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Chapter 1

Synthesis of New Biocompatible Polymers and Fabrication of Nanosheets

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Additional information is available at the end of the chapter

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1. Introduction

Synthetic polymers have been investigated for the applications in the medical field as biomaterials, and used for processing biomedical devices and artificial organs which could be used in living organs. However, most of the polymers are not suitable for a long-term implantation when the materials contact with flowing blood or internal organs, because the material surface could not avoid the initiation of the process leading to thrombosis. Therefore, the development of the materials, which are continuously showing a stable biocompatibility during the long-term use, is desired for the advanced medical devices. For example, segmented polyurethanes have been widely used in practical applications for medical devices due to their high mechanical strength and biocompatibility [1, 2].

On the other hand, the phosphorylcholine (PC) group is a polar component of phospholipid molecules, which cover the surface of cell membranes. It has been well known that synthetic polymer materials containing PC group exhibit biocompatibility including nonthrombogenicity. Firstly, Ishihara *et al.* has been developed 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer as an excellent biocompatible material, which efficiently reduces the adhesion of cells and proteins to the polymer surface [3 – 7]. The design of the MPC polymer was inspired by the chemical structure of the phospholipid polar group in biomembranes. Then, in recent years, the MPC polymer has been widely applied in biological and medical fields. Furthermore, the applications of MPC polymer to medical devices and other uses have been greatly advanced in these years [8 – 15]. However, most of MPC polymers do not possess the thermal stability and the mechanical strength, which were derived from the polymethacrylate type main chain. Then, if these physical properties of MPC polymers improved satisfactorily while maintaining the excellent biocompatibility, novel biocompatible polymer materials could be developed.

In these years, we have succeeded in the syntheses of novel diamine and diol monomers containing PC moiety for the preparations of polyamides, polyimides, polyesters and polyurethanes from these monomers [16 – 23]. It was found that the obtained polymers exhibited the excellent biocompatibility derived from PC unit in addition to the processability, the durability to solvents, the thermal stability and the mechanical strength, which were derived from the main chain components.

By the way, the development of practical biomaterials will desire the collaborations among chemists, biologists, material scientists. We focused in the field of nanotechnology, especially the processing for free-standing ultrathin films consisting of polymers with a thickness less than 100 nm (often called nanosheets), which exhibited the unique properties such as high adhesive strength, flexibility, transparency and smoothness [24-26]. If the nanosheets could be fabricated from such PC-containing polymers, the applications as new biomaterials would be significantly advanced. Then, we attempted to prepare the nanosheets from the obtained copoly(ester-urethane)s and to investigate the physical properties and the biocompatibility of the nanosheet surface.

This chapter covered the subject of our recent study to develop new biomaterials containing a phospholipid moiety. We describe the preparations of aromatic polyimides and segmented polyurethanes containing PC group, which are obtained by polycondensation or polyaddition using PC-containing diamine and diol monomers. In addition, the fabrication of ultra-thin films, so called nanosheets, composed of these PC-containing polymers is described in detail. The obtained nanosheets exhibited the high adhesive strength, indicating that the nanosheets could conform closely to the desired surfaces due to their exquisite flexibility and low roughness. In this chapter, the physical properties such as thermal stability, biological function as blood compatibility, and surface property of the obtained polymers and nanosheets are discussed to reveal the possibility of a new biocompatible polymer material.

2. Preparations of polymer materials

2.1. Syntheses of monomers containing PC group

In order to prepare polyamides, polyimides and polyurethanes, we have investigated the syntheses of diamine and diol monomers containing PC moiety. At first, the synthesis of 2-(3,5-diaminophenylcarbonyloxy)ethyl phosphorylcholine (DAPC) was carried out to prepare PC-containing polyamide [16]. Then, copolyamides were prepared by the polycondensation of DAPC with isophthaloyl chloride and another diamine comonomer. It was revealed that the obtained copolyamide films exhibited the excellent blood compatibility. These results would be due to the PC unit located at the surface of the polymer film, where the surface is covered with PC unit, and the interaction between the polymer surface and blood ingredients such as cells and platelets is very weak. However, the molecular weight and the PC content of copolyamides from DAPC were not enough to produce a self-standing film and to exhibit the higher biocompatibility, respectively, which would be due to the low reactivity and also the highly hygroscopic property of DAPC. Thus, we have developed a new structure of high

