Surface Modified Poly(methyl methacrylate) Microspheres with the *O*-Methacryloyl-L-serine Moiety

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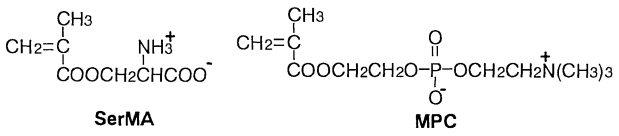
The poly(methyl methacrylate) microspheres [poly(SerMA-co-MMA)], modified their surface with novel zwitterionic *O*-methacryloyl-L-serine (SerMA) were prepared by emulsifier-free emulsion copolymerization of SerMA and MMA. Poly(SerMA-co-MMA) suppressed the adsorption of serum protein and the activation of platelet.

The biocompatibility of polymer materials has been improved by the modification of their surface with the phosphoryl choline moiety which exists on the extracellular surface of lipid bilayer.[1-4] For instance, the copolymers containing 2-(methacryloyloxy)ethyl phosphorylcholine (MPC) are found applications in variety of biomaterials because they suppress drastically the protein adsorption and exhibit an excellent nonthrombogenicity.[5-7] The biocompatibility with human cells is considered as being caused by a hydrogel structure formed at the surface based on the zwitterionic MPC moiety, that is, the mimic interface characteristic of the body's cell membrane. On the other hand, poly(α-amino acid) containing benzyl-L-glutamate or leucine,[8] polymer containing acylated L-lysine,[9] and poly(*O*-acyl-hydroxy-L-proline)[10] are also found to be biocompatible materials. L-Serine is amino acid found abundantly in sericin, fibroin, and serine protease. Thrombin which takes part in thromb-formation is one of serine proteases, in which the serine residue acts as an active site. In this communication, in order to develop a novel biocompatible material, *O*-methacryloyl-L-serine (SerMA) having the zwitterionic structure like as MPC was prepared and emulsion copolymerized with methyl methacrylate (MMA). The resulting polymer microspheres were characterized and were evaluated their biocompatibility by the adsorption behavior of serum protein on particles and the effect of addition of particles on the aggregation of platelet rich plasma (PRP).



SerMA was prepared from the esterification of the hydroxyl group in *N*-t-butoxycarbonyl-L-serine using methacryloyl chloride, followed by the treatment with trifluoroacetic acid as a deblocking reagent.[11] The emulsifier-free emulsion copolymerization of SerMA and MMA initiated with 2,2'-azobis[2-(2-imidazolin-2-yl)propane]dihydrochloride (VA-044) was carried out according to the method described previously.[12] The comparable poly(AlaMA / AlaEMA-co-MMA) was also prepared from the ternary copolymerization of *N*-methacryloyl-L-alanine (AlaMA)[13] with the terminal COOH group, L-alanine 2-methacryloyloxyethyl ester (AlaEMA)[14] with the terminal NH_2 group, and MMA in a similar manner as above. The results of polymerization and characterization of microspheres obtained are summarized in Table 1. The XPS analysis described the SerMA moiety and fragments of initiator being located on the surface of the particles. From the comparison of particle diameter from SEM with that from light scattering method, it was estimated that the hydrogel layer was formed on the surface of microspheres.[12] ζ-Potential of the particles was near zero due to the zwitterionic SerMA.

Interaction between poly(SerMA-co-MMA) and serum protein such as bovine serum albumin (Alb), human serum γ-globulin (Glo), and bovine serum fibrinogen (Fib) was studied in order to obtain basic information for biomedical applications.[7]

Table 1. Preparation and characterization of poly(methyl methacrylate) microspheres

Polymer microspheres[a]	comonomer / mmol	f [b]	Ymic[c] / %	Diameter[d] / nm	XPS analysis[e] / 10^3N / C	ζ-potential[f] / mV
Poly(MMA)	0	0	90.2	290 ± 40 (220 ± 10)	2.83 (0.80)	+ 27
Poly(SerMA-co-MMA) I	2.4	1	82.5	750 ± 140 (460 ± 30)	5.50 (2.75)	− 5
Poly(SerMA-co-MMA) II	7.2	3	80.4	800 ± 100 (450 ± 30)	11.17 (6.51)	− 8
Poly(AlaMA / AlaEMA-co-MMA)	1.2 / 2.4	1.5	73.9	340 ± 140 (250 ± 20)	7.30 (3.70)	≅ 0

[a]MMA ; 240 mmol, VA-044 ; 0.24 mmol, Water ; 250 mL, 70°C, 6h. [b]f represents mol% of comonomer to MMA. [c]Ymic represents the yield of polymer microspheres. [d]Calculated from particle size distribution. The values in parenthesis are calculated from SEM. [e]The values in parenthesis are the calculated values of N / C in feed. [f]Measured at pH 5.6.

