

Poly (urethane-g-N-vinyl pyrrolidone) 의 혈액적합성

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Blood Compatibilities of Poly(urethane-g-N-vinyl pyrrolidone)

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Abstract: The reactive double bonds were introduced to the backbone of polyurethane (PU) and then N-vinyl pyrrolidone (NVP) which has hydrophilicity and biocompatibility was grafted to improve the blood compatibility. Also, the mechanical properties were varied by adjusting the amount of double bonds introduced. When a few double bonds were introduced to the backbone of PU, mechanical strength was little weakened in spite of the increase of grafting efficiency. Blood compatibility was evaluated by the film depositing method and the number of platelets was measured by Coulter Counter. Appearance of platelets adhered on the polymer surfaces was observed by scanning electron microscopy. Contact angles were measured by means of captive bubble technique and interfacial free energies of water-solid interfaces were calculated to estimate the thromboresistance. It was consequently found that the interfacial free energy approached conspicuously to zero with the increase of degree of swelling.

INTRODUCTION

There are various factors that cause the thrombus formation when a foreign material comes in contact with the blood. The most important problem with the foreign material is platelet adhesion, followed by blood coagulation. When a material is inserted into the human body, the first event which takes place is the adsorption of plasma proteins from the blood onto the material surfaces. Subsequently, platelets adhere to the proteinated surface and then thrombus formation takes place¹.

Development of antithrombogenic materials has been generally empirical, because the precise clotting mechanism between blood and foreign materials is not known well. Therefore, there are a number of hypotheses for blood compatibility. Among them, an important method to obtain more thromboresistant polymer is the modification of

polymers by grafting into them with hydrophilic monomers which have biocompatibilities. The improvement of blood compatibilities has reported for such materials and the interactions between blood and surfaces of materials have also reported². Hydrophilic polymers like poly(vinyl pyrrolidone) are known to be biocompatible, but they have poor mechanical properties when they are swollen in water. Therefore, many researches have been done about the grafting of hydrophilic monomers into polymers with good mechanical properties³⁻⁵.

In this work, blood compatibilities were studied on grafting NVP onto PU which has good mechanical properties. At first, reactive substituents with double bond were introduced into PU backbones and then graft copolymerization was carried out. In order to evaluate the blood compatibilities of graft copolymers, platelet adhe-

sion experiment was performed *in vitro* by the film depositing method in fresh blood.

We investigated the platelet adhesion according to variation of water contents and also measured the interfacial free energies between polymer surfaces and water by a captive air and octane method⁶. The variations of mechanical properties of polymers were studied with the water contents and the amount of substituents and graft monomers, respectively.

EXPERIMENTAL

Materials Purified polyurethane (PU; Estane 5707, soft type, Goodrich) was used for graft copolymerization as backbone polymer and dried in vacuum oven for 2 days. N-vinyl-2-pyrrolidone (NVP; Nakarai Chemicals) was purified by distillation under reduced pressure. Azobisisobutyronitrile (AIBN; Wako Pure Chemicals) was purified by recrystallization. Dimethyl formamide (DMF; Shinyo Pure Chemicals) was dried by CaH₂ and distilled under reduced pressure. Allyl bromide (Aldrich) and sodium hydride (Wako Pure Chemicals, abs. 60% in oil) were used without further treatment.

Synthesis of Allyl-substituted Polyurethane For the purpose of introducing the reactive double bonds to PU, the carbamate protons of PU backbones were substituted by allyl groups through a two-step reaction. The substitutions were carried out as follows.

The substitution reactions were carried out in a solvent medium of DMF under dried nitrogen⁷. The required amount of sodium hydride was added to the DMF solution of PU cooled to about 0°C. Sodium hydride was used as a strong base to remove a hydrogen from the urethane bond. The metallated PU formed homogeneous solutions in DMF and the end of the metallation reaction was noted by appearance of yellowish green colour of polymeric anions. Allyl bromide was then added to form allyl-substituted PU.