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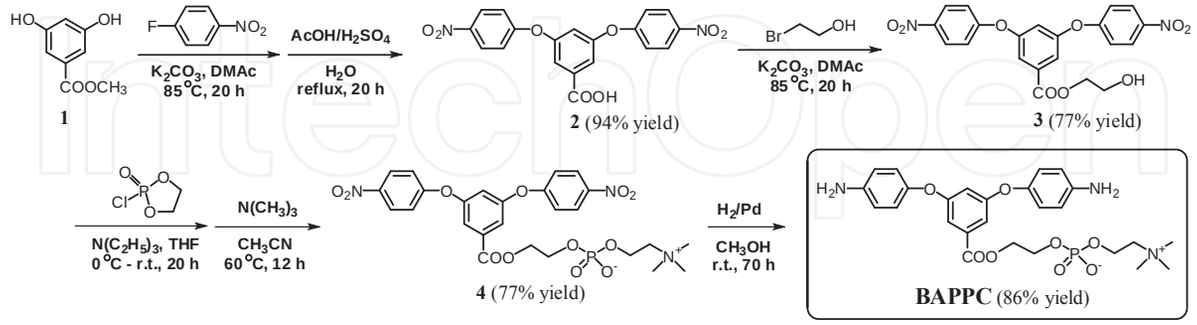
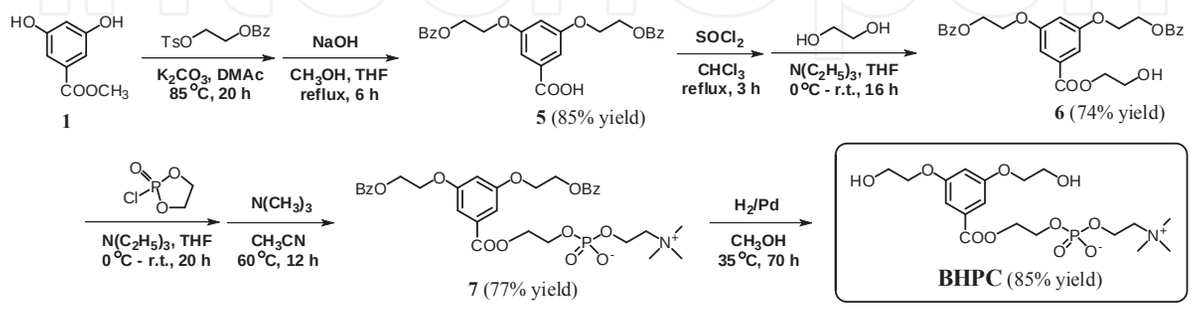
molecular weight polymer in order to create the practical biomaterials for several applications, which exhibit the excellent biocompatibility in addition to the processability, the durability to solvents, the thermal stability and the mechanical strength [19].



Sheme 1. Synthesis of diamine monomer containing PC moiety (BAPPC).

For this purpose, we designed a new diamine monomer containing PC group, 2-[3,5-bis(4-aminophenoxy)phenylcarbonyloxy]ethyl phosphorylcholine (BAPPC). BAPPC is expected to show the higher reactivity in the polymerization than DAPC, which would be due to the relatively higher reactive amino groups on *p*-position of phenoxy groups. The synthetic route of BAPPC is outlined in Scheme 1. The compound **3** was prepared as an intermediate, which were obtained by the esterification of **2** with 2-bromoethanol. Then, the reaction of **3** with 2-chloro-2-oxo-1,3,2-dioxaphospholane (COP) yielded a phospholane compound. The purification of the product by a silica-gel column chromatography was difficult because it was easily hydrolyzed. Therefore, the extraction of the crude products with chloroform followed by washing with distilled water gave the pure phospholane compound. Finally, BAPPC (melting point=109°C) was obtained by opening the cyclic phosphoric ester moiety with trimethylamine, followed by the reduction of the nitro groups of **4** with H_2 catalyzed by Pd. Although the several reaction steps are necessary to prepare this monomer, all of the reaction steps proceeded smoothly in high yields [18].



Sheme 2. Synthesis of diol monomer containing PC moiety (BHPC).

On the other hand, the synthesis of diol monomer containing PC unit was investigated to prepare polyurethanes or polyesters [21]. The synthetic route of the desired diol monomer, 2-(3,5-bis(2-hydroxyethoxy)benzoyloxy)ethyl phosphorylcholine (BHPC), is outlined in Scheme 2. The terminal benzyl groups of compounds **5**, **6** and **7** were introduced as a protection group of the diol moiety. The key intermediate, **6**, was synthesized by esterification of **5** with ethylene glycol, and the incorporation of the PC group was achieved by the reaction of **6** with COP, followed by the ring-opening reaction of the cyclic phosphoric ester moiety with trimethylamine. Finally, deprotection of the benzyl groups of **7** by Pd-catalyzed hydrogen reduction with H_2 gas to afford the desired diol monomer, BHPC. This reaction proceeded quantitatively to give the pure product of BHPC as a white solid (melting point=34°C), although it was so hygroscopic that the obtained solid softened when exposed to moisture.

2.2. Syntheses and properties of polyimides

In these years, we have achieved the syntheses of polyamides, poly(urethane-urea)s and poly(amide-ester)s containing PC moiety by polycondensation or polyaddition using the novel diamine monomer, BAPPC, and investigated the physical and biological properties of the obtained polymers, as described in our literatures [18 – 20]. These aromatic polymers containing PC group showed the thermal stability up to *ca.* 250°C, where the thermal degradation of PC component would started over 200°C that was confirmed by the thermogravimetric analysis of polymers. The heat resistance of these PC-containing polymers over 200°C is enough to use for biomaterial devices, for example, for the thermal sterilization process over 150°C. In addition, the tough films could be prepared by solvent casting from poly(urethane-urea)s and poly(amide-ester)s, which were copolymerized with polycarbonate diol as the soft segment, and the elastomeric property was observed in these films [20]. Furthermore, it has been found that these PC-containing aromatic polymers efficiently reduced the adhesion of proteins and platelets, where the number of adhered platelets of PC-containing polymers was reduced in nearly one-tenth amount as compared with that of polymers without PC group. These results indicate that the PC unit plays an important role for the blood compatibility of the polymers. The amount of adhered proteins and platelets decreased as the increase of the content of PC unit in the copolymers, therefore, the composition of the PC unit was a dominant factor in the reduction of the adhesion of proteins and platelets.

In this chapter, we will describe our recent study for the synthesis of polyimide containing PC group as a biocompatible hard material. The desired copolyimides were carried out by the polycondensation of BAPPC and bis(*p*-diaminophenyloxy)benzene (BAPB) with 4,4'-hexafluoroisopropylidene diphthalic anhydride (6FDA), followed by the chemical imidization with triethylamine and acetic anhydride, as shown in Scheme 3. As the acid anhydride, 6FDA was used to make the polyimide soluble in some solvents. Table 1 summarizes the compositions and molecular weights of the obtained copolyimides. Four kinds of copolyimides with PC content were prepared by changing the ratio of BAPPC and BAPB in the feed of polymerization. The obtained copolyimides showed the number-average molecular weights (*M*n) at around 1×10^4.

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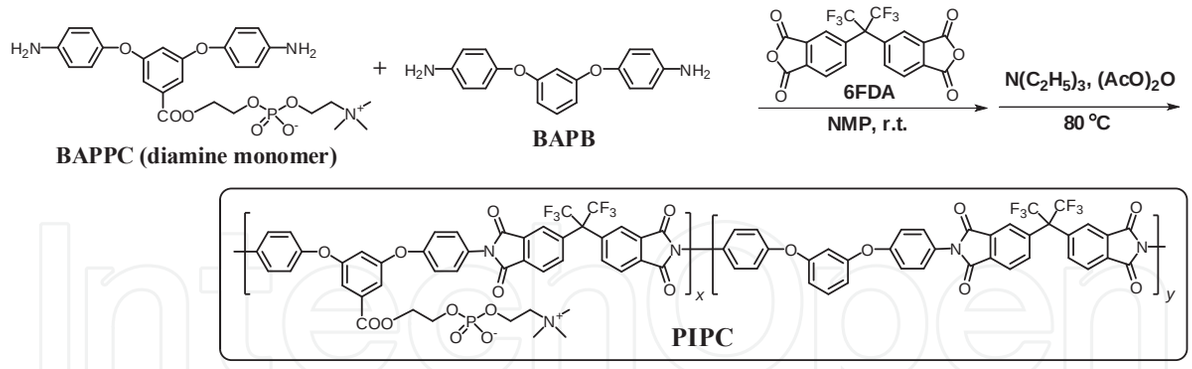


Sheme 3. Preparation of copolyimides containing PC moiety (PIPC).

	BAPPC/BAPB [a]	PC content [b]	*M*n [c]	
Code	(mol %)	(mol %)	(x 10^3)	*M*w/*M*n
PIPC-1	20/80	14	17.4	1.44
PIPC-2	25/75	24	10.4	1.37
PIPC-3	30/70	40	10.1	1.52
PIPC-4	50/50	48	7.29	1.30

[a] The molar ratio of monomers, BAPPC and BAPB, in the polymerazition.

[b] PC contents in the copolymers were estimated by ^{1}H-NMR spectra.

[c] Number-avarage and weight-avarage molecular weights, *M*n and *M*w, were determinated by GPC using 20mM LiBr solution in DMF as an eluent.

Table 1. Composition and molecular weight of polyimides (PIPC).

These copolyimides were soluble in aprotic polar solvents, such as dimethylformamide (DMF), dimethylsulfoxide (DMSO) and *N*-methyl-2-pyrrolidinone (NMP), at room temperature, whereas they were insoluble in water, methanol, ethanol and acetone. This solubility in specific solvents is advantageous in the processing for medical devices, and the insolubility in other solvents enables the material durable to these solvents. For the solubility in some solvents, the solubility of these copolyimides depended on the PC content of polymers. For example, only PIPC-1 in Table 1 was soluble in chloroform and tetrahydrofuran (THF), but PIPC-2, 3 and 4 were insoluble in these low boiling point solvents. By the way, polyimide without PC unit, which was prepared from 6FDA and BAPD, was soluble in chloroform and THF. Therefore, the solubility of these copolyimides decreased with increasing PC content, where the maximum PC content that allowed the solubility in chloroform and THF was 15-20 wt. %. It was speculated that polar PC groups in the side chains would have a strong interaction, which would make the polymer insoluble in these solvent.

On the other hand, it was found by the thermogravimetric analysis that the weight loss of the PIPC series started at *ca.* 250°C, similar to polyamide and poly(urethane-urea) containing PC

group, whereas the decomposition temperature of the polyimide without PC group was over 400°C. In addition, the hard but brittle films were prepared from these polyimides by solvent casting method.

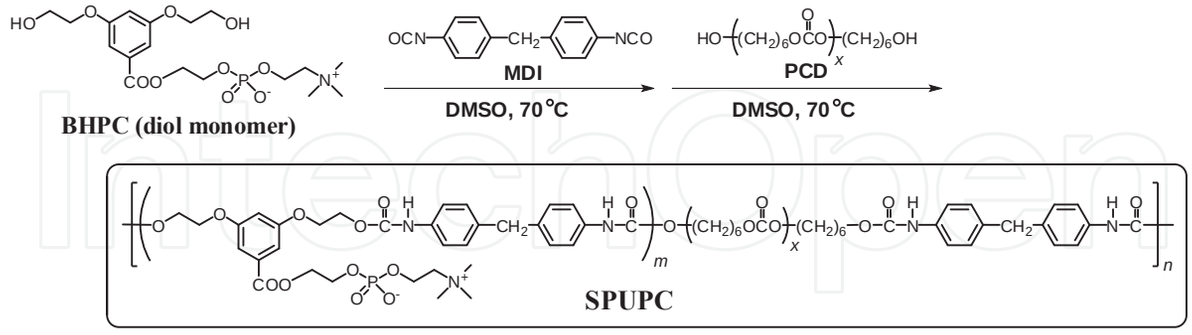
2.3. Syntheses and properties of segmented polyurethane

Segmented polyurethanes generally consist of short alternating blocks of soft and hard segments, and exhibit an elastomeric property. The biocompatibility of segmented polyurethane is thought to arise from the microphase separation of the soft and hard segments. However, the biostability of segmented polyurethane is not suitable for long-term implantation. It has been suggested that the biodegradation and cracking of polyurethane that occurred in vivo was due to the adsorption of proteins, adhesion of macrophages and peroxide formation [27 – 29], which resulted in the reduction of the mechanical strength of segmented polyurethane. Moreover, the soft segment of segmented polyurethane was reportedly degraded by oxygen radicals produced by adherent macrophages [30]. Therefore, several studies of surface or chemically modified segmented polyurethanes have been conducted to improve biostability by reducing the adhesion of cells and proteins [31 – 35]. Ishihara *et al.* have also investigated a polymer composite consisting of segmented polyurethane and MPC polymer to reduce protein adsorption to the polymer surface and to improve the biocompatibility of segmented polyurethane [36 – 41].

In our previous studies, we have prepared segmented poly(urethane-urea)s containing PC group by using the PC-containing diamine monomer, BAPPC, as a coupling reagent in the polyaddition of diols with diisocyanate [19]. The obtained polymers exhibited excellent biocompatibility, the film surface of which efficiently reduced the adhesion of human platelets. In addition, stress-strain measurements revealed that the poly(urethane-urea) films exhibited high elastic mechanical properties, where the Young's modulus increased with increasing PC content. The aim of the next study was to prepare another type of PC-containing polyurethane from diol monomer (BHPC). Cooper and his co-worