Journal of Macromolecular Science, Part A
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597274

Design and Synthesis of Biocompatible Polymeric Materials

Yukio Imanish[a]; Yoshihiro Ito[a]; Lin-Shu Liu[a]; Masako Kajihara[a]
[a] Department of Polymer Chemistry, Kyoto University, Sakyo-ku, Kyoto, Japan

To cite this Article Imanish, Yukio , Ito, Yoshihiro , Liu, Lin-Shu and Kajihara, Masako(1988) 'Design and Synthesis of Biocompatible Polymeric Materials', Journal of Macromolecular Science, Part A, 25: 5, 555 — 570
To link to this Article: DOI: 10.1080/00222338808053384
URL: http://dx.doi.org/10.1080/00222338808053384

J. MACROMOL. SCI.—CHEM., A25(5-7), pp. 555-570 (1988)

DESIGN AND SYNTHESIS OF BIOCOMPATIBLE POLYMERIC MATERIALS

YUKIO IMANISHI, YOSHIHIRO ITO, LIN-SHU LIU, and MASAKO KAJIHARA

Department of Polymer Chemistry
Kyoto University
Yoshida Honmachi, Sakyo-ku, Kyoto 606, Japan

ABSTRACT

For one of the approaches to antithrombogenic materials, the combination of synthetic polymers with physiologically active substances seems to be promising. One of these ways involves crosslinked urokinase (HM-UK), in which urokinases are linked with side chains of poly(2-hydroxyethyl methacrylate) (PHEMA) by 4,4′-diisocyanatodiphenylmethane, and encapsulated urokinase (HMe-UK), in which urokinases are encapsulated in the network of crosslinked PHEMA. The HMe-UK with a low degree of crosslinking was found to be highly antithrombogenic. For another way, Val-Pro-Arg-immobilized polyetherurethaneurea (PEUU) was synthesized. The tripeptide, Val-Pro-Arg, is a substrate of thrombin. The tripeptide- immobilized PEUU was found to complex specifically with thrombin and inactivate it, and hence to prolong the thrombin time and the active partial thromboplastin time. For a further approach to antithrombogenic materials, the design of materials which are covered with a living tissue formed by cell proliferation seems to be promising. For one of the ways in this approach, Arg-Gly-Asp-Ser-immobilized silicone films were synthesized. The tetrapeptide constitutes the active site of cell-adhesion proteins. A number of fibroblast cells were found to grow on the tetrapeptide-immobilized silicone film.

INTRODUCTION

Three important ways of approach have been advocated in the design of biocompatible materials. The first way is to acquire the biocompatibility through regulated interactions with living systems by control of the surface properties of the polymeric material [1].

The second way is to acquire the biocompatibility by a combination of physiologically active substances with the polymeric material. The present authors have synthesized polyetherurethaneureas, their ionic complex with heparin [2], and their covalent composite with heparin [3], and have observed their excellent *in vitro* and *in vivo* [4] antithrombogenicities. Furthermore, *in vitro* blood-clotting tests were carried out with polyetherurethaneureas which were linked with heparin molecules with intervening spacer linkages. It was found that long-term antithrombogenicity was obtained with a controlled release of heparin when polyallylamine was used for the spacer linkage [5].

The third way is to develop polymeric materials that stimulate biological functions to assimilate foreign materials or to recover the living tissue from injury. The validity of this way was suggested in 1979 by Van Kampen et al. [6]. In the *in vivo* blood-clotting test of vascular prostheses made of polyurethane and various kinds of polypeptides, they observed that the vascular prostheses were quickly embolized by the formation of a large amount of thrombus if leucocytes were not spread over the surface of the material, and they also observed that the vascular prostheses showed the formation of little thrombus if leucocytes were well spread over the surface of the material. They proposed a mechanism of thrombogenesis that stops at a certain stage before the embolism with a competing attachment and spreading of leucocytes. Burkel et al. [7] succeeded in the use of crosslinked fibrin of thrombus for the matrix of the attachment and growth of endothelial cells. They precipitated fibrin networks on Teflon or polyester cloth by preclotting of blood and seeded endothelial cells in advance of the *in vivo* use for artificial blood tubes. They observed the formation of a layer of endothelial cells on the luminal surface and a high percentage of thrombus-free surface.

The present authors spun a suture of the polyetherurethaneurea with covalently linked heparins, placed it in the femoral vein of an adult dog for 90 d, and observed the state of the surface with an electron micrograph [4]. A layer of endothelial cells was found to have grown on the matrix of fibrin which was formed to some extent at the puncture point of the vein. With these experimental findings as background, the idea has become prevalent that the stimulation of biological function to assimilate foreign materials

and to recover living tissue from injury is the best way to acquire real biocompatibility of materials.

Recent research achievements of the present authors in the design and synthesis of biocompatible, in particular, antithrombogenic materials along the lines of the second and third ways of the above approach, will be briefly described.

ANTITHROMBOGENICITY OF UROKINASE-IMMOBILIZED POLYMERS

Urokinase is a kind of fibrinolytic enzyme and inhibits thrombus formation by decomposing the crosslinked polymer of the fibrin. Highly antithrombogenic polymeric materials may be obtained by immobilizing urokinase with poly(2-hydroxyethyl methacrylate) (PHEMA) or polyurethanes.