Introduced allyl groups served as polyinitiating sites for the growth of graft copolymers. The

amount of substituted allyl group was controlled by the concentrations of NaH added. The proof of substitution was established by IR spectroscopy. The absorption band of the -NH bond at 3340 cm⁻¹ is clearly reduced in intensity with substitution degree.

Preparation of Graft Copolymer Grafting reactions were carried out in a three neck flask, placed in a constant temperature bath (70°C) under nitrogen atmosphere. The appropriate amounts of NVP were graft copolymerized onto allyl-substituted PU using AIBN as an initiator. The concentration of AIBN was 8.85 × 10⁻³ mole/L and the reactions were allowed to proceed for 80 and 120 minutes, respectively.

After the completion of reaction, the ingredients were poured into cold methanol/water mixture and precipitated. The product was filtered and homopolymer was extracted with water by the Soxhlet extraction apparatus for 2 days.

The various conditions which were performed in graft copolymerization and grafting efficiencies are shown in Table 1. Grafting efficiency was estimated gravimetrically from the weight of grafted monomer and base polymer.

Grafting efficiency (%) =

$$\frac{\text{weight of grafted monomer}}{\text{weight of base polymer}} \times 100$$

Degree of Swelling Degree of swelling Hw (%) of membranes were measured in pseudo-extracellular fluid (PECF) at 37°C and defined by

$$Hw(\%) = (W_1 - W_0)/W_0 \times 100$$

where W₀ is the weight of dried sample and W₁ is the weight in fully swollen state. The composition of PECF is shown in Table. 2.

Mechanical Properties in Wet State Tensile strength and elongation of the membranes in wet state at 37°C were measured by Instron (Toyo Baldwin UTM-4-100) at a cross head speed of 5 mm/min. Every specimen with 10mm width, 40 mm span length and about 40 μm thickness was used for measurement after fully swollen in PECF solution.

Platelet Adhesion Test Platelet adhesion test for

Table 1. Preparation of Graft Copolymers

	NaH (mol)/1g PU	NVP (mol/L)	reaction time (min)	grafting eff. (%)
PU-1-GV-1		0.80	80	11.25
PU-1-GV-2	1.04×10^{-3}	0.96	80	10.25
PU-1-GV-3		1.05	80	21.25
PU-1-GV-4		1.20	80	40.00
PU-2-GV-1	0.52×10^{-3}	0.75	120	19.70
PU-2-GV-2		1.20	120	27.50
PU-3-GV-1	0.26×10^{-3}	0.75	120	30.25
PU-3-GV-2		1.20	120	35.50

Table 2. Preparation of Pseudo-Extracellular Fluid (PECF)

	*Extracellular fluid of blood (meqv./L)	Pseudo-extracellular fluid (meqv./L)
Na	142	145
K	4	5
Ca	5	—
Mg	3	—
NH ₄	3	—
Cl	103	118
HCO ₃	27	30
HPO ₄	3	2

*Extracellular fluid; Textbook of Surgery, Sabiston

graft copolymers was performed by the film depositing method⁸ with whole human blood. The area of film used was 2.25 cm². After fresh human whole blood was poured into EDTA coated bottle, the fully swollen films by PECF were dipped into whole blood. After the contact time of 45 seconds, the films were washed carefully with saline and dipped into 1.25% glutaraldehyde solution for fixation. After fixation with glutaraldehyde, they were freeze-dried. And then, the shapes of adhered platelets on the surfaces were investigated by the scanning electron microscopy. And the number of platelets which remained in the sampling bottle was calculated by Coulter Counter (Model-S-Plus).

Contact Angle Measurements An Elma Model G-1 goniometer was used for the contact angle measurement⁶. The captive bubble technique was used to measure the contact angles of air and octane bubbles in water. All polymer samples were

swollen in distilled water before the contact angle measurements. Bubbles of air or octane were formed and distributed on the underwater polymer surfaces using a microsyringe. The bubble volume was 1-2 μ l and ten measurements were taken for each specimen with a deviation of $\pm 1^\circ$ deg. at room temperature.

