Cellular Affinity of Polyamides Having Various Functional Groups. I. Platelet Adhesion

NAOYA OGATA, KOHEI SANUI, HOZUMI TANAKA, and YOICHI TAKAHASHI, *Department of Chemistry, Sophia University, 7 Kioi-cho, Chiyoda-ku, Tokyo, Japan 102*, and YASUHISA SAKURAI, TOSHIHIRO AKAIKE, TERUO OKANO, and KAZUNORI KATAOKA, *Heart Institute of Japan and Institute of Medical Engineering, Tokyo Women's Medical College, 10 Kawada-cho, Ichigaya, Shinjuku-ku, Tokyo, Japan 162*

Synopsis

Various polyamides having a different density of hydrogen bonding, of hydrophilic character, and containing ionic groups have been synthesized and their blood compatibilities were evaluated in terms of the adhesion behavior of blood platelets on polyamide by the microsphere column method. Polyamides containing anionically charged groups such as carboxylate or sulfonate groups adsorbed fewer blood platelets than those with undissociated carboxylate groups. Polyamides having thioether groups adsorbed fewer platelets than those having ether groups. Introduction of a rigid piperazine unit caused an increase in platelet adhesion.

INTRODUCTION

There are a number of significant applications of polymeric materials in the medical field including vascular prostheses for large, high flow-rate arteries, artificial kidneys for hemodialysis, scaffoldings for cell culture, cell fractionation by cell adhesion chromatography, and so on. When such polymeric materials are implanted in a living body or contacted with body fluids, acute as well as chronic inflammatory responses take place. Such "foreign" materials can induce vascular responses of hyperemia or cellulitis development or produce blood coagulation. Within the cardiovascular system, thrombotic or embolytic events are also encountered. Therefore, the biocompatibility of polymeric materials is very important for medical applications.

There is a wide variety of *in vitro* and *in vivo* methods for evaluating the biocompatibility of polymeric materials, and many reports have been published. Most of polymeric materials which had been evaluated before are related to poly(ethylene terephthalate),[1] poly(ether urethane)s,[2] or poly(tetrafluoroethylene).[3] Although there were some attempts to use commercial polyamides (nylon 6 and nylon 66) for biomaterials, few made-to-order polyamides for medical application have been produced. Few reports on polyamides having various functional groups have been published in terms of the biocompatibility.

Polyamides are essentially the same polymer as polypeptides in respect to amide linkages to form long polymer chains. Therefore, it is expected that polyamides having various functional groups may have good biocompatibility

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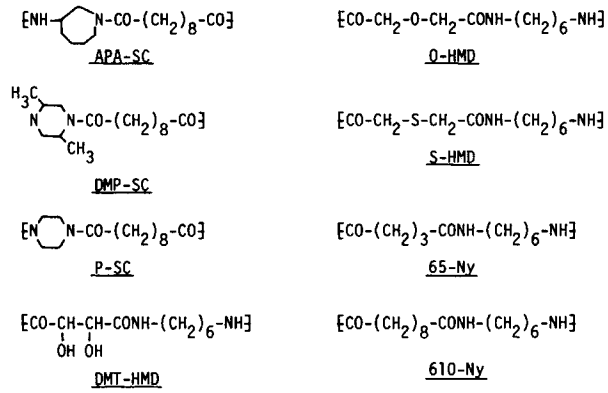


Fig. 1. Structures of polyamides having various hetero atom groups.

in the living body. On the other hand, it is also true that materials with structures similar to the living body often induce immune reactions or unfavorable biodegradation reaction. Therefore, it is interesting to study bioreactions of polyamides. From these viewpoints, polyamides having a different density of hydrogen bonding, hydrophilic character, and containing ionic groups were synthesized, and their blood compatibilities were investigated in terms of adhesion of blood platelets. This was evaluated by contacting polyamides with fresh blood of a dog.

