NOTE

Chemical Modification of Silk Fibroin with 2-Methacryloyloxyethyl Phosphorylcholine I. Graft-Polymerization onto Fabric Using Ammonium Persulfate and Interaction Between Fabric and Platelets

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INTRODUCTION

Much work with vinyl monomers graft-polymerized onto silk fibers was done from the early 1960s to increase silk weight and improve silk textile performance, such as crease recovery, dimensional stability, rub resistance, photoyellowing resistance, oil, and water repellency, and colorfastness.[1] Biomedical applications such as surgical sutures are developed based on natural biopolymers with outstanding physicomechanical properties. If biocompatibility is given to silk by chemical modification, the range of medical fields could be broadened. 2-Methacryloyloxyethyl phosphorylcholine (MPC) effectively inhibits blood cell–platelet,[2–4] monocyte,[5] and macrophage[5] adhesion. Ishihara et al.[6] found that if the free water fraction on the MPC-related polymer surface is kept, proteins contact the surface reversibly without being significantly conformationally changed. MPC-related polymers containing a phosphorylcholine group thus effectively provide a biocompatible surface.

To improve the biocompatibility of silk fabric, MPC was grafted onto silk fabric by free radical initiation. Platelet adhesion was preliminarily tested measuring biocompatibility to confirm the effect of grafted MPC on silk fabric.

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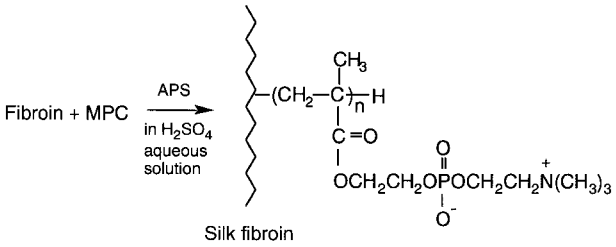
EXPERIMENTAL

Degummed habutae fabric made of silk from *Bombyx mori* was cleaned by Soxhlet extractor with *n*-hexane for 24 h, rinsed with distilled water, and dried for 24 h in a vacuum at ambient temperature. MPC monomer was donated by NOF Co. (Tokyo, Japan), and used without further purification. Ammonium persulfate (APS), an initiator, was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and used without further purification. All other solvents were used after distilling.

Infrared spectra using KBr pellets to analyze reactants were recorded by FT/IR-350 (Jasco Co., Tokyo, Japan). Released lactic dehydrogenase (LDH) from platelets adhering to samples was quantified by UV spectrophotometry (UV-1200, Shimadzu Co., Kyoto, Japan).

MPC was grafted onto silk fabric using APS as an initiator; 1.8 mmol of MPC monomer and APS—2.0 mol % per monomer—were dissolved in 5.0 ml of sulphuric acid aqueous solution (pH 3.0). Six dried pieces of original silk fabric 1.5 cm diameter and 9.7 mg/piece were immersed into solutions in 10 ml thick-walled polymerization tubes. Tubes were degassed by freezing, evacuated four times and sealed. Graft polymerization was conducted at 50°C for different periods. MPC-grafted fabrics were collected from the reaction system and washed with large amounts of water and freeze dried for 24 h. Weight gain (add-on) was calculated from the increase in weight of the dried original silk fabric after graft polymerization as follows:



Scheme 1 Graft polymerization of silk fibroin with MPC using APS as an initiator.

$$\text{add-on (wt \%)} = (W_2 - W_1)/W_1 \times 100$$

where W_1 indicates the weight of the dried original silk fabric and W_2 the weight of the dried MPC-grafted fabric.