RESULT AND DISCUSSION

Mechanical Properties of Membranes Tensile strength and elongation of graft copolymers were measured as a type of membrane swollen in PECF at 37°C. Mechanical properties in wet state of membranes are listed in Table 3. In this study, the number of grafting sites was controlled by degree of sodium metallation. The length of the grafted chain was also varied with the amount of monomer added.

If NVP content of graft copolymer increases, the degree of swelling is increased and tensile strength is decreased greatly. Elongation is also diminished to some extent. From the result, it was revealed that PU-3-GV-1 (wt. fraction; 0.232) had relatively better mechanical properties than others. The influence of the degree of swelling to the mechanical properties is illustrated in Fig. 1.

When the degree of sodium metallation was lower, polymer had better mechanical properties. Especially, in the case of PU-3-GV-2, its mechanical properties were much more superior to those of PU-1-GV-4 even though its grafting efficiency was as much as that of PU-1-GV-4.

Table 3. Mechanical Properties of Polymer Films Swollen in PECF at 37°C

	Wt. Fraction of NVP in copolymer	Degree of swelling (%)	Tensile strength (Kg/cm ²)	Elongation (%)
PU-1-GV-1	0.093	13.8	69	262
PU-1-GV-2	0.101	14.2	67	260
PU-1-GV-3	0.175	20.6	63	255
PU-1-GV-4	0.286	35.9	37	160
PU-2-GV-1	0.165	16.8	72	239
PU-2-GV-2	0.216	22.3	68	231
PU-3-GV-1	0.232	17.4	109	278
PU-3-GV-2	0.262	26.9	82	225
PU	—	0.8	220	280

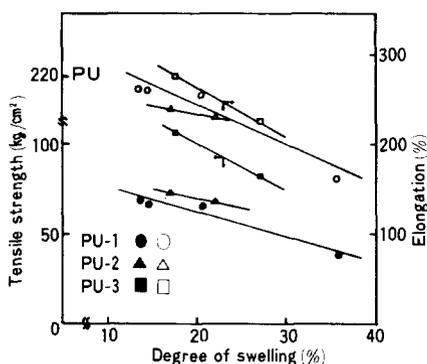


Fig. 1. Mechanical properties of graft copolymers as a function of degree of swelling.

From the result, we found that mechanical properties of the samples were influenced especially by the number of grafting sites though its strength was weakened a little as its grafting efficiency was increased. Therefore, optimum state that shows large degree of swelling and good mechanical properties is obtained by diminishing the number of grafting sites and by increasing the amount of grafted monomers.

Platelet Adhesion The result of blood tests carried out by the film depositing method was shown in Fig. 2. In this experiment, blood was used in a moment after extraction from man. The number of platelets of normal human blood was about 320,000/mm³. We found that, as shown in Fig. 2,

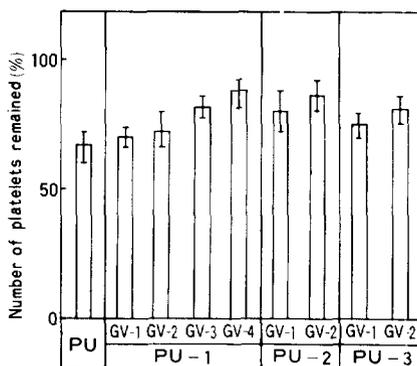


Fig. 2. Relative number of platelets remained in the sampling bottle for the various samples.

graft copolymers were more improved than PU in thromboresistance, and that PU-1-GV-4, PU-2-GV-2 and PU-3-GV-2 were a little superior to others. The relative number of platelets adhered on the polymer surfaces according to the degree of swelling was shown in Fig. 3. In the case of PU-1-GV-4 (36%) whose degree of swelling was large, platelet adhesion happened in a little degree. As hydrophilicity of polymer was increased, platelet adhesion was decreased comparatively.

