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Platelet Adhesion to Microcapsules with Different Potentials

Nobuhiro Muramatsu, Yukio Goto, and Tamotsu Kondo

Faculty of Pharmaceutical Sciences, Science University of Tokyo, Ichigaya Funagawara-cho, Shinjuku-ku, Tokyo 162, Japan

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Microcapsules having the same surface composition but different surface potentials were prepared, and the effect of the surface potential of the microcapsules on platelet adhesiveness was studied. It was found that platelet adhesion was facilitated by an increase in the surface potential of the microcapsules, and that coating of the microcapsules with plasma caused no change in this trend of facilitated platelet adhesion, though the difference in surface potential disappeared after plasma coating. Therefore, the surface potential of the microcapsules was concluded not to affect the platelet adhesion directly but to influence the adsorption of plasma components on the microcapsules, thereby producing changes in platelet adhesiveness.

Keywords——microcapsule; surface potential; blood compatibility; platelet adhesion; adsorption of plasma

Introduction

A promising possible application of microcapsules in the medical field is as artificial red blood cells (ARBC), to enclose a mammalian hemolysate or a hemoglobin solution within an ultrathin polymer membrane. However, it was revealed in our previous studies[1] that platelets adhered easily to ARBC made of carboxylated or sulfonated poly(1,4-piperazinediylterephthaloyl) membrane and, moreover, the platelet adhesion was facilitated by increase in the surface negative charge on the ARBC; *i.e.*, the surface negative charge was found to have an adverse effect on the platelet adhesion properties. In these previous experiments, various amounts of L-lysine or 4,4′-diaminostilbene-2,2′-disulfonic acid were introduced into the polymer chains of ARBC membrane in order to vary the surface negative charge on the ARBC. However, this inevitably caused changes in the surface composition concurrently with those in the surface charge, so that the results obtained could hardly be explained merely in terms of surface negative charge on the ARBC.

There are many papers[2] demonstrating that the surface composition of substrates greatly affects the platelet adhesiveness. In fact, platelet adhesion onto ARBC was found to be considerably affected by the surface composition.[3] Therefore, in the present work, in order to study the effect of surface potential on platelet adhesion, poly(1,4-piperazinediylterephthaloyl)microcapsules containing different amounts of an anionic polyelectrolyte (dextran sulfate) were prepared and platelet adhesion was examined on these microcapsules. These microcapsules could be assumed to have the same surface composition but different surface potentials depending on the amount of dextran sulfate enclosed within them.

Experimental

Three kinds of microcapsules containing 0, 3 and 5% w/v dextran sulfate (Pharmacia, $\bar{M}$w-500000) solutions were prepared by making use of the interfacial polycondensation reaction between terephthaloyl dichloride and piperazine according to the procedures described earlier.[1] These microcapsules are designated hereafter as MC-1, -2 and -3, respectively. Measurements of platelet adhesion were carried out by counting the number of platelets not adhered to the microcapsules after mixing platelet and microcapsule suspensions for various times. The details of the method were reported in the previous paper.[1] The platelet suspension

used was rabbit platelet-rich plasma (PRP) or gel-filtered rabbit platelets suspended in Tris–HCl buffer saline (pH 7.4) (GFP). Electrophoresis was performed with a microelectrophoresis apparatus (Rank Brothers) at pH 7.4 and ionic strength 0.01, at 25°C.

Results and Discussion

The electrophoretic mobilities of the MC prepared above are listed in Table I. As they were prepared under identical conditions except for the concentration of dextran sulfate entrapped, each MC could be regarded as having the same surface composition. It was repotred[4] that microcapsules containing an aqueous solution of cationic or anionic polyelectrolyte move towards the anode or cathode, depending on the sign of the charge of the polyelectrolyte encapsulated. This is interpreted as showing that the counter ions of the polyions encapsulated diffuse out of the microcapsules through their semipermeable membrane to form double layers around the microcapsules. Thus, the differences in mobility observed would be due to the differences in the concentration of dextran sulfate encapsulated; dextran sulfate will be adsorbed on the inner surface of the MC membrane, and an increase in the dextran sulfate concentration will cause an increase in the amount adsorbed, thereby raising the mobility of the MC.

Table I. Electrophoretic Mobility of Microcapsules

Microcapsules	Mobility (μm/s/V/cm)
MC-1	−0.97±0.07
MC-2	−1.23±0.04
MC-3	−1.98±0.06

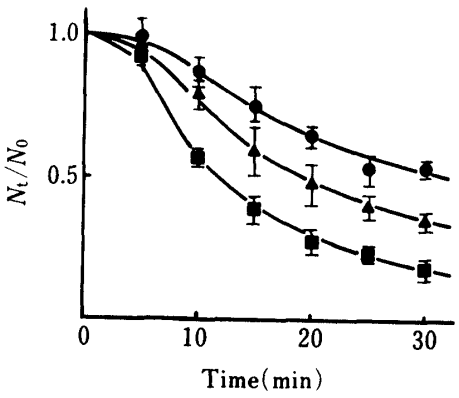

Fig. 1. Platelet Adhesion to MC in Platelet-rich Plasma

The ordinate represents the ratio of platelet number at a given time, N_t, to the initial value, N_0, before mixing the platelet suspension and MC.
●, MC-1; ▲, MC-2; ■, MC-3,
Each plot shows the mean value of 6 experiments and each bar the standard deviation.