A platelet adhesion experiment was conducted based on the method of Tamada et al.[7] The number of platelets adhering sample fabrics was calculated from the amount of LDH released from adhering platelets. Briefly, platelet rich plasma (PRP) was prepared from normal human blood using sodium citrate as an anticoagulant. Platelet suspensions (1.0 ml) with a concentration of 1.80×10^6 platelets/ml prepared by dilution of PRP with Ca^{2+}-free phosphate-buffered saline [PBS(−)] were placed on sample fabrics and a glass plate of 1.8 cm^2 and incubated at 37°C for 60 min. Fabrics were rinsed gently with PBS(−) and immersed in 1.0 ml of 0.5% Triton X-100 in PBS(−) to rupture the cell membrane. The amount of LDH released was measured using an LDH Monotest™ kit (Boehringer Mannheim Co., Mannheim, Germany) and was transformed to the number of adhered platelets onto sample fabrics.

RESULTS AND DISCUSSION

Graft polymerization with MPC onto silk fabric was conducted by free radical initiation (Scheme 1). The grafting mechanism is explained by free radical polymerization initiated at radicals formed on silk fibroin molecules by a peroxide initiator. The macroradical reacts with the monomer, propagating a graft polymer chain. The polymerization solution of acidic pH generally accelerates the decomposition of the initiator and the formation of active species, leading to a large number of initial free radicals.[1] APS as an initiator and the acidic solution were attempted in our grafting system. The weight gain of silk fabric by MPC grafting is plotted as a function of reaction time (Fig. 1). The weight gain of poly MPC increased with increasing reaction time, and reached plateau at about 10 wt %. FT-IR spectra of MPC-grafted silk prepared by using APS as an initiator (Fig. 2) showed absorptions at 970 cm^{-1} attributed to $-N^+(CH_3)_3$ and that at 1240 cm^{-1} attributed to —$POCH_2$ groups in poly MPC, increased with proceeding graft polymerization. Several monomers—methacrylamide (MAm),[8] methyl methacrylate,[9] and styrene[10]—were grafted onto silk fiber and fabric using peroxide as an initiator, and the weight gain with these graft polymers onto silk substrates was ≈ 120 wt %. The maximum weight gain, however, was approximately 10 wt % in our MPC graft polymerization. Freddi and Tsukada[1] reported that the drop in weight gain depended on suppression of monomer diffusion, oxidation product formation on the silk fibroin backbone, homopolymerization, and vinyl monomer reactivity to silk. In our graft polymerization, graft efficiency was theorized to be lower due to steric hindrance between the side chains of the MPC and silk substrate. We are now studying ways to increase graft efficiency with MPC onto silk fabric. Graft polymerization with MPC onto silk fabric can thus be well controlled.

To confirm preliminary biocompatibility of MPC-grafted silk, we conducted a platelet adhesion test (Fig. 3) using MPC-grafted silk fabrics after contact with human PRP (1.00×10^6 platelets/cm^2) for 60 min. Platelets were counted by LDH. The number of adhered platelets on original silk fabric was about half that on a glass plate ($2.05 \pm 0.70 \times 10^5$ platelets/cm^2) used as the positive control substrate in the platelet adhesion test. The number of platelets adhering to the fabric appeared to be higher because the surface area of the fabric was comparatively larger than that of the plate. The interaction between human platelets and the MPC-grafted silk fabrics having different grafting ratios—2.8, 5.2, and 10.2 wt %—was also studied. The number of platelets adhering to MPC-grafted silk dras-