The shapes of adhered platelets on the polymer surfaces were investigated at magnification of 5000 by SEM and were illustrated in Fig. 4. We could see, in the case of PU, the process that inner materials of platelets were released and thrombus

formed, while in the case of PU-1-GV-4, initial step of thrombus formation.

Surface Properties and Thromboresistance Table 4 shows the contact angle data obtained from PU and graft copolymers. And also the surface free

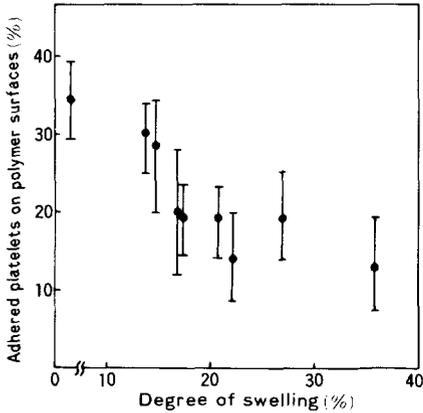


Fig. 3. Relative number of adhered platelets on polymer surfaces as a function of degree of swelling.

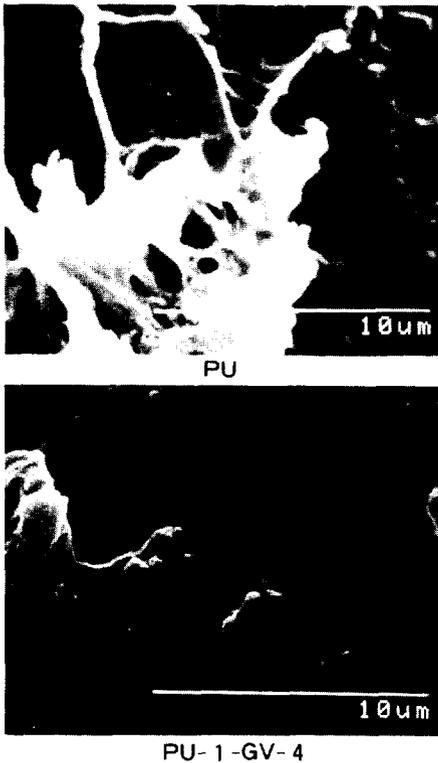


Fig. 4. SEM views of the platelets at the polymer surfaces.

energies listed calculated using the harmonic mean equation as a definition of Andrade, et al⁹. For the calculation of γ_{sw} interface free energy between water and solid surface, contact angles of octane and air bubble were measured by captive bubble technique in the water.

From the Young's equation,

$$\gamma_{sv} - \gamma_{sw} = \gamma_{wv} \cos \theta \dots\dots\dots (1)$$

where γ_{sv} and γ_{sw} are the interfacial free energies between solid surface and water vapor and between solid surface and water, respectively. θ is the contact angle of air and $(\gamma_{sv} - \gamma_{sw})$ is called the adhesion tension. From the contact angle of octane, I_{sw} , polar interaction between solid surface and water is

$$I_{sw} = 50.5 (1 - \cos \theta) \dots\dots\dots (2)$$

If surface free energy between solid surface and water vapor consists of only two components, i.e., dispersive (γ_{sv}^d) and polar (γ_{sv}^p), then

$$\gamma_{sv} = \gamma_{sv}^p + \gamma_{sv}^d \dots\dots\dots (3)$$

And γ_{sv}^d and γ_{sv}^p are

$$\gamma_{sv}^d = \left(\frac{(\gamma_{sv} - \gamma_{sw}) - I_{sw} + 72.1}{9.3} \right) \dots\dots\dots (4)$$

$$\gamma_{sv}^p = I_{sw}^2 / 4 \gamma_{wv}^p \dots\dots\dots (5)$$

Thus γ_{sw} is calculated as follows,

$$\gamma_{sw} = \gamma_{sv} + \gamma_{wv} - 2(\gamma_{sv}^d \cdot \gamma_{wv}^d)^{\frac{1}{2}} - I_{sw} \dots\dots\dots (6)$$

where γ_{wv} is 72.1 erg/cm², γ_{wv}^d is 21.6 erg/cm² and γ_{wv}^p is 50.5 erg/cm².

