique with the amphiphilic water-soluble PMB as the emulsifier [6,30]. The diameter of the nanoparticles was approximately 200 nm that was determined by atomic force microscopy and dynamic light scattering. The XPS indicated that the surface of the nanoparticles was fully covered with the PMB (see Fig. 10). The amount of plasma protein, BSA adsorbed on the nanoparticles coated with the MPC polymer was significantly smaller compared with that on the polystyrene
nanoparticles. The PLA nanoparticles coated with the PMB are suggested to be a safer drug carrier in the bloodstream. One of the anticancer drugs, adriamycin (ADR), could be adsorbed on the nanoparticles through a hydrophobic interaction. Even when the nanoparticles were stored in PBS for five days, 40% of the ADR still remained on the surface. It was concluded that the nanoparticles coated with the MPC polymer are useful as a novel adsorption-type drug carrier, which could be applied through the bloodstream.

9. Conclusions

The MPC polymers are useful not only for artificial organs but also medical devices due to their excellent properties, that is the resistance to protein adsorption and cell adhesion. The MPC polymer chemistry is one of the triggers for opening surface modifications of the substrate with phospholipid derivatives and these surfaces also demonstrated the usefulness of the improvement of protein adsorption and cell adhesion resistance. Therefore, the construction of the biomembrane-like surface has become a general concept to obtain not only blood-contacting medical devices which can be safely and clinically applied for longer periods, but also devices or equipment for bioengineering and tissue engineering. It is evident that the MPC polymers will become an important material not only in biomedical and pharmaceutical fields, but also every field of biotechnology.

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