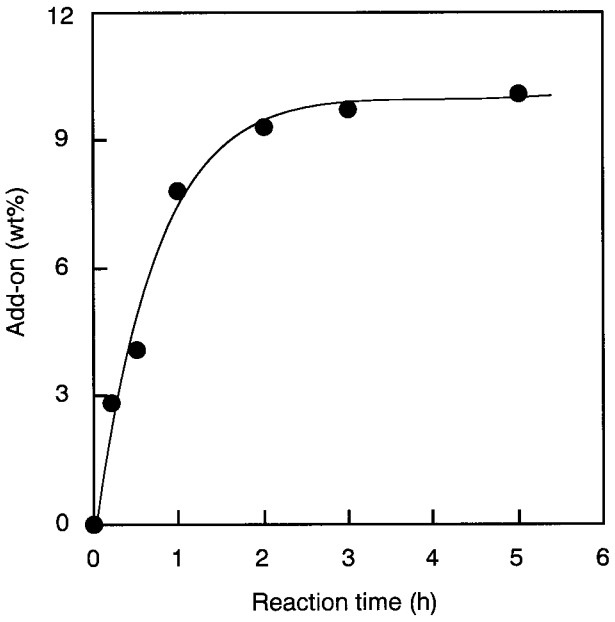


Figure 1 Weight gain of MPC on silk fabric as a function of reaction time.

tically decreased to about one tenth of that of the original silk. No significant difference was seen in the number of platelets adhering among the three types of MPC-grafted silk fabric. Even grafting a low amount of MPC onto fabric imparted sufficient biocompatibility. Relationships between blood cells and MPC-grafted materials were shown in studies on MPC-grafted cellulose membrane[11–14] and poly(*n*-butyl methacrylate) backbones.[3] The mechanism of blood compatibility observed in MPC-grafted materials suggests the construction of a self-assembled biomembrane by adsorbing phospholipid molecules from plasma. The weak interaction with platelets on MPC-grafted silk fabric may thus be due to this mechanism, i.e., grafted MPC branches on the silk fabric functioned well.

In conclusion, graft polymerization with MPC on silk fabric using ammonium persulfate in an acidic system is well controlled. The interaction between human platelets and grafted fabric was very weak in the preceding experiment checking biocompatibility. Thus, a higher biocompatible silk material was developed.

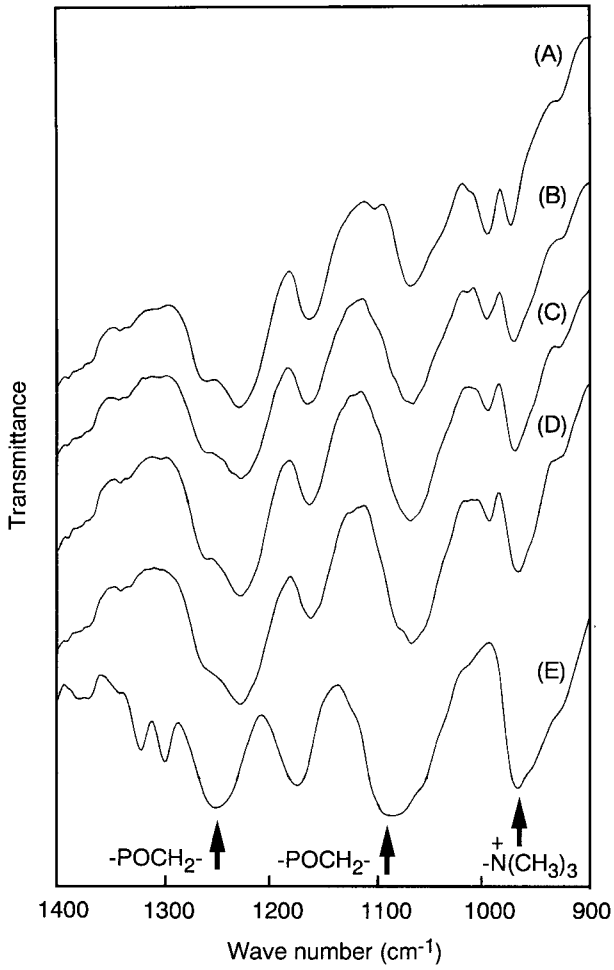


Figure 2 FT-IR spectra of MPC-grafted silk fabrics prepared using APS. (A): original silk; (B): MPC-*g*-silk (weight gain = 2.8 wt %); (C): MPC-*g*-silk (7.8 wt %); (D): MPC-*g*-silk (10.2 wt %); and (E): MPC monomer.

