# Preparation of Biocompatible Materials and Their Evaluation

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**Summary:** Biomedical application of polymers is an important topicin polymer society. Firstly, we have applied polymers developed for general usage. There has also been a need to prepare speciallydesigned polymers to substitute or assist lost or diseased function of tissues and organs to improve quality of patients' lives. There was a big demand for non-thrombogenic materials to develop artificialheart. 2-Methacryloyloxyethyl phosphorylcholine (MPC) was prepared and functions of MPC copolymers were evaluated. MPC is a promising methacrylate to develop several advanced artificialorgans and therapeutic instruments requiring fouling-free, blood-compatibility, permeability, biocompatibility, etc.

**Keywords:** biocompatible; blood compatible; fouling free; permeablemembrane; phospholipid polymer

## Introduction

There are many disposable devices, such as plasticized polyvinyl chloride tubing, polyolefin syringes and several plastic bags etc. to make medical treatments easier and safer. Inter-ocular and contact lenses, dentures, filling materials for tooth cavities, artificial kidney, heart and lungs, hip and knee joints are important, for instance.

The purpose of this presentation is to show new types of biomaterials having several functions applicable to various fields in medical and biological science and technology. 2-Methacryloyloxyethyl phosphorylcholine (MPC) was designed first to prepare reliable non-thrombogenic surface suitable for blood contacting artificial organs.[1] Many researchers attempted to develop non-thrombogenic materials for long time but we have not had reproducible methods. For instance, it was believed that non-thrombogenic elastomers were essential to fabricate reliable artificial hearts but they were introduced in clinics without them. They are still desired. Nakabayashi interested in the reason simply why blood vessel surface does not induce blood coagulation. He hypothesized when he could introduce such surface by mimicking the vessel surface, he could prepare non-thrombogenic surface. His first aim was to prepare such polymers having affinity with phospholipids, which could accumulate them from blood stream. Formation of a biomimetic membrane on MPC copolymer surfaces was beyond his expectation but one could think about characteristics of phospholipid molecules it is reasonable they could rearrange to form a biomimetic membrane by themselves once accumulated on the surface. And it might be beneficial to discuss relation between chemical structures and nonthrombogenicity to develop non-thrombogenic materials. This is relevant to long-term implantable cardiovascular devices, external circulation, intervenous catheters and sensors, etc. He did not try to introduce phosphorylcholine groups on biomaterials surface by a polymer reaction[2] as he intended to collect reliable informative results, as blood is very sensitive. Our analytical technology must be not so sharp as biological sensing systems. We have to obey nature always and not to

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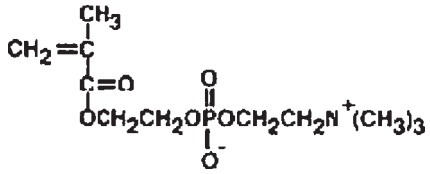


*Figure 1.*
Chemical Structure of MPC.

challenge aggressively the nature with biomaterials. Their biocompatibility is evaluated by natural biological systems and they inform their conclusion to us later. The best biocompatible biomaterials would not give stimuli to the biological systems such as not denature peptides, prevent accumulation of peptides, not give adverse effect on cells, not induce blood coagulation, etc.[2]

Phosphatidylecholine is a major component of cell membranes. He thought polymerizable phosphorylcholine was a candidate and MPC was prepared first, as it was not so complicated. Copolymers of MPC with methyl methacrylate showed good blood compatibility.[1] Ishihara et al. modified the synthetic route to increase yield and characterization of MPC copolymers (phospholipid polymers) were widely carried out.[3]

### Adsorption of Phospholipids and Plasma Proteins on MPC Copolymers

Adsorbed amount of phospholipids was increased with increasing MPC units in poly(butyl methacrylate-co-MPC) upto the 30% MPC copolymer. On the other hand, those of plasma proteins decreased with increasing the MPC units as shown in Fig. 2. These results suggested that MPC copolymer surfaces have good affinity with phospholipids as hypothesized and they could suppress protein adsorption when they contact with blood.[4]

DPPC liposome structure was distracted when the DPPC solution was mixed with a polystyrene emulsion. On the other hand, the structure was maintained when it was mixed with emulsion of poly (styrene-block-MPC). Transition temperature of DPPC liposome at 42 °C disappeared in the former but it was maintained in the latter.[6] This means that MPC copolymers could stabilize the liposome structure.[4]