EXPERIMENTAL

Polyamides Having Functional Groups

Polyamides having various hetero atom groups such as ether,[4] thioether,[5] or hydroxyl group[6] or those having ionic groups such as carboxyl or sulfonic groups were synthesized according to interfacial or solution methods.[7] The chemical

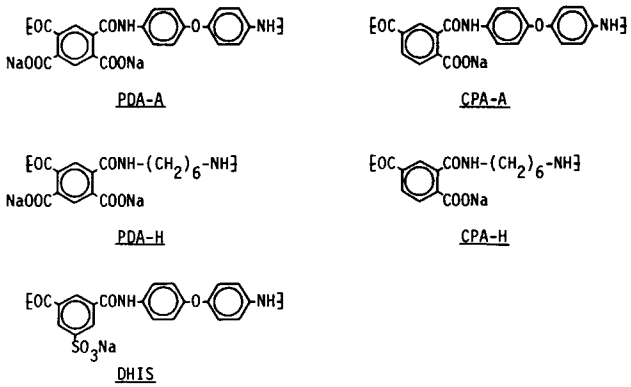


Fig. 2. Structures of polyamides having various ionic groups.

TABLE I
Properties of Polyamides

Polyamides	Yield %	η_{sp}/C[a]	Solubility H_2O	$CHCl_3$	MeOH	DMSO	DMAc	MeOH (10% $CaCl_2$)
O—HMD	72.8	0.63	–	–	–	+[b]	–	+
S—HMD	68.9	0.73	–	–	–	+[b]	–	+
P—SC	73.6	3.08	–	+	–	–	–	+
DMP—SC	60.5	0.84	–	+	+	+[b]	+[b]	+
APA—SC	76.7	1.29	–	+	+	+[b]	+	+
DMT—HMD	55.2	0.43	–	–	–	+	–	+
Nylon 65	73.7	0.97	–	–	–	–	–	+
Nylon 610	82.3	1.13	–	–	–	–	–	+

[a] 0.1 g/10 cm^3 in H_2SO_4 at 30°C.
[b] Hot.

structures of the synthesized polyamides are summarized in Figures 1 and 2, in which their abbreviation are also listed. The physical properties of these polyamides are summarized in Tables I and II.

Evaluation of Platelet Adhesion[8]

The procedure for the coating of various polyamides on glass beads was the precipitation method.

Precipitation method. A 30-cc beaker was charged with 20 g of glass beads with a size of 40–60 mesh, and the glass beads were immersed in 20 cc solution which was prepared by dissolving polyamides having ionic groups in *N,N*-dimethylacetamide (DMAc) or dissolving polyamides having hetero atom groups in methanol (10 wt % $CaCl_2$) at a concentration of 0.25 wt %.

The contents were then poured onto a microfilter mounted in a suction funnel to separate the glass beads from the solution. Immediately, the glass beads on the microfilter were treated with a large amount of a nonsolvent for the polyamide such as acetone in order to coat the bead surface with the polyamide. No influence of coating conditions on the platelet adhesion was observed.

TABLE II
Properties of Polyamides

Polyamides	Yield, %	η_{SP}/C[a]	Solubility H_2O	$CHCl_3$	MeOH	DMSO	DMAc	$Cl_2CHCOOH$
PDA-A	78.7	1.00	–	–	–	+[b]	+	+
PDA—H	63.5	0.64	–	–	–	+	+	+
CPA—A	72.1	0.53	–	–	–	+[b]	+	+
CPA-H	58.3	0.41	–	–	–	+	+	+
DHIS	61.5	0.52	–	–	–	–	+	+

[a] 0.1 g/10 cm^3 in H_2SO_4 at 30°C.
[b] Hot.

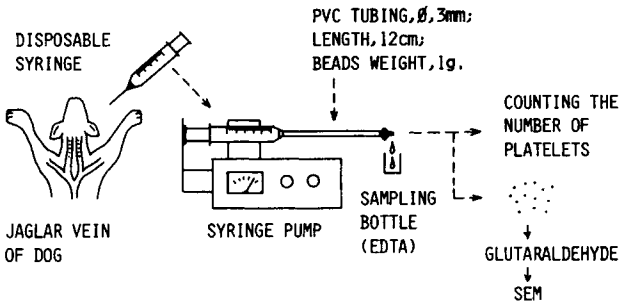


Fig. 3. Illustration for the evaluation of platelet retention.