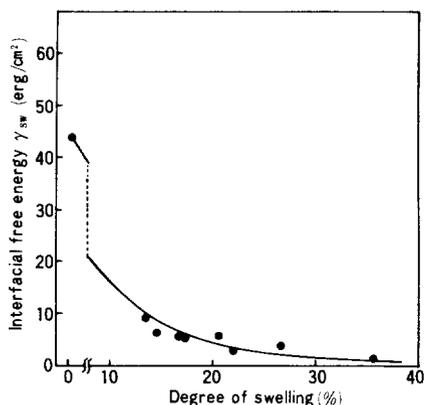
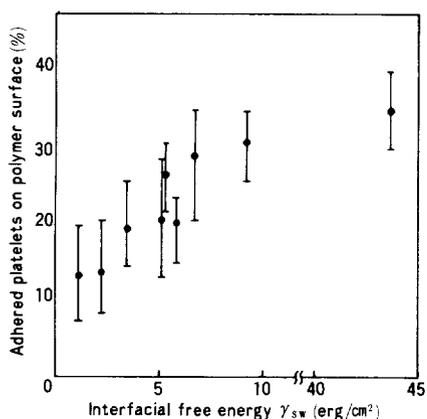
From the result, we can see that the interfacial free energy, γ_{sw} depends on the degree of swelling. And the dependency is shown in Fig. 5. γ_{sw} promptly approach to zero for the degree of swelling of 20%. Therefore, it was suggested that the surfaces of graft copolymers may have low interfacial free energies with water.

Relative number of adhered platelets on polymer surfaces were investigated as a function of interfacial free energy γ_{sw} and presented in Fig. 6. When blood contacts with polymer surfaces, platelet adhesion was diminished with the decrease of interfacial free energy γ_{sw} .

In this paper, it was shown that the smaller the interfacial free energy between water and poly-

Table 4. Contact Angles and Interfacial Parameters for Graft Copolymers and PU

Sample	Degree of swelling (%)	θ (deg) (octane)	θ (deg) (air)	$\gamma_{sv} - \gamma_{sw}$	I_{sw}	γ_{sv}^d	γ_{sv}^p	γ_{sv}	γ_{sw}
PU	0.8	75.5	50	18.1	18.0	60.1	1.6	61.7	43.7
PU-1-GV-1	13.8	40.5	108.5	54.8	66.5	42.2	21.9	64.1	9.3
PU-1-GV-2	14.2	29.8	118.5	62.6	74.6	41.7	27.6	69.3	6.7
PU-1-GV-3	20.6	49.3	110.8	47.0	68.4	29.7	23.2	52.9	5.9
PU-1-GV-4	35.9	29.7	136	62.6	86.8	26.5	37.3	63.8	1.2
PU-2-GV-1	16.8	30.3	121	62.3	76.5	38.7	29.0	67.7	5.4
PU-2-GV-2	22.3	47.5	140	48.7	89.2	11.6	39.4	51	2.2
PU-3-GV-1	17.4	40.6	116	54.7	72.6	34.0	26.1	60.1	5.4
PU-3-GV-2	26.9	29.0	82.3	65.9	82.3	35.9	33.5	69.0	3.5

**Fig. 5.** Interfacial free energy (γ_{sw}) between polymer surfaces and water as a function of degree of swelling.**Fig. 6.** Relative number of adhered platelets on polymer surfaces as a function of interfacial free energy (γ_{sw}).

mer surface, the better the blood compatibility. Mechanical properties were also investigated. As a result, the fewer grafting sites graft copolymer has, the better mechanical properties.

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