Figure 1 shows the amount of Fib adsorbed on various polymer microspheres as a typical instance. Poly(SerMA-co-MMA) suppressed the adsorption of Fib much less than poly(MMA) (ζ = + 27 mV) did. The amount of Fib adsorbed decreased with increasing the content of SerMA moiety. Poly(AlaMA / AlaEMA-co-MMA) (ζ = 0 mV) modified with both AlaMA and AlaEMA moieties adsorbed Fib as much as poly(MMA) did. The similar behavior was observed for the adsorption of Alb and Glo. It is, therefore, worth noting that the introduction of the SerMA moiety with a zwitterionic structure similar to MPC on the surface of microspheres is considered to be effective for the suppression of protein adsorption, but is nevertheless of the influence of ζ -potential. Since the platelet aggregation occurs as a results of the addition of adenosine 5'-diphosphate sodium salt (ADP) to PRP,[15] the transmission change of PRP in the presence of poly(SerMA-co-MMA)II, comparing with poly(MMA) and poly(MPC)[16] was studied as shown in Figure 2.[17] The transmission of PRP increased as the control when platelet was aggregated by the addition of ADP. The addition of poly(SerMA-co-MMA)II to PRP showed the inhibition of aggregation of platelets much more than poly(MPC) case, while poly(MMA) showed no inhibition.

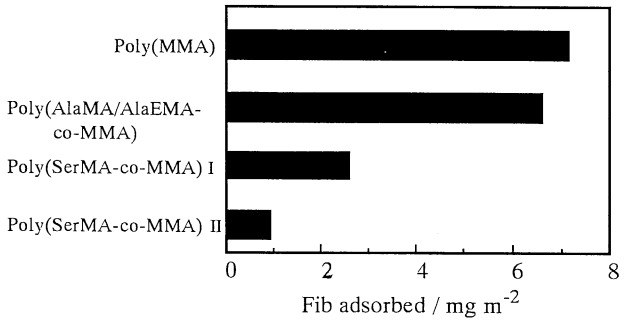


Figure 1. Amount of Fib adsorbed on polymer microspheres ; initial Fib concentration, $[Fib]_0$= 50 mgL^{-1} 25 ℃, ionic strength 0.01, pH 5.6.

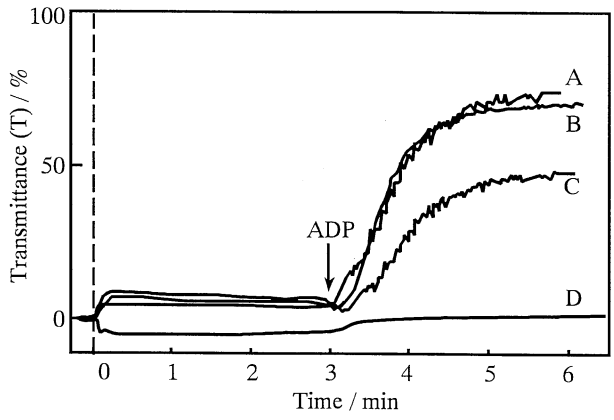


Figure 2. Platelet aggregation test. [ADP]= 9.0 μmolL^{-1}, A : control, B : [Poly(MMA)] = 15 mgL^{-1}, C : [Poly(MPC)] = 2.5 mgL^{-1} ([MPC]= 8.3 μmolL^{-1}), D : [Poly(SerMA-co-MMA)II]= 15 mgL^{-1} ([SerMA]= 4.4 μmolL^{-1}).

As a conclusion the modification of the surface of poly(MMA) with the SerMA moiety is useful for improving its biocompatibility.

References and Notes

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11 SerMA, mp 114 ~ 119 ℃ (decomp.), $[\alpha]_w^{25} = -10^\circ$ (H_2O), ^{1}H-NMR (D_2O) δ (ppm) : 1.86(s ; 3H, C$\underline{H}_3$), 4.07(m ; 1H, C$\underline{H}$), 4.53(m ; 2H, C$\underline{H}_2$), 5.70, 6.11(m; 2H, C = C$\underline{H}_2$). ^{13}C-NMR (D_2O) δ (ppm) : 17.9 (C$\underline{H}_3$), 54.5 (C$\underline{H}$), 64.0(C$\underline{H}_2$), 128.3(C=C$\underline{H}_2$), 136.0(C$\underline{=}$CH$_2$), 169.5(C$\underline{O}$O), 171.3(C$\underline{O}$OH). Found: C, 52.57; H, 6.71; N, 4.91%. Calcd for $C_7H_{11}O_4N$(173.169): C, 52.74; H, 7.01; N, 5.13%.
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16 The homopolymer of MPC, poly(MPC), was obtained by radical polymerization of MPC initiated with 2,2'-azobisisobutyronitrile as an initiator and purified by reprecipitation from ethanol / diethylether system.
17 Ninety milliliter of fresh whole human blood was collected in a disposable syringe containing 10 mL of a 3.8 wt / v % aqueous sodium citrate solution. The citrated whole blood was centrifuged immediately for 10 min at 1000 rpm to obtain citrated PRP. The transmittance (T) of PRP was recorded with a NKK Hema Tracer equipped with temperature controller. Four hundred microliters of PRP was incubated in glass cell at 37 ℃ for 3 min with stirring magnetically by the addition of 40 μL of 0.018 wt / v % polymer microspheres in physiological NaCl solution, followed by the addition of 44 μL of ADP (SIGMA, 100 μmolL^{-1}) in Owren buffer solution (pH 7.35).