Evaluation. A 1-g portion of the coated glass beads was packed in a tubing of poly(vinyl chloride) and subjected to the following platelet adhesion test. Fresh blood, 3 cm^3, was collected from a jugular vein of a mongrel dog by a disposable syringe without using anticoagulant. The collected blood was immediately passed through the column packed with the polyamide-precoated glass beads for 60 sec at a flow rate of 1.2 cm^3/min with the use of a Precidol model 5003 syringe pump. The procedure is illustrated in Figure 3.

After all blood was passed through the column, the eluted blood was collected together with the primed saline to a sample bottle the inner surface of which was covered with EDTA as anticoagulant. Then, the column was washed with saline solution at a flow rate of 1.7 cm^3/min for a period of 120 sec. The rinsed column was divided into three parts, namely, upper, middle, and lower parts. The beads situated in each of the three parts of the column were placed in a saline solution containing 1.25% glutaraldehyde in order to fix adhered platelets. The beads were then freeze-dried, followed by coating with gold. The coated glass beads were also observed by scanning electron microscopy (SEM). Scanning electron micrographs were taken with a Hitachi-Akashi MSM-101 scanning electron

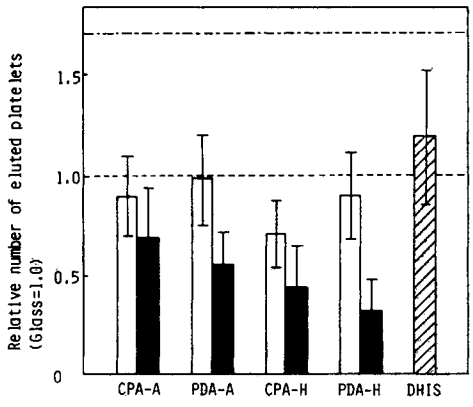
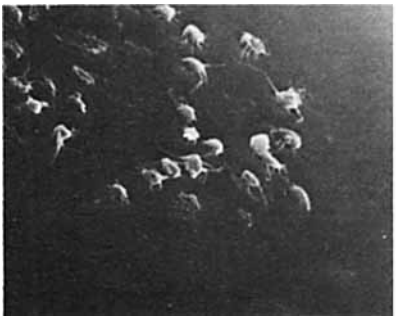


Fig. 4. Platelet retention on surface precoated with polyamides having charges: (□) —COONa; (■) —COOH; (▨) —SO_3Na. Five dogs were used in this series of experiments. Data points indicate mean value ± SD; (—·—) mean number of platelets in control blood.

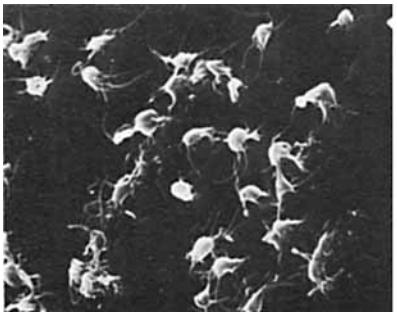
DHIS (×3000)
(a)

PDA-H (×3000)
(d)

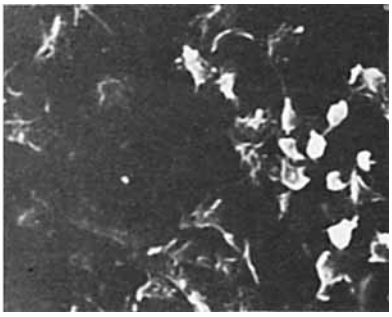


PDA-A (×3000)
(b)

CPA-H (×3000)
(e)

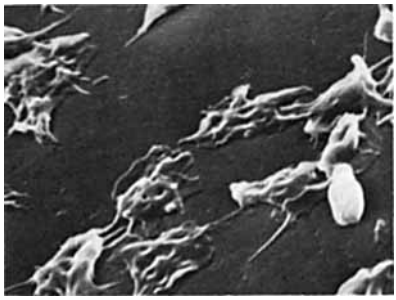


CPA-A (×3000)
(c)