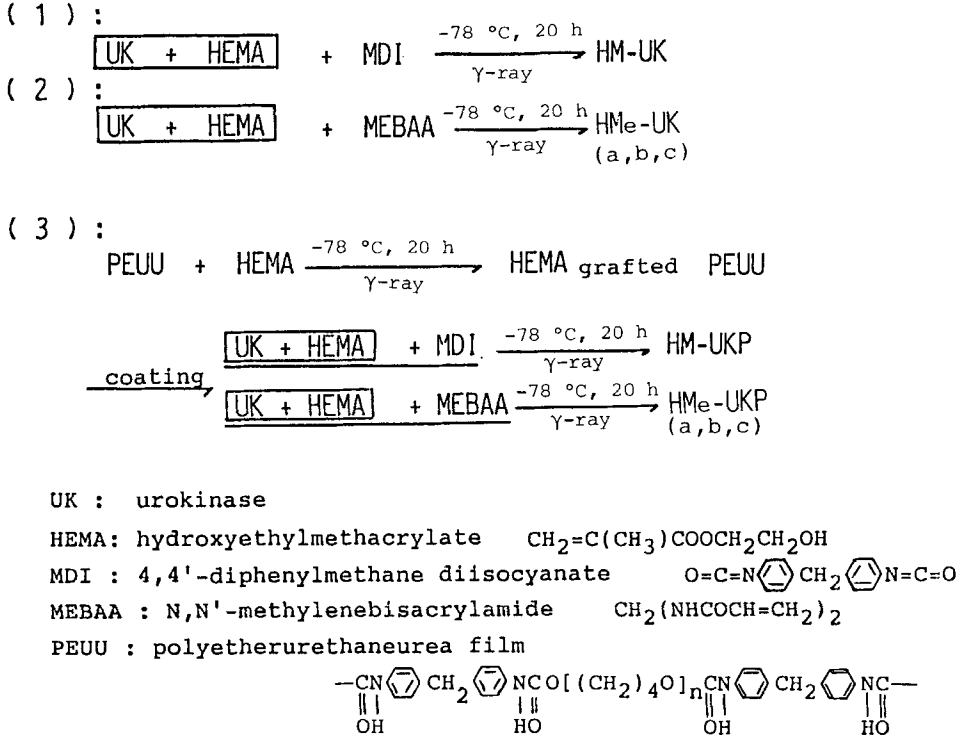
Synthesis of Urokinase-Immobilized Polymeric Materials

The scheme of urokinase immobilization to PHEMA is illustrated in Fig. 1.

The method of synthesizing crosslinked urokinase (HM-UK) is as follows. 2-Hydroxyethyl methacrylate (HEMA) (200 μL) was added to a tris-buffered solution (pH 8.0, 200 μL) containing 300 I.U. urokinase. After adding an acetone solution of 4,4′-diisocyanatodiphenylmethane (MDI) (20 mg/mL, 20 μL) and bubbling a nitrogen stream, the mixture was irradiated by ^{60}Co γ-rays (2×10^4 rd/h) for 20 h at -78°C. The reaction product was washed repeatedly with distilled water until the urokinase activity disappeared from the washed liquid.

Encapsulated urokinase (HMe-UK) was synthesized as follows. HEMA (200 μL) and various amounts of *N,N′*-methylenebisacrylamide (MEBAA) were added to a tris-buffered solution (pH 8.0, 200 μL) containing 300 I.U. urokinase. The mixture was bubbled with a nitrogen stream and irradiated with ^{60}Co γ-rays (2×10^4 rd/h) for 20 h at -78°C. The reaction product was purified in the same way as HM-UK. The symbols HMe-UK(a), HMe-UK(b), and HMe-UK(c) are given to the reaction products obtained in the presence of 15, 10, and 5 mg of MEBAA, respectively. On going from (a) to (c), the degree of crosslinking in the PHEMA network decreases.

Polyurethane films coated with the urokinase-immobilized PHEMA were prepared as follows. A urethane prepolymer produced from poly(oxytetramethylene) (molecular weight 870) and MDI in a molar ratio of 1:2 was chain-extended by reaction with 1,2-diaminopropane (molar ratio 1) to produce polyetherurethaneurea. A film of the polyetherurethaneurea (1 cm diameter,



UK : urokinase

HEMA: hydroxyethylmethacrylate $CH_2=C(CH_3)COOCH_2CH_2OH$

MDI : 4,4'-diphenylmethane diisocyanate $O=C=N-C_6H_4-CH_2-C_6H_4-N=C=O$

MEBAA : N,N'-methylenebisacrylamide $CH_2(NHCOCH=CH_2)_2$

PEUU : polyetherurethaneurea film

FIG. 1. Scheme of urokinase immobilization.