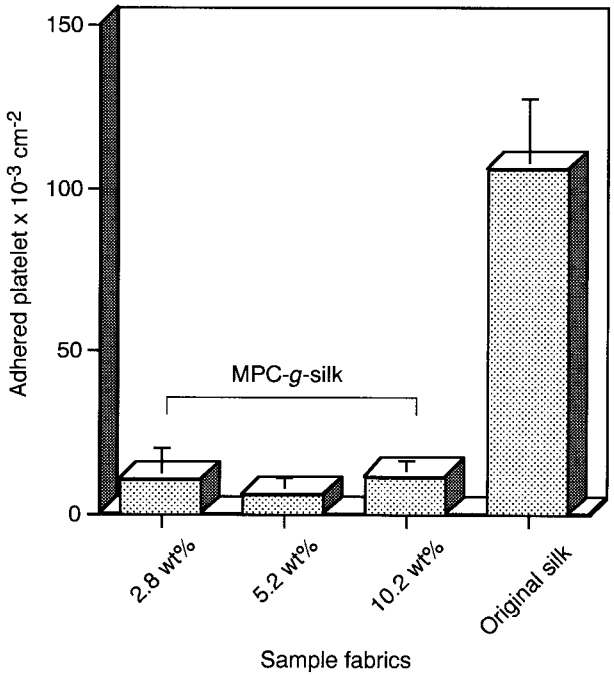


Figure 3 Number of platelets adhering on silk fabrics grafted with MPC. Standard error is shown $n = 3$. The number of platelets adhering onto a glass plate was $2.05 \pm 0.70 \times 10^5$ platelets/cm^2.

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REFERENCES

1. Freddi, G.; Tsukada, M. In Polymeric Materials Encyclopedia; Salamone, J. C., Ed.; CRC Press: Boca Raton, 1996; p. 7734.
2. Ishihara, K.; Oshida, H.; Endo, Y.; Ueda, T.; Watanabe, A.; Nakabayashi, N. J Biomed Mater Res 1992, 26, 1543.
3. Ishihara, K.; Tsuji, T.; Kurosaki, T.; Nakabayashi, N. J Biomed Mater Res 1994, 28, 225.
4. Ishihara, K.; Hanyuda, H.; Nakabayashi, N. Biomateirals 1995, 16, 873.
5. DeFife, K. M.; Yun, J. K.; Azeez, A.; Stack, S.; Ishihara, K.; Nakabayashi, N. J Biomed Mater Res 1995, 29, 431.
6. Ishihara, K.; Nomura, H.; Mihara, T.; Kurita, K.; Iwasaki, Y.; Nakabayashi, N. J Biomed Mater Res 1998, 39, 323.

7. Tamada, Y.; Kulik, E.; Ikada, Y. Biomaterials, 1994, 16, 259.
8. Tsukada, M.; Freddi, G.; Ishiguro, Y.; Shiozaki, H. J Appl Polym Sci 1993, 50, 1519.
9. Tsukada, M.; Gotoh, Y.; Freddi, G.; Yamamoto, T.; Nakabayashi, N. J Appl Polym Sci 1992, 44, 2197.
10. Tsukada, M.; Freddi, G.; Monti, P.; Bertoluzza, A. J Appl Polym Sci 1993, 49, 1565.
11. Ishihara, K.; Fukumoto, K.; Aoki, J.; Nakabayashi, N. Biomaterials 1992, 13, 145.
12. Fukumoto, K.; Ishihara, K.; Takayama, R.; Aoki, J.; Nakabayashi, N. Biomaterials 1992, 13, 235.
13. Ishihara, K.; Fukumoto, K.; Miyazaki, H.; Nakabayashi, N. Artif Organs 1994, 18, 559.
14. Ishihara, K.; Miyazaki, H.; Kurosaki, T.; Nakabayashi, N. J Biomed Mater Res 1995, 29, 181.