Journal of the Korea Society of Dyers and Finishers
Vol. 7, No. 3, pp. 38~43(1995. 9)

〈研究論文(學術)〉

생체적합성 고분자의 개발과 응용(III)

- Phosphoryl choline기를 가진 견피브로인막의 생체적합성 -

이미경 · 이영희 · 최해욱* · 박수민

부산대학교 섬유공학과
*밀양산업대학교 견섬유학과
(1995년 7월 1일 접수)

Development and Application of Biocompatible Polymers(III)

- Biocompatibility of Silk Fibroin Membranes with Phosphoryl Choline Groups -

Mi Kyung Lee, Young Hee Lee, Hae Wook Choi*, and Soo Min Park

Dept. of Textile Eng., Pusan National Univ., Pusan, Korea
**Dept. of Silk Textile Miryang National Univ., Miryang, Korea*
(Received July 1, 1995)

Abstract—To improve the biofunctional properties of silk fibroin membranes, 2-(methacryloyloxy)ethyl-2-(trimethylammonium)ethyl phosphate(MTP), which is a methacrylate with phospholipid polar groups grafted and poly(MTP-co-BMA) was coated on the surface of silk fibroin membranes. The permeability and biocompatibility of silk fibroin membranes with phosphoryl choline group were investigated. The permeability of a salt(NaCl) was increased with grafting by MTP. Futhermore, the poly(MTP-co-BMA)-coated silk fibroin membranes displayed less blood cell adhesion than the silk fibroin membranes.

Introduction

The design of biomedical materials must be based on normal contributions to the maintenance of a desirable interaction between the materials and living organisms, since the biomedical materials are used in contact with blood and tissues. The simplest and common feature of biomembranes which constitutes cell wells is the high content of electrically neutral phospholipids which contain the phosphoryl choline head group. Therefore, it could be an interesting approach to prepare biomedical materials having phosphoryl choline groups.

We previously found that the copolymers from a monomer having a phospholipid polar groups, the 2-(methacryloyloxy)ethyl-2-(trimethyl ammonium) ethyl phosphate could be prepared at high yields and that copolymers obtained exhibited excellent antithrombogenic properties[1-4].

Silk is a natural protein fiber widely used as biostable suture in surgery. A number of properties of silk have been improved by grafting or by simple coating with monomers[5,6]. Several workers have grafted hydrophilic monomers onto the surface since greater hyd-

rophilicity generally leads to better compatibility[7].

In this study, to improve the bio functional properties of silk fibroin membranes, 2-(methacryloyloxy)ethyl-2-(trimethylammonium) ethyl phosphate(MTP) with phospholipid polar groups was grafted, O.K poly(MTP-co-BMA) was coated on the silk fibroin membranes. and The permeability and antithrombogenicity of the membranes were investigated.

Experimental

Materials

Vinyl monomer containing phospholipid, MTP was synthesized according to the procedures previously reported[1]. Poly(MTP-co-BMA) (MTP 50%) was prepared conventionally using the radical polymerization method. The chemical formula of the poly(MTP-co-BMA) is shown in Figure 1.

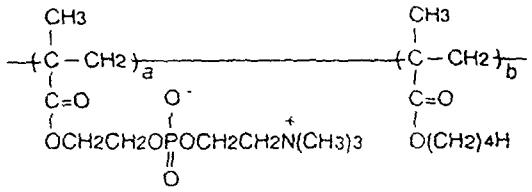

Fig. 1. The chemical formula of poly (MTP-co-BMA).

The degummed silk fibers were dissolved in the 9.3M LiBr aqueous solution at 40 ℃ for 1 hr. After removing a small amount of undissolved, the solution was dialyzed against water for 3 days at atmosphere temperature using cylinderical cellulose dialysis tube. The silk fibroin aqueous solution obtained was casted on the acrylic plate and dried at the atmosphere temperature for 3 days. By the treatments in saturated water vapor at 30℃, the insoluble membrane was obtained and the thickness was 25μm.

Coating and grafting on the silk fibroin membranes with phosphoryl choline groups

The coating of the poly(MTP-co-BMA) on the silk fibroin membranes was carried out using a 5 wt% ethanol solution of the poly (MTP-co-BMA). The silk fibroin membrane was immersed in the solution for 3 min and the membrane was removed and dried under atmospheric conditions for 3 days. The structure of the coated poly(MTP-co-BMA) on the silk fibroin membrane was confirmed using IR spectroscopy and SEM observation.