0.5 mm thickness) cast from a 10 wt% dimethylformamide solution was immersed in HEMA (0.5 mL). After bubbling with a nitrogen stream, HEMA containing the film was irradiated by ^{60}Co γ-rays (2×10^4 rd/h) for 24 h at -78°C. The PHEMA-grafted polyetherurethaneurea film was immersed in a urokinase/HEMA/MDI solution with the same composition as in the synthesis of HM-UK, or in a urokinase/HEMA/MEBAA solution with the same composition as in the synthesis of HMe-UK, and irradiated by γ-rays in the same way as before to yield a polyurethane film coated with HM-UK (HM-UKP) or with HMe-UK(a), (b), and (c) (HMe-UKP(a), (b), and (c)].

Activities of Immobilized Urokinases

The activity of immobilized urokinase was determined by the caseinolytic method or by the peptide method.

In the caseinolytic method, plasminogen is decomposed by urokinase to yield plasmin, which is in turn decomposed by α-casein to yield tyrosin, which is determined [8]. Urokinase (10-50 I.U.) was dissolved in a tris-buffered solution (0.01 *M* Tris-HCl, 0.05 m*M* NaCl, pH 8.0) containing 1 wt% gelatin. To the solution (1 mL), 2 wt% tris-buffered saline of plasminogen (1 mL) and tris-buffered saline (0.5 mL) were added. The mixture was kept at 37.5°C for 10 min and incubated for 2 or 32 min with 1.4 wt% tris-buffered saline of α-casein (2.5 mL). A part of the incubating solution (2.0 mL) was taken out and mixed with 0.5 *M* $HClO_4$ (3.0 mL). After standing at room temperature for 30 min, the mixture was centrifuged at 2000 *g* for 15 min, and the absorption at 275 nm of the supernatant was measured. The sample incubated for 2 min was used as the reference, and the difference absorption was plotted against the amount of urokinase to obtain a calibration curve.

In the peptide method, Glu-Gly-Arg-MCA (4-methyl-7-coumarin amide), which is a substrate of urokinase, is used, and the fluorescence intensity of the hydrolytic product, 7-amino-4-methylcoumarin (AMC), is determined. A dimethylsulfoxide solution of Glu-Gly-Arg-MCA (5.03 mg/mL, 20 μL) was diluted to 1 mL by adding a tris-buffer (50 m*M* Tris-HCl, 100 m*M* NaCl, 10 m*M* NaCl, pH 8). The diluted substrate solution (1 mL) was kept at 37°C for ~3 min and then mixed with urokinase (less than 30 I.U.) for reaction under stirring. After 10 min, 17% acetic acid (1.5 mL) was added to stop the reaction. The solution was excited with a 380-nm light and the fluorescence at 460 nm was measured to determine the ACM produced.

The reactivities of immobilized urokinases are shown in Table 1. It was found that the urokinase activity was decreased to 85% of the native enzyme by the irradiation. The activity of urokinase crosslinked with HEMA and MDI (HM-UK) was found to decrease to ~10% of the original level and that of encapsulated urokinases [HMe-UK(a), (b), and (c)] to the 40-70% level, as determined by the caseinolytic method, and to the ~65% level, as determined by the peptide method. The apparently lower activities of immobilized urokinases as determined by the caseinolytic method may be due to difficulty of access of the high molecular weight plasminogen to the urokinase immobilized in the network. This consideration explains the increasing activities of urokinases immobilized in looser networks [HMe-UK(a) → HMe-UK(c)].

Thrombus Formation on Urokinase-Immobilized Polymer Materials

Blood of an adult dog was collected through a scalp vein needle into a polypropylene bag which contained 1 part of acid-citrate-dextrose (ACD) for 9 parts of blood. The resultant ACD blood was placed on a glass plate, gel membranes of HM-UK and HMe-UK, and polyurethane films coated with

TABLE 1. Activity of Immobilized Urokinase at 37°C[a]

	Caseinolytic method		Peptide method	
Sample	Activity, I.U.	Ratio, %	Activity, I.U.	Ratio, %
N-UK[b]	300	100	300	100
R-UK[c]	255	85	255	85
HM-UK	33	11	35	11
HMe-UK(a)[d]	123	41	192	64
HMe-UK(b)	165	55	198	66
HMe-UK(c)	198	66	196	65

[a]Concentration of urokinase in all samples is 300 I.U./0.44 mL.
[b]Free urokinase.
[c]Irradiated urokinase.
[d]Content of MEBAA: (a) 15 mg, (b) 10 mg, (c) 5 mg.

HM-UK and HMe-UK. Clotting was initiated by adding aqueous $CaCl_2$ solution, and the weight of thrombus formed during 20 min was weighed according to the procedure reported by Imai and Nosé [9]. The relative weights of thrombus formed on different samples were determined, that formed on a glass plate being taken as 100%. The experimental results are presented in Fig. 2.

It is shown in Fig. 2 that PHEMA (HM and HMe) is more thromboresistant than glass and that the antithrombogenicity becomes higher on immobilization of UK. Coating immobilized urokinase on polyurethane film does not seem to influence the antithrombogenicity of the hybridized material, since only a very low difference was observed between HM-UK and HM-UKP or between HMe-UK(a), (b), (c) and HMe-UKP(a), (b), (c). Encapsulated urokinases were more thromboresistant than crosslinked urokinases, as judged by the greater thrombus formation on HM-UK than on HMe-UK(a), (b), (c). This difference may be a reflection of the higher urokinase activity of the encapsulation type than of the crosslinking type (Table 1). The decreases of thrombus formation, in the order HMe-UK(a) > (b) > (c), may also be a result of increasing activity of the encapsulated urokinases with decreasing crosslinking density in the same order.

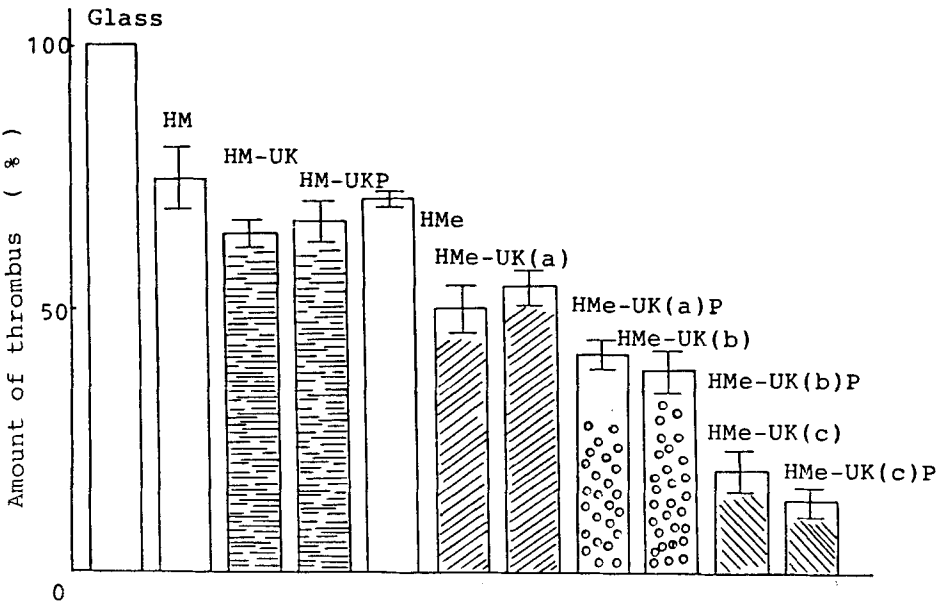


FIG. 2. Amount of thrombus formed on films of urokinase-immobilized polymers. The initial concentration of urokinase was 300 I.U./0.44 mL.

ANTITHROMBOGENICITY OF THROMBIN SUBSTRATE-IMMOBILIZED POLYMERS

The present authors found that polyetherurethaneureas with ionically or covalently bound heparins were highly antithrombogenic and confirmed that heparins which are covalently linked to the material activate antithrombin III, which in turn deactivates thrombin [3]. Furthermore, polyurethanes carrying sulfonic acid groups, which are considered to be most essential for the antithrombogenic activity of heparin, were found to be antithrombogenic due to the inactivation of thrombin [10]. Therefore, antithrombogenic materials may be obtained by controlling their thrombin activity. Antithrombogenic materials were expected by immobilizing a tripeptide Val-Pro-Arg, which is a substrate of thrombin. The tripeptide-immobilized material may take up thrombin from the activation of fibrinogen.

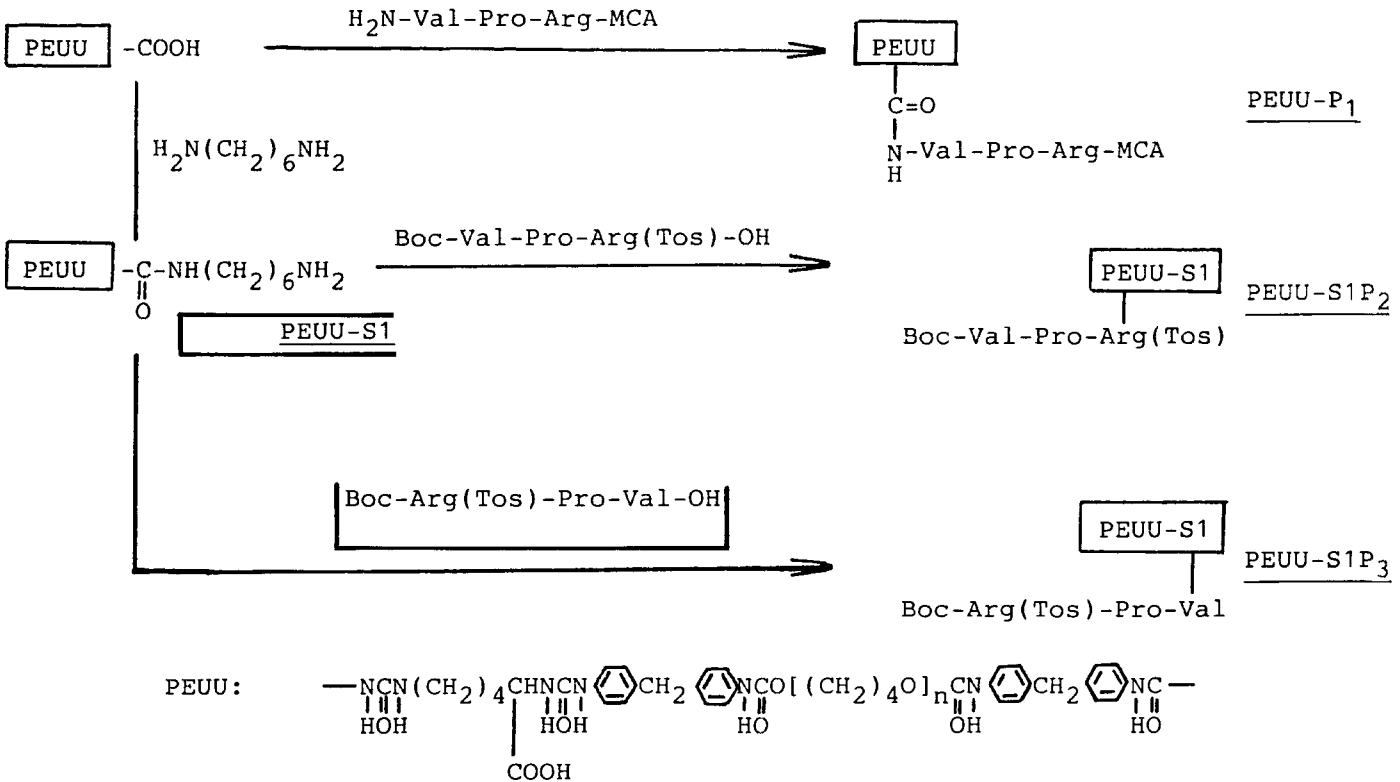


FIG. 3. Scheme of tripeptide immobilization onto polyetherurethaneurea film.

Synthesis of Val-Pro-Arg-Immobilized Polymeric Materials

The scheme of Val-Pro-Arg immobilization to polyurethane is shown in Fig. 3. Boc-Val-Pro-Arg-MCA was purchased from the Peptide Institute. Boc-Val-Pro-Arg(Tos)-OH and Boc-Arg(Tos)-Pro-Val-OH were obtained by solid-phase synthesis.

The synthesis of polyetherurethaneurea (PEUU) carrying carboxyl groups in side chains has been reported [3].

Boc-Val-Pro-Arg-MCA (5 mg) was dissolved in 4 *N* HCl/dioxane (2 mL), and the solution was left standing at room temperature for 30 min. After evaporating dioxane, petroleum ether was added to the residue. The solidified product was dried under vacuum. Complete removal of the *t*-butoxycarbonyl (Boc) group was confirmed by infrared spectroscopy. The PEUU film (5 mm × 5 mm × 10 μm) was immersed in an MES buffer (0.1 *M* MES, pH 4.75, 2 mL) containing the tripeptide (1 wt%) and a water soluble carbodiimide (WSC, 1 wt%), and left overnight at 4°C. The film was then washed with distilled water until the tripeptide was not detected in the washed liquid by ultraviolet spectroscopy. The product is designated PEUU-P_1.

The PEUU film was immersed in an MES buffer (2 mL) containing 1,6-diaminohexane (10 wt%) and WSC (10 wt%) and left overnight at 4°C. The film was then washed with distilled water to yield PEUU with spacer arms (PEUU-S1). By the same procedure as that for PEUU-P_1, the reaction of Boc-Val-Pro-Arg(Tos)-OH yielded PEUU-S1-P_2, and the reaction of Boc-Arg(Tos)-Pro-Val-OH led to PEUU-S1-P_3. The absence of free carboxyl groups on the surface of PEUU-P_1 film was confirmed by determination of carboxyl groups on the surface of PEUU film [3].

Absorption of Thrombin to Val-Pro-Arg-Immobilized PEUU Film and Activity of Adsorbed Thrombin

PEUU or PEUU-P_1 film (0.25 cm × 0.2 cm) was immersed in phosphate-buffered solutions (PBS, pH 7.2) of known amounts of thrombin. The adsorption of thrombin to the film was determined by the decreased fluorescence intensity of the solutions (excitation at 280 nm, emission at 340 nm) and is shown in Fig. 4.

With the same initial concentration of thrombin in solution (0.22 mg/mL), the amount of equilibrium adsorption was the same on PEUU and PEUU-P_1 films, but the adsorption to PEUU-P_1 film was much faster than to PEUU film. This indicates a strong interaction of thrombin with the tripeptide immobilized to the surface of PEUU film.

PEUU or PEUU-P_1 film which adsorbed thrombin was immersed in a ~0.1

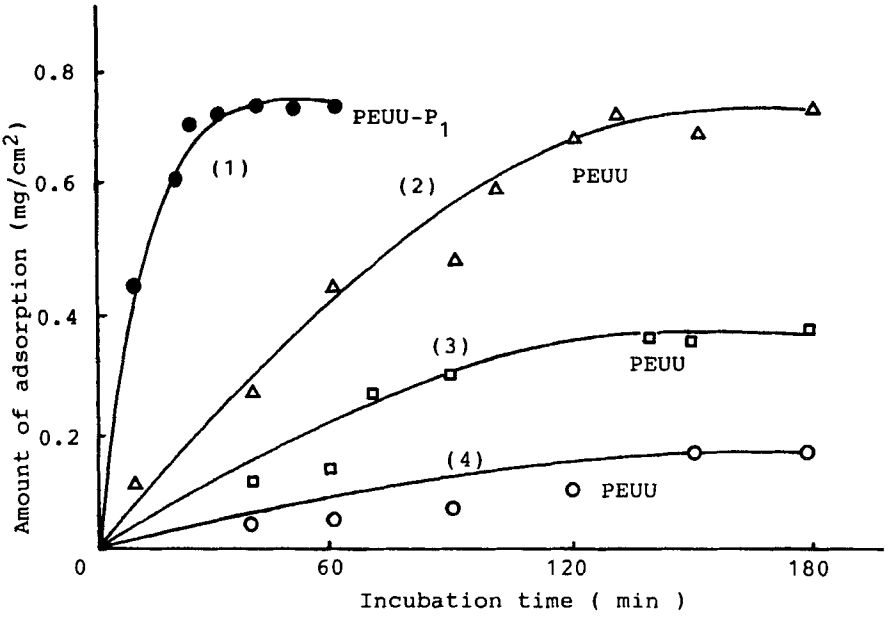


FIG. 4. Thrombin adsorption onto PEUU or PEUU-P_1 film. Thrombin concentration: (1) and (2), 0.22 mg/mL; (3), 0.11 mg/mL; (4), 0.02 mg/mL.

m*M* PBS (pH 7.4) of Boc-Val-Pro-Arg-MCA (1.0 mL) and incubated at 37°C for 10 min. Acetic acid (17%, 1.5 mL) was then added to stop the reaction. The change of thrombin activity before and after adsorption to the films was determined and is shown in Table 2.

Thrombin adsorbed to PEUU film had an activity higher than 70% of that before adsorption, while thrombin adsorbed to PEUU-P_1 film had only ~10% of that before adsorption. This indicates that the binding of thrombin by the PEUU-P_1 film occurs via a specific interaction between the enzyme and the substrate.

Antithrombogenicity of Val-Pro-Arg-Immobilized PEUU

Platelet-poor plasma (PPP) was prepared from canine blood. To PPP (1 mL), PEUU films (0.25 cm X 2 cm) immobilized by different amounts of the tripeptide and a PBS (0.1 mL) containing thrombin of 1.0 NIH U. were added, and

TABLE 2. Activity of Thrombin Before and After Adsorption

Sample	Amount of adsorption, mg/cm^2	Activity before incubation, NIH U.	Remaining activity after incubation, NIH U.	Ratio, %
PEUU	0.73	33.18	24	73
PEUU-P_1	0.73	33.18	4	12

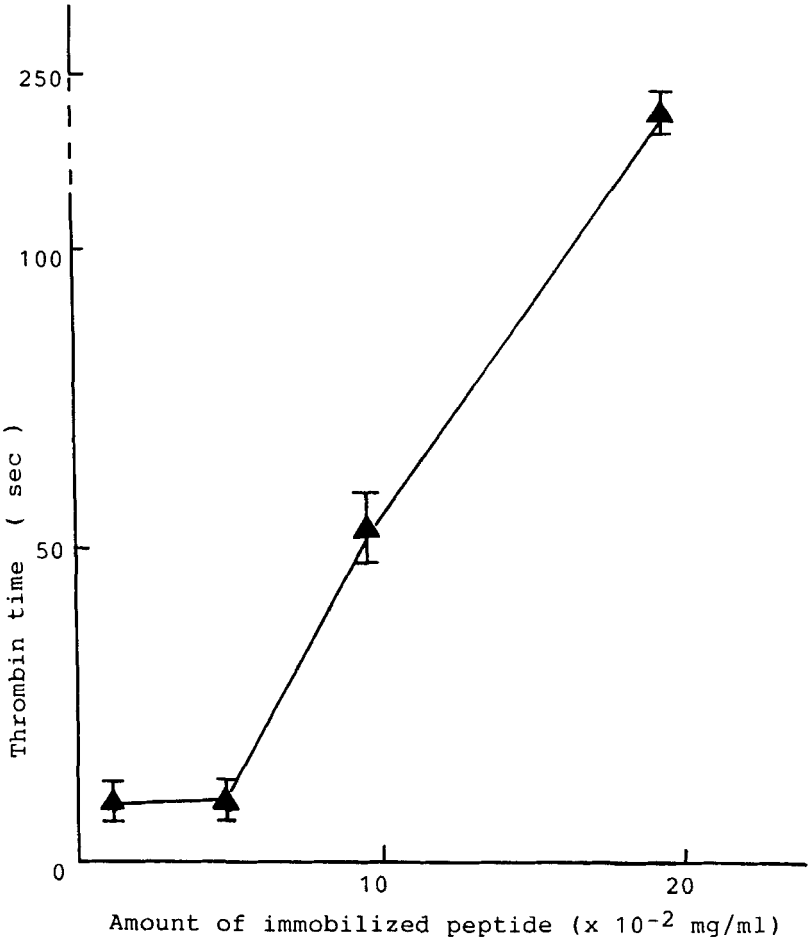


FIG. 5. Thrombin time of PEUU-P_1.

the mixture was kept at 37°C. The time required for clotting was determined and is shown in Fig. 5.