MC-1, MC-2 and MC-3 were each mixed with PRP and the number of platelets not adhered to the MC was counted at various times. The results are shown in Fig. 1. This figure indicates that platelets adhered readily to the MC, and platelet adhesion was facilitated by increase in the surface potential of the MC. This trend is very similar to that found for the carboxylated and sulfonated polyamide microcapsules in the previous studies.[1] These results suggest that platelet adhesiveness is dependent solely on the magnitude of surface potential of the MC.

As many substances exist in plasma, it can be assumed that some of them are adsorbed

Table II. Electrophoretic Mobility of Plasma-coated Microcapsules

Microcapsules	Mobility (μm/s/V/cm)
MC-1	−1.15±0.07
MC-2	−1.16±0.07
MC-3	−1.17±0.09

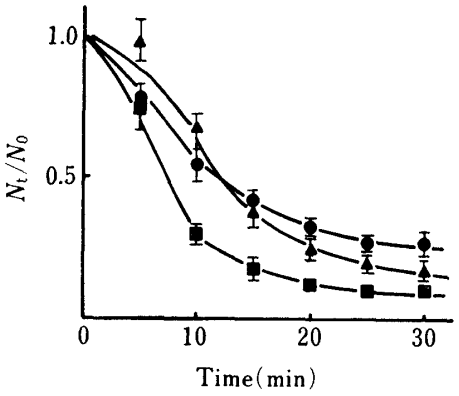

Fig. 2. Platelet Adhesion to Plasma-coated MC in Platelet-rich Plasma

The ordinate and the symbols used here are the same as those in Fig. 1. Each plot shows the mean value of 6 experiments and each bar the standard deviation.

on the MC in advance of platelet adhesion. Accordingly, the MC were premixed with an autologous platelet-free plasma and the electrophoretic mobilities of and platelet adhesion on the plasma-coated MC were measured. The results are shown in Table II and Fig. 2, respec tively. It can be seen that the differences in the magnitude of surface potential of the MC almost completely disappeared after this treatment, which may indicate that considerable amounts of plasma components are adsorbed to shield the electric field on the surface of bare MC. Platelet adhesion on the plasma-coated MC, however, was quite similar to that on the bare MC.

In order to investigate the role of plasma components in platelet adhesion more clearly, PRP was filtered on Sepharose 2B gel and the platelet adhesiveness on the bare and plasma-coated MC in the buffer solution was examined. The results are shown in Figs. 3 and 4, respectively. In the absence of plasma, almost no difference was found in platelet adhesiveness on the bare MC, while it was again found for the plasma-coated MC that platelet adhesion depended strongly on the surface potential initially present on the MC before plasma coating. Therefore, we conclude from these findings that platelet adhesion is seriously affected by the surface potential of the MC. However, the surface potential does not affect platelet adhesion in a direct manner but through the adsorbed layer of plasma components on the MC surface formed in advance of platelet adhesion; the pattern of adsorption and/or the composition of the adsorbed plasma layer would depend on the surface potential of the MC, which would in turn affect platelet adhesiveness.

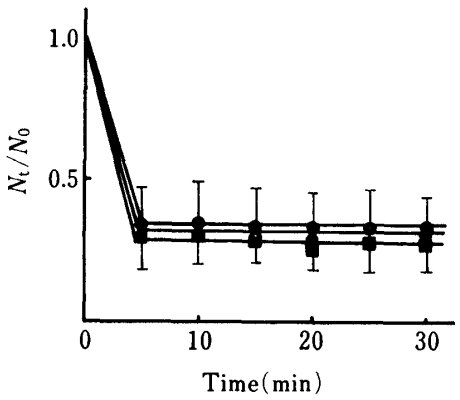

Fig. 3. Platelet Adhesion to MC in Gel-filtered Platelet Suspension

The ordinate and the symbols used here are the same as those in Fig. 1. Each plot shows the mean values of 3 experiments and each bar the standard deviation.

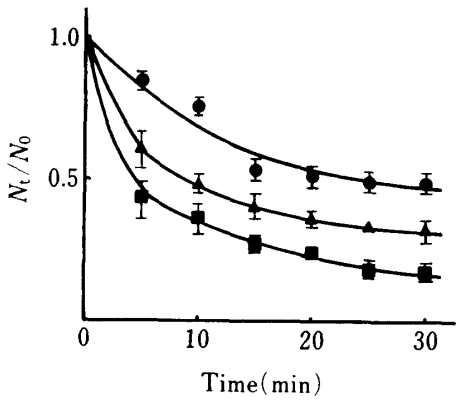

Fig. 4. Platelet Adhesion to Plasma-coated MC in Gel-filtered Platelet Suspension

The ordinate and the symbols used here are the same as those in Fig. 1. Each plot shows the mean value of 3 experiments and each bar the standard deviation.

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