The desired amounts of MTP against silk fibroin membrane were placed into a polymerization tube and ammonium persulfate(APS) as initiator and water were added to the tube, respectively. After nitrogen gas was bubbled through the mixture for 10 min to eliminate any oxygen in the solution, the mixture was heated at 60℃ for 1hr under nitrogen atmosphere. The reaction mixture was then poured into distilled water and the resulting membrane was dried and identified by IR spectroscopy and the graft ratio.

Equilibrium sorption and permeation

Equilibrium sorptions were measured in glass stoppered pyrex tubes at 25℃. The temperature was kept constant in a thermostated bath. After equilibration at a certain concentration, the membrane was taken out of the solution and carefully blotted with a filter paper. The membrane was desorbed in distilled water. The concentration of the desorbing solution was determined conductometrically using conductometer.

The partition coefficient, K defined as the ratio of the salt concentration in the membrane, C_{in}(mol/l in membrane) to the outer solution, C_{ex}(mol/l) at equilibrium was determined using equation (1).

$$K = C_{in}/C_{ex} \quad (1)$$

The permeability was measured by a conventional cell consisting of two chambers separated by a membrane. The cell was placed in a thermostated water bath(25℃) throughout the experiments. The solutions of eachside were stirred powerfully during the measurements. The concentration change in the downstream cell was monitored by measuring the conductivity of the solution.

Evaluation of thrombogenicity of the polymer surface

Scanning electron microscopic(SEM) observation of the adhered blood cell and PRP was used for the evaluation of thrombogenicity of the coated polymer surface. After the membrane was treated in whole blood cell and PRP containing 3.8wt% phosphate buffer(pH 7.4) solution of sodium citrate, they were placed in a phosphate buffer solution containing 2.0wt% glutaraldehyde to fix the adhered cells.

The membrane was rinsed with phosphate buffer(pH 7.4), freezed and dried *in vacuo* for 5hr~10hr, and coated with gold. The morphology of cells adhered on the membrane surface was observed with SEM.

Results and Discussion

Characterization of silk fibroin membrane with phosphoryl choline groups.

The cross-section of poly(MTP-co-BMA)-coated silk fibroin membrane was observed by SEM pictures. The coated layer with the thickness of 5μm was observed onto the silk fibroin membrane which average diameter was 25μm.

The chemical structure of the silk fibroin membrane with phosphoryl choline groups was identi fied by IR spectra which are shown in Figure 2. When the IR spectrum of the membrane was compared with that of the original silk fibroin membrane, adsorption at 1720, 970, and 800 cm^{-1} appeared after coating. These IR adsorptions were assigned to C=O, $N^{-}(CH_3)_3$, and C-O-P in phosphoryl choline moiety, respectively.

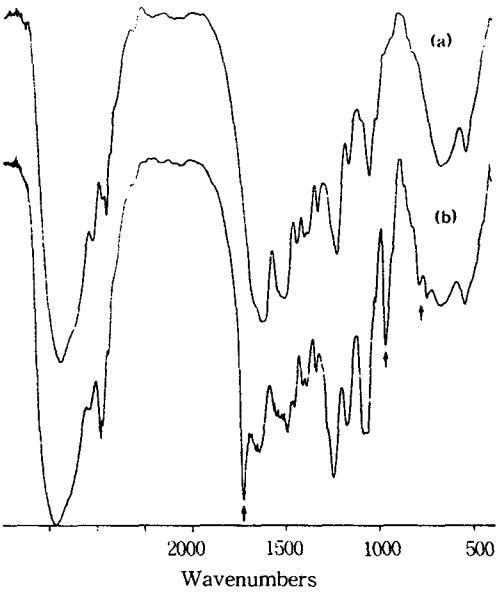

Fig. 2. IR spectra of silk fibroin membrane(a) and silk fibroin membrane with phosphoryl choline group(b).

Adsorption and permeability of the salt

The salt concentration on the membrane

C_{in}(mol/l in membrane) to the outer solution concentration C_{ex}(mol/l) at equilibrium was plotted in Figure 3. It is indicated that the grafted membrane absorbs much more salts than the silk fibroin membrane and the shapes of the plots were partition types. The partition coefficients of the silk fibroin membrane and MTP-g-silk fibroin membrane, which were calculated using eqn (1) from Figure 3, were 1.01 and 1.10, respectively.

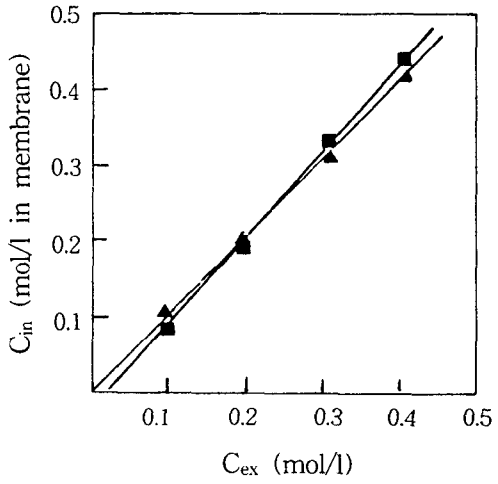

Fig. 3. Binding Isotherm of sodium chloride into fibrion and MTP-g-fibroin membrane at 25℃ and pH 7.0
▲ Fibroin membrane
■ MTP-g-fibroin membrane

We obtained the straight lines from the permeation experiments from the slopes against the concentrations of the salt solutions which were given in table 1 and Figure 4. They were shown that the permeability coefficients are small due to Donnan exclusion in the lower concentration and the permeability of the salt through MTP-g-silk fibroin membranes was higher than that of the silk fibroin membranes. This result suggests that the phosphoryl choline moieties on the silk fibroin surfaces play an important role in increasing the permeability of the salts.

Table 1. Permeation coefficient of sodium chlorides through fibroin and MTP-g-fibroin membrane at 25℃ and pH 7.0

	P(cm^2/sec)	
C(mol/l)	Fibroin membrane	MTP-g-Fibroin membrane
1.0×10^{-3}	1.87×10^{-6}	1.96×10^{-6}
4.0×10^{-3}	3.86×10^{-6}	4.12×10^{-6}
1.0×10^{-2}	4.93×10^{-6}	5.30×10^{-6}
2.0×10^{-2}	5.54×10^{-6}	5.80×10^{-6}
4.0×10^{-2}	5.59×10^{-6}	6.29×10^{-6}
8.0×10^{-2}	5.65×10^{-6}	6.40×10^{-6}

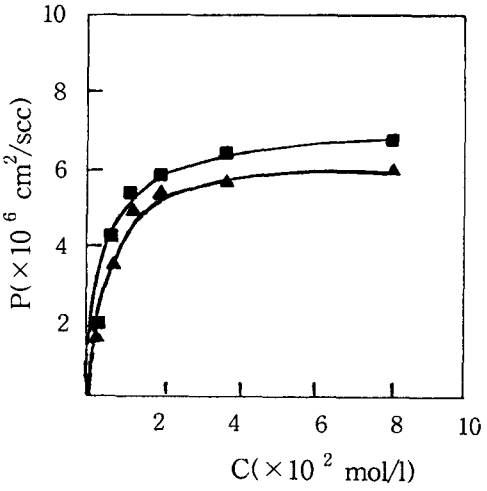

Fig. 4. Relation between concentration and permeation coefficient at 25℃ and pH 7.0
▲ Fibroin membrane
■ MTP-g-fibroin membrane

Nonthrombogenicity

SEM pictures of poly(MTP-co-BMA)-coated silk fibroin membranes and silk fibroin membrane after contacting with whole blood cell for 60 min and 120 min were shown in Figure

5. It was confirmed that the poly(MTP-co-BMA)-coated silk fibroin membrane displayed less blood cell adhesion than the silk fibroin membrane. Futhermore, Figure 6 shows SEM pictures of the poly(MTP-co-BMA)-coated silk fibroin membrane and the silk fibroin membrane after contacting with PRP for 60 and 120 min, respectively. When PRP came in contact with the original silk fibroin membrane for 60 min, numerous platelets were adherent and deformed on the surface. On the other hand, although some platelet adhesion was observed on the surface of the membrane, platelet adhesion was effectively reduced by coating with poly(MTP-co-BMA). This may be due to the formation of biomembrane-like surface by the adsorption and arrangement of phospholipid molecules from serum onto the poly(MTP-co-BMA)-coated silk fibroin membranes.

Conclusions

The biofunctional properties of the silk fib-

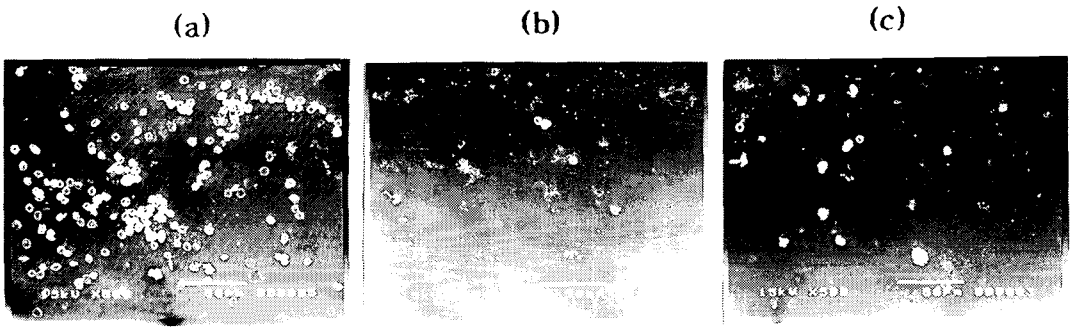

Fig. 5. SEM pictures of membranes after contacting citrated whole blood cell
(a) Silk fibroin membrane(60min)
(b) Poly(MTP-co-BMA)-coated silk fibroin membrane(60min)
(c) Poly(MTP-co-BMA)-coated silk fibroin membrane(120min)

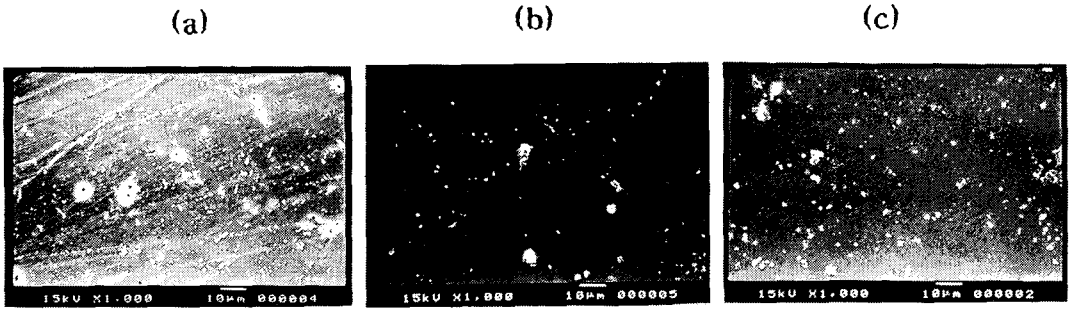

Fig. 6. SEM pictures of membranes after contacting citrated PRP
(a) Silk fibroin membrane(60min)
(b) Poly(MTP-co-BMA)-coated silk fibroin membrane(60min)
(c) Poly(MTP-co-BMA)-coated silk fibroin membrane(120min)

roin membranes with phosphoryl choline groups were studied. It was indicated that the phosphoryl choline moieties displayed an important role in increasing the permeability and adsorption of the salts and the poly(MTP-co-BMA)-coated silk fibroin membranes displayed high antithrombogenicity than silk fibroin membranes. Therefore, these results suggest that the MTP copolymer will be useful as a biomedical material for improving blood compatiblity of the surface of biomaterials.

Acknowledgment

This papes was supported by NON DIRECTED RESEARCH FUND., Korea Research Foundation, 1993.

References

1. S. M. Park, *J. Kor. Fiber Soc.*, **29**, 32(1992).
2. M. K. Lee, S. K. Jung, H. D. Kim, H. H. Cho, K. H. Kim, and S. M. Park, *J.Kor. Fiber Soc.*, **30**, 823(1993).
3. M. K. Lee, M. S. Kim, S. K. Jung, and S. M. Park, *J. of Kor. Soc. of Dyers and Finishers*, **6**, 324(1994).
4. M. K. Lee, H. S. Park, E. Y. Kim, and S. M. Park, *J. of Kor. Soc. of Dyers and Finishers*, **7**, 63(1995).
5. G. W. Hasting, *Polymer*, **26**, 1337(1985).
6. R. D. Ratner and A. S. Hoffmen, *J. Appl. Polym. Sci.*, **18**, 3183(1974).
7. M. Toller, M. Stol, and K. Kliment, *J. BioMed. Mater. Res.*, **3**, 305(1969).