The time required for the formation of fibrin network was prolonged with increasing amount of the tripeptide immobilized. It was found that the antithrombogenicity of PEUU-P_1 film increased due to thrombin inactivation by binding.

TABLE 3. APTT of Several Materials

Sample	APTT, s
Glass	930 ± 17
PEUU	1079 ± 10
PEUU-P_1	1139 ± 7
PEUU-P_2	1068 ± 11
PEUU-P_3	1077 ± 7

A glass plate, a PEUU film, and PEUU films (0.25 cm × 2 cm) immobilized by various amounts of the tripeptide were placed in a mixture of the canine PPP (0.1 mL), which was diluted to 4-fold with tris-buffer, and a thromboplastin buffer solution (containing rabbit brain phospholipid, 0.003 wt% elaidic acid) (0.1 mL) and the mixture was incubated at 37°C for 5 min. $CaCl_2$ solution (0.02 *M*) was then added, and the time until the beginning of clotting, active partial thromboplastin time (APTT), was measured and is shown in Table 3.

APTT was slightly prolonged on PEUU-P_1 film. It was again shown that the antithrombogenicity of PEUU-P_1 film increased due to thrombin inactivation by the specific interaction between the tripeptide and thrombin. On the other hand, APTT's of PEUU-P_2 and PEUU-P_3 films were not much different from that of PEUU. The most important difference among PEUU-P_1, P_2, and P_3 could be the blocking of the Arg side chain. It was considered that the Arg side chain should be free from blocking for the tripeptide to be a substrate of thrombin.

CELL ADHESION ON POLYMERIC MATERIALS IMMOBILIZED BY ACTIVE-SITE PEPTIDE BY CELL-ADHESION PROTEIN

It had been extremely difficult to grow cells and to construct tissues under nonphysiological conditions before the first success of Rose [11]. Since then, a number of successful instances of proliferation of cells directly on synthetic polymers have been reported [12, 13]. It has also been reported that endothelial cells and fibroblast cells grow efficiently on synthetic polymers with fibrin [7], collagen [14, 15], and albumin [16]. Recently, it was discovered

that the active site of cell-adhesion proteins, such as fibronectin and laminin, is constituted of a tetrapeptide, Arg-Gly-Asp-Ser [17]. It occurred to us that truly biocompatible materials may be obtained through tissue construction by cell proliferation on polymer materials on which the tetrapeptides are immobilized in place of the cell-adhesion proteins themselves.

Immobilization of Cell-Adhesion Peptide to Silicone Film

A silicone rubber film (5 mm × 5 mm × 1 mm), produced by Orion Co., was immersed in aminoalkylated polydimethylsiloxane (Chisso Co., PS054, MW 7600) at room temperature for 1 h and then washed with distilled water. The content of amino groups in the blend film was determined by the method reported by Gisin [18] to be 0.509 μmol/cm^2. The blend film was immersed in an aqueous solution containing oxalic acid (10 wt%) and a WSC (3 equivalent) and kept at 4°C overnight. The blend film was then washed thoroughly with distilled water and immersed in 10 wt% aqueous solution of polyallylamine (Nitto Spinning Co., MW 60 000) at 4°C overnight. After this treatment the content of amino groups in the blend film became 0.713 μmol/cm^2. Various amounts of tetrapeptide, Arg-Gly-Asp-Ser, purchased from the Peptide Institute, were dissolved in 10 wt% aqueous WSC solution, and the silicone blend film modified as above was dipped in the peptide solution at 4°C overnight. The film was then washed with distilled water until no extract was obtained.

The amount of immobilized peptide was determined using a calibration curve which was obtained for Boc-Ser-immobilized silicone film. Various amounts of Boc-Ser were linked to amino groups on the surface of the modified silicone film, and unreacted amino groups were determined by the Gisin method. The amount of immobilized Boc-Ser groups was also determined by Fourier-transform attenuated total reflection infrared (FT-ATR-IR) spectroscopy. A KRS-5 prism was used in the ATR measurement. From the ratio of intensities of amide I absorption (1652 cm^{-1}) and polydimethylsiloxane absorption (1002 cm^{-1}), the molar extinction coefficient was determined (1.21×10^{-5} cm^2/mol). Using this value of the molar extinction coefficient, the amount of immobilized peptide was calculated.

Coating of Silicone Film with Cell-Adhesion Proteins

The modified silicone film was dipped at 37°C for 1 h in PBS of bovine Achilles tendon collagen (Type I, 1 mg/mL) or bovine fibronectin (plasma type, 1 mg/mL), then washed with PBS for 10 s and subjected to measurements.