Speculative mechanism of blood compatibility of MPC copolymers could be explained as illustrated in Fig. 3. When blood contacts with a MPC surface, phospholipids in blood are adsorbed and accumulated first on the surface and then they rearrange by themselves forming a biomi-

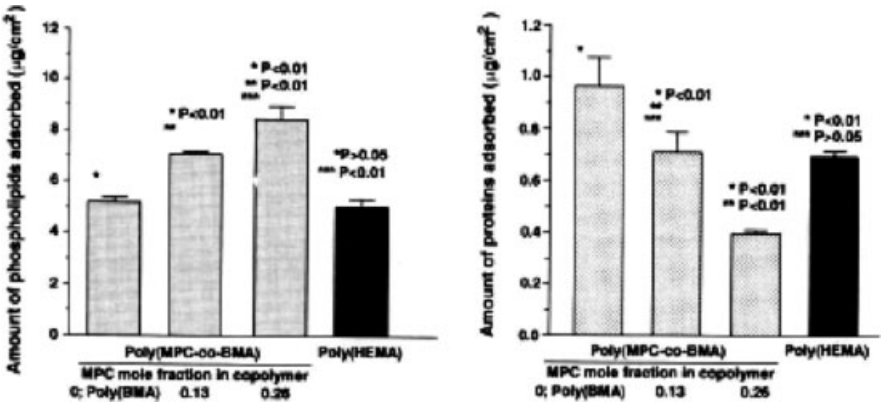
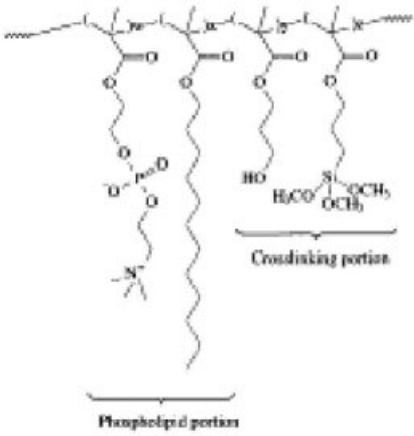



*Figure 2.*
Adsorption of phospholipid and protein from human plasma.[5]




Chemical Structure of cross-linkable MPC copolymer[7].

several limitations to make such measurements. It has been very popular to examine deformation of platelets after they were contacted with the substrates. These results are not so informative to predict their performance in clinical usage. Also we have to take in mind blood is changing dynamically when it is exposed outside. Nakabayashi et al. tried to make serial blood compatibility tests, *in vitro*, *ex vivo* and *in vivo* (studied with rabbits) without any systemic anticoagulation. And successful results were obtained by the *in vivo* long-term (four weeks) non-thrombogenicity test. It was concluded that the phospholipid polymer surfaces are excellent in the blood compatibility. These data could support good results in the stent application of a MPC copolymer in clinics.[7]

metic membrane there. Then plasma proteins come contact with the membrane but it could inhibit further their adsorption, which might induce blood coagulation. Then blood cells recognize that the biomimetic membrane rearranged might be epithelium of natural vessel and blood cells can flow fluently as in natural blood vessels.[4]

**Evaluation of Non-Thrombogenicity**

As blood is very sensitive and unstable, it is too hard to make absolute characterization in the absence of anticoagulants. Almost of all blood compatibility studies were carried out *in vitro*. The long-term blood compatibility data are required but there are

*In vitro Blood Compatibility*

Non-thrombogenicity of MPC-butyl methacrylate copolymers (BMP) was evaluated by microcolumns packed with PMMA beads coated with poly(BMP)s. Whole blood was passed through the column in the absence of anti-coagulant for 30 min. There was not identified deposition of blood elements on the BMP-coated surfaces examined by SEM. Platelets counts did not decreased during the chromatography (Fig. 4). This experiments showed that column chromatography of whole blood is possible not giving adverse effect on blood and we could conclude that 10 and 30 % MPC copolymer surfaces have excellent blood compatibility.[3] Platelet adsorption and their activation were comple-