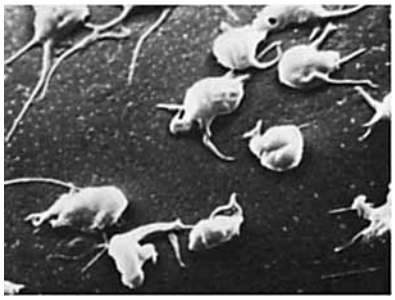


Glass (×3000)
(f)

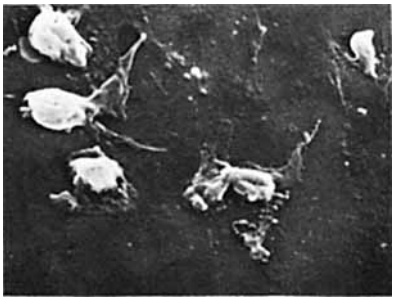
Fig. 5. Scanning electron micrographs of platelets adsorbed on the surface of polyamides having ionic groups.



(g)



(h)



(i)

Fig. 5. (*Continued from previous page.*)

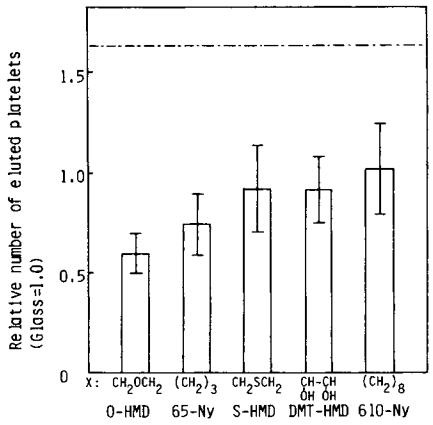


Fig. 6. Platelet retention on surface precoated with polyamides having hetero atom groups. Polyamide structure is —CO–X—CONH—$(CH_2)_6$—NH—, where X is indicated on abscissa. Four dogs were used in this series of experiments. Data points indicate mean value ± SD; (—·—) mean number of platelets in control blood.

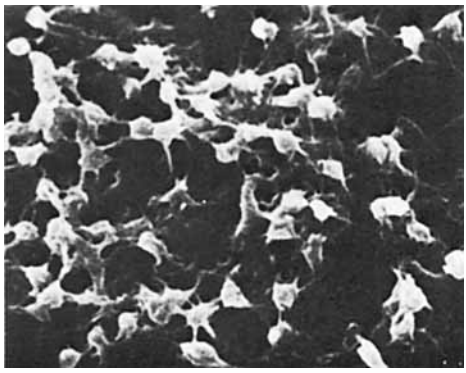
microscope. More than 10 views per each sample were observed, and typical representative scanning electron micrographs of various samples are shown in Figures 5, 7, and 9. The number of platelets in the eluted blood was counted according to the method of Brecher and Cronkite.[9]

RESULTS AND DISCUSSION

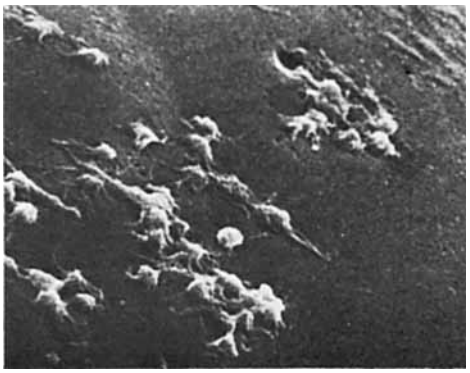
Effect of Ionic Groups on Platelet Adhesion