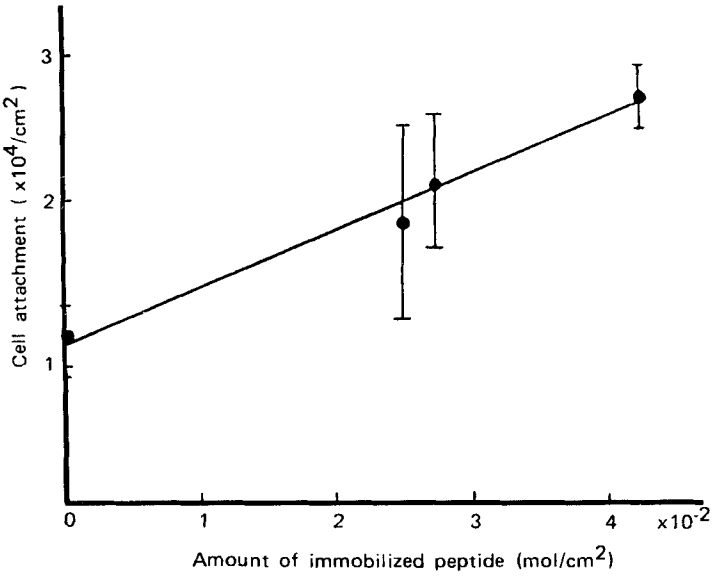


FIG. 6. Cell attachment onto the tetrapeptide-immobilized silicone film.

Cell Adhesion to Peptide- or Protein-Immobilized Silicone Film

Mouse fibroblast cells STO, which were subcultured for 10 d in advance, were collected with PBS (pH 7.2) containing 0.02% trypsin and 0.02% ethylenediamine tetracetate, and radio-labeled with $Na_2\ ^{51}CrO_4$. The radio-labeled cell suspension was brought into contact with a silicone film and incubated in Eagle MEM at 37°C for 1 h. Thereafter the film was washed with PBS for 10 s, and the radioactivity of γ-rays from the film surface was measured to obtain the number of cells adhered. The experimental results are shown in Fig. 6.

The number of cells adhered increased with increasing concentration of peptides linked to the surface of silicone film. It reached $2.73 \times 10^4/cm^2$ when the concentration of immobilized tetrapeptide was 4×10^{-2} mol/cm^2. When collagen or fibronectin was immobilized as described above, the number of cells adhered was $2.25 \times 10^4/cm^2$ or $2.27 \times 10^4/cm^2$, respectively. It was therefore suggested that the number of cells adhered would be increased further with a further increase of immobilized peptides.

The advantage of immobilizing biologically active peptides in place of proteins would be threefold. First, the accumulation in a high density of biologically active site on a film surface is possible. Second, immobilized peptides are

more resistant to changes of temperature and pH than proteins. Third, novel functions such as cell separation are expected, according to the nature of the carrier macromolecule.

REFERENCES

[1] S.-K. Liu, Y. Ito, M. Sisido, and Y. Imanishi, *Polym. Prepr., Jpn., 35,* 534 (1986).
[2] Y. Ito, M. Sisido, and Y. Imanishi, *J. Biomed. Mater. Res., 20,* 1017 (1986).
[3] Y. Ito, M. Sisido, and Y. Imanishi, *Ibid., 20,* 1157 (1986).
[4] Y. Ito, Y. Imanishi, and M. Sisido, *Biomaterials,* In Press.
[5] C. L. Van Kampen, D. F. Gibbons, and R. D. Jones, *J. Biomed. Mater. Res., 13,* 517 (1979).
[6] L. S. Liu, Y. Ito, M. Sisido, and Y. Imanishi, *Polym. Prepr., Jpn., 35,* 536 (1986).
[7] W. E. Burkel, D. W. Vinter, J. W. Ford, R. H. Kahn, L. M. Graham, and J. C. Stanley, *J. Surg. Res., 30,* 305 (1981).
[8] A. J. Johnson, D. L. Kline, and N. Alkjaersig, *Thromb. Diath. Haemorrh., 21,* 259 (1969).
[9] Y. Imai and Y. Nosé, *J. Biomed. Mater. Res., 6,* 165 (1972).
[10] Y. Iguchi, Y. Ito, Y. Imanishi, and M. Sisido, Paper Presented at the 8th Annual Meeting of Japanese Society for Biomaterials, Tokyo, November 13, 1986.
[11] G. G. Rose, *J. Cell Biol., 32,* 89 (1967).
[12] W. L. Chick, A. A. Like, V. Lauris, P. M. Galletti, P. D. Richardson, G. Panol, T. W. Mix, and C. K. Colton, *Trans. Am. Soc. Artif. Intern. Organs, 21,* 8 (1975).
[13] S. Nagaoka, T. Suzuki, S. Kobayashi, M. Itoga, and H. Tanzawa, *Maku, 3,* 59 (1978).
[14] I. V. Yannas and J. F. Burke, *J. Biomed. Mater. Res., 14,* 65 (1980).
[15] M. Matsunobu, Y. Shimizu, H. Wada, H. Yamamoto, T. Teramatsu, H. Hino, and S. Okamura, *Jinko Zoki, 7,* 168 (1978).
[16] M. F. Sigot-Luizard, D. Domurado, M. Sigot, R. Guidoin, C. Gosselin, M. Marois, J. F. Girard, M. King, and B. Badour, *J. Biomed. Mater. Res., 18,* 895 (1984).
[17] M. D. Pierschbacher and E. Ruoslahti, *Nature, 309,* 30 (1984).
[18] B. F. Gisin, *Anal. Chim. Acta, 58,* 248 (1972).