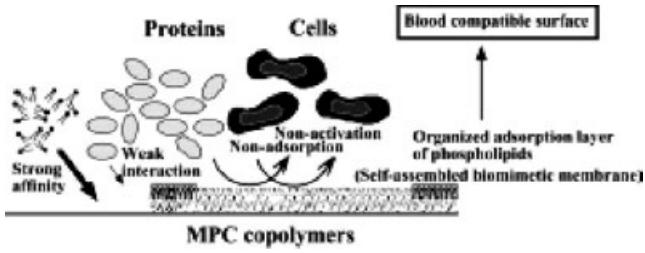



***Figure 3.***
Schematic illustration of blood compatibilityof MPC copolymer surface.[4]

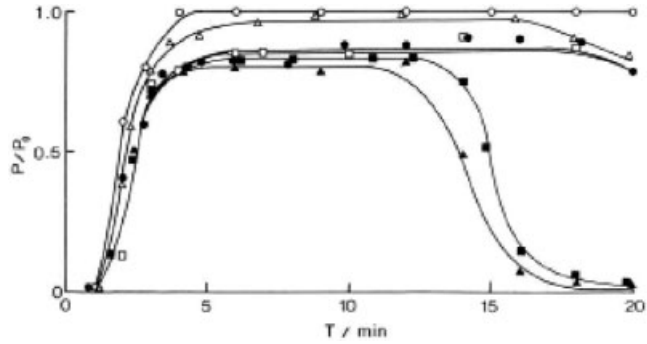



***Figure 4.***
[3] The representative elution profile of platelets from the column packed with poly(MP-co-BMA) (MB) coated beads and poly(HEMA)-coated beads when $Ca^{2+}$-re-added PRP was injected into the column. (▲) poly(BMA), (●)MB5, (□)MB10, (○)MB30, (■)poly(HEMA).

tely inhibited on MPC copolymers when MPC units were higher than 10% (Fig. 5).[4]

*Ex vivo Blood Compatibility Studied by MPC Modified Hollow Fiber*

Hemodialysis is important to keep lives of chronic kidney patients and it is operated in the presence of heparin to inhibit blood coagulation. Non-thrombogenic dialyzers, which do not require systemic heparinization, are desired but combination of both permeability and nonthrombogenicity have been difficult. But there are such patients who cannot accept anticoagulant therapy and non-thrombogenic dialyzers are needed to treat such patients. Poly-MPC is soluble in water and good candidate to develop non-thrombogenic dialyzers. Cellulose[8–10] and polysulfone[11,12] membranes were modified by MPC copolymers to improve their blood compatibility not giving adverse effect on the permeability and mechanical properties. Graft polymerization of MPC onto cellulose membranes and hollow fibers,[8] coating with water soluble MPC grafted methyl cellulose on cellulose hollow fibers,[9] chemical reaction of reactive phospholipid polymers to cellulose,[10] and coating of MPC copolymers onto polysulfone hollow fibers[11] were

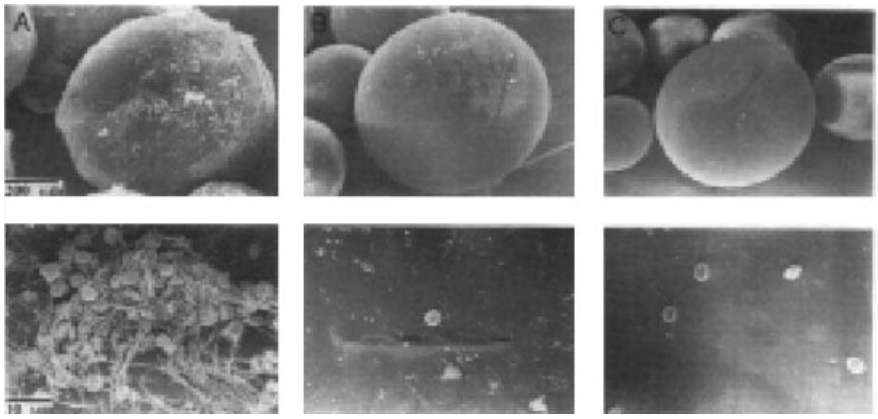



***Figure 5.***
[4] SEM pictures of poly(MPC-co-BMA)-coated beads after contact with human hole blood without an anticoagulant, MPC mole fraction: (A) 0(poly(BMA)), (B) 0.13, (C) 0.32.

studied. Mini-module hemodialysers were fabricated and *ex vivo* blood compatibility tests were carried out in the absence heparin on rabbits for 2 hours. Hollow fiber surfaces were characterized and concluded that blood compatibility were improved but properties of membranes were not decreased by the modification. Thinking about the small size of hollow fibers, 20 mμ in the diameter, blood compatibility of MPC modified surfaces is excellent. They suppressed plasma proteins adsorption on the surface and platelets adsorption and activation. These results suggested that protein fouling free process could be possible by application of phospholipid polymers (Fig. 6).[13]

But it is very difficult to introduce these modified dialyzers into clinics as present dialyzers are well accepted with systemic anticoagulation to avoid risk and there is not so strong demand to put them on the market at this moment. But it is true that there is a demand for non-thrombogenic dialyzers to treat such patients who are risky to take a hemodialysis with systemic anticoagulation. And we do not know the adverse effect of repeated hepalinization to avoid blood coagulation for long time.

This technology was applicable to treat **diabetic patients** easily to keep their glucose concentration percutaneously monitered ferrocene-mediated needle-type[14,15] and microdialysis probe[14] glucose sensors for two weeks. It was suggested that two weeks durability is good enough to measure glucose concentration reliably with a sensors coated with fouling free MPC copolymers, as accumulation of proteins on the sensor surface decreases the permeability of glucose severely. Replacement of the sensors every two weeks is beneficial to avoid infection at the interface.[13,14]

### Long Term *in vivo* Blood Compatibility Studied by Small Diameter Artificial Vessels

Two hours blood compatibility test was much informative for clinicians but patients cannot satisfy to be treated by such modified devices and artificial organs. So long-term blood compatibility test was strongly required and designed by fabricating small diameter vascular graft. This research could open new field in small diameter vascular prostheses, which have been rather hard, as we did not have excellent blood compatible materials like MPC copolymers. Many researches have tried to introduce tissue-engineering technology to mimic epithelium of natural blood vessel to prevent initial blood coagulation to keep them open. But we have to keep in mind pannus formation at the tissue side interface in the

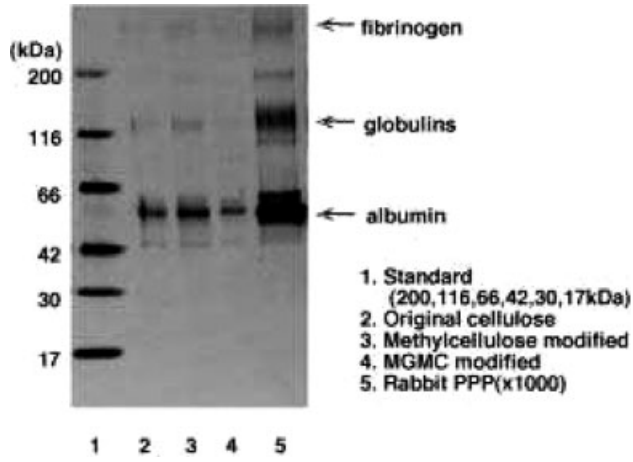



***Figure 6.***
SDS-PAGE of proteins adsorbed on the membrane.

extended usage, which is very severe problem even today in vascular prostheses.

Porous polyester woven fabrics were dip-coated with a blend polymer solution of segmented polyurethane and poly(MPC-co-2-ethylhexyl methacrylate) to fabricate fiber-reinforced tubings, non-thrombogenic small diameter vascular grafts. They were implanted to rabbit artery by conventional way and checked their patency.[16] The control specimens fabricated by the polyurethane only instead of the blend occluded within 90 min but the blend group was excellent to keep them open for four weeks. The surfaces were clear and pseudointima was not identified on the surface[17] due to fouling free characteristics.[4,13]

We could conclude through these serial blood compatibility tests that blood biomaterial interaction of phospholipid polymer surfaces is the most biocompatible among existing non-thrombogenic materials.

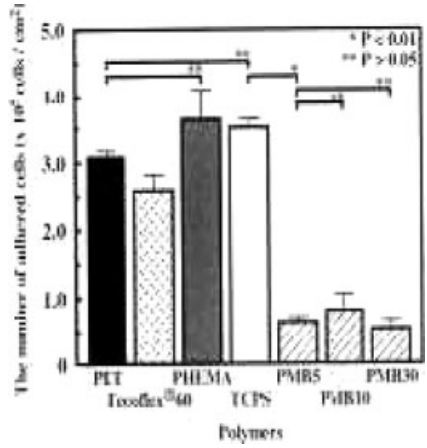



*Figure 7.*
Number of HL-60 cells adhering to the polymer surfaces after a 24-h incubation. The mean values of three measurements and standard deviations are indicated.18).

### Milder Interaction of MPC Surfaces with Biological Systems

Biocompatible materials must not give stress to cells when they contact with such surface as described previously. Proliferation of cells on several conventional biomaterials, PET (polyester), Tecoflex (polyurethane), poly(HEMA), TCPS (tissue culture polystyrene) and MPC- butyl methacrylate copolymers (BMP) of 5, 10 and 30% was evaluated (Fig. 7). They stuck many cells on their surfaces but PMB copolymers suppressed the phenomena. We tried to evaluate given degree of stress by measuring IL-1β m-RNA accumulated in the cells. The data shown in Fig. 8 suggests that MPC copolymer, PMB-10 and -30 give much less stimuli than conventional biomaterials. TCPS is widely used to evaluate biocompatibility of materials. We could say that the cell culture test to access safety of materials might be not enough as it gives stimuli to cells in some extent. We could conclude that MPC copolymers could be very mild biomaterials to living cells. This is also true that blood-MPC copolymers interaction must be less and they show better blood compatibility. Further, we could say MPC copolymers coated surfaces are low cell binding surface including platelet (Fig. 4).

PMB higher than 30% MPC copolymers are soluble in water. They do not give adverse effect on cells suspended in the solution and the retrieved cells showed

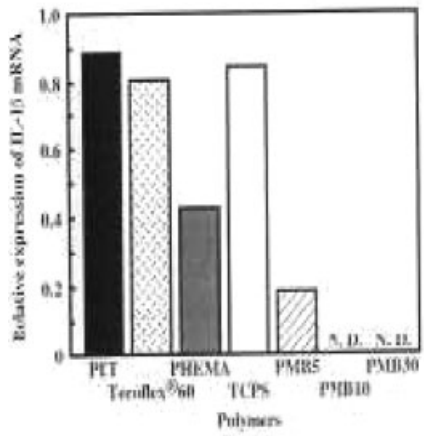



*Figure 8.*
Expression of IL-1_ mRNA transcripts in HL-60 cells on the polymer surfaces as a standard of β-actin by RT-PCR after a 24-h incubation. N.D. indicates that the expression was not detected.18).

same proliferation as the original ones. They do not induce denaturation of enzymes, as they had higher concentration of free water than conventional so-called hydrogels.[19] Furthermore water soluble PMB-30 is an excellent synthetic blocking agent to increase stability of antibody conjugats.[20]

### Several Applications of Biocompatible MPC Polymers

MPC copolymers have good affinity with skins and the skin-covered membrane could protect the tissue by preventing dehydration and can keep them moist as skin-care agents.[21] Stainless transparent hydrogels of MPC copolymers are good for contact lenses and MPC copolymer solutions can prevent staining by minimizing accumulation of proteins in the tear on contact lenses. Non-thrombogenic permeable membranes of MPC copolymers are useful for dialyzers[8–13], oxygenators[22] and sensors[14,15], small diameter vascular prostheses[16,17] and stents[7] are good place for MPC applications. Lubrication of joints must be interesting to support joint fluids.[23] Milder abrasion at tissue-catheter interface[24] is very beneficial to minimize tissue damage by the lubrication with MPC copolymers. Several diagnostic applications using biological elements are promising and good place for their application.[20,25]

Fouling free protein processing is interesting and promising in industries, which could be suggested by the data studied hollow fiber membranes.[9,12,26] During cultivation of cells and tissues, it is very important not to induce denaturation of proteins. When it is taken place, implanted host cells and tissues must remove or activate them.[27] How to minimize these adverse effects must be our research targets. We do not know these problems well. But we have not had such smart materials as MPC copolymers to suppress denaturation of peptides.

How to make intimate attachment between biomaterials and natural tissues without inducing infection at the tissues side interface is very severe target for biomaterials researchers. Tissues have wound healing process and can heal by themselves generally. But we have many experiences that medical devices induce infection in the subjacent tissues. Blood access for chronic kidney patients is very severe problems but we do not have reliable science to develop good blood access for them. Blood vessel and vascular graft interface has same problems as the blood access.

## Conclusion

Through these studies we could conclude that MPC is good methacrylate to produce many varieties of medical devices, which contact with blood in the absence of anticoagulants. They can suppress adsorption of proteins and prevent their conformational change both on the surface and in the solution. We would like to encourage researchers to improve biocompatibility and blood compatibility of biomaterials, to minimize random adsorption of proteins, and to increase stability of unstable enzymes. New membranes having good permeability, non-thrombogenicity and fouling-free could be prepared. They could open new various fields in biological science and technology.

NOF Corporation (Shibuya, Tokyo 150-6019, Japan) is a manufacturer of MPC and the copolymers.

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