Polyamides having one or two carboxylic acids in each repeating unit were synthesized, and the platelet adhesion on these polyamides was compared with that of sodium salt derivatives. The platelet adhesion on polyamides having sodium sulfonate groups was also determined. These results are summarized in Figure 4, where a dotted line indicates the platelet adhesion on glass microspheres as a relative standard. It is seen that the platelet adhesion was greatly decreased by neutralizing carboxylic acid with sodium hydroxide. The polyamide having two neutralized carboxylate groups was superior to that having one neutralized carboxylate group in each repeating unit, and an anionic charge on the surface of a polyamide may be effective in preventing the adhesion of platelets. On the other hand, it was found that the polyamide with a nonneutralized carboxylate group adsorbed more platelets than that with neutralized carboxylate anion. The adhesive effect was enhanced by the introduction of undissociated carboxylate groups. It is very suggestive to design biomedical polymer molecules so that the interaction of carboxylate groups can contribute to the weak interaction with platelets as well as strong interaction by the process of neutralization. Figure 4 also indicates that the polyamide having sodium sulfonate groups was superior to the polyamide having carboxylate groups. These results suggest that anionically charged polyamides show better platelet adhesion.

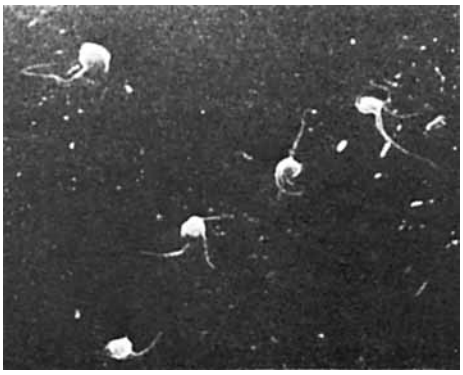
The scanning electron microscopic examinations of platelets attached to the surface of anionic polyamides and to the control glass surface are shown in Figure



OHMD (×3000)
(a)



S-HMD (×3000)
(b)



610-Ny (×3000)
(c)

Fig. 7. Scanning electron micrographs of platelets adsorbed on the surface of polyamides having hetero atom groups.

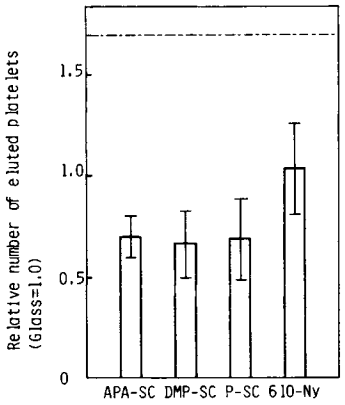


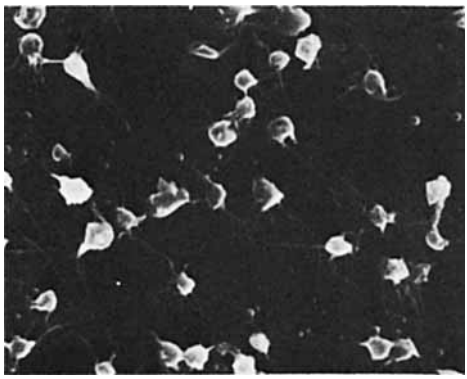
Fig. 8. Platelets retention on surface precoated with polyamides having secondary or tertiary amide linkages. Five dogs were used in this series of experiments. Data points indicate mean value ± SD; (—·—) mean number of platelets in control blood.

5. The glass surface without polyamide was covered with flatly deformed platelets. On the surface of polyamides with carboxylate or sulfonate groups, the deformation and aggregation of adherent platelets are not so significant compared with those on the bare glass. Strictly speaking, the platelets had a tendency to adhere to polyamide with undissociated carboxylate groups more mildly than to polyamide with dissociated carboxylate anions. Platelet attached on the latter surface sometimes took a flat or spindlelike shape. These results indicate that the chemical properties of carboxylate groups of polyamides affect not only the number of adhesions but also the morphological changes of the platelets.

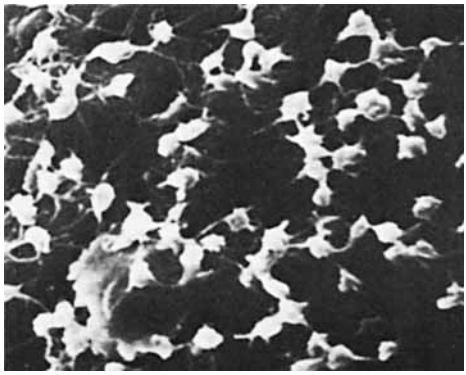
Effect of Hetero Atom Groups on Platelet Adhesion

Hetero atom groups such as ether, thioether, or hydroxyl groups are polar and modify properties of polyamides in terms of flexibility of polymer main chains or hydrophilicity. Therefore, various polyamides containing ether, thioether, or hydroxyl groups were synthesized, and the platelet adhesion on these polyamides was investigated. Results are summarized in Figure 6. In the series of experiments hexamethylenediamine was chosen as amino component to simplify the effect of hetero atoms. Figure 6 indicates that the polyamide having ether groups adsorbed platelets, while that having thioether groups reduced the platelet adhesion when compared with results of nylon 65. It is interesting that three polyamides having the same repeating unit except for a single atom (S, O, CH_2) showed differences in platelet adhesion. The results of Figure 6, including those of other polyamides, nylon 610, and DMT–HMD, indicate interesting variations in platelet adhesion by polyamides with hetero atoms.

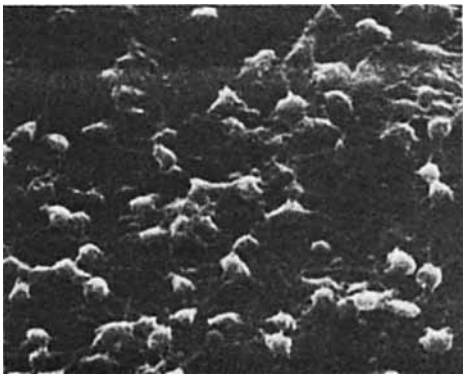
Figure 7 indicates scanning electron micrography of the platelets attached to the surface of the polyamides. In this case the degree of deformation of adherent platelets is consistent with the results of the column method. The deformation and aggregation of the platelets attached to the surface of polyamides with ether groups is greater than that having thioether groups.



APA-SC (×3000)
(a)



DMP-SC (×3000)
(b)



P-SC (×3000)
(c)

Fig. 9. Scanning electron micrograph of the platelets adsorbed on the surface of polyamides having secondary or tertiary amide linkages.

Effect of Amide Groups on Platelet Adhesion

Polyamides having saturated cyclic structures were synthesized in order to investigate the effect of rididity of the main chain and distance between amide groups on the platelet adhesion in comparison with nylon 65 or 610. Results of the platelet adhesion for these polyamides are summarized in Figure 8, where a dotted line indicates platelet adhesion on glass microsphere as a relative standard. It is seen that those polyamides having a rigid cyclic structure adsorbed platelets with the same relative values of about 0.7, and no influence of minor difference in cyclic structure was observed.

From the scanning electron micrograph (Fig. 9), the difference in morphology of adhering platelets was not observed.

CONCLUSIONS

Polyamide is characterized by a combination of high strength, elasticity, toughness, and abrasion resistance and thus is expected to be applicable to the biomedical field. The preliminary survey on the biocompatibility of polyamide revealed that the polyamide with nonneutralized carboxylate groups adsorbed more platelets than that with neutralized carboxylate anions. The adhesive effect of nonneutralized carboxylate groups and the nonadhesive effect of neutralized carboxylate groups were respectively enhanced by the number of carboxylate groups. It was also found that replacement of backbone carbon atoms by such hetero atoms as oxygen and sulfur changed the adhesiveness of platelets. These results should serve as guidelines for the molecular design of polyamides of good compatibility in terms of biomedical applications. Polyamides examined in this report show a relatively high affinity for platelets. These polyamides may have a potential use in the field of cell culture and cell fractionation. Further studies on the preparation of polyamides showing low platelet adhesiveness are now in progress. These results will be reported on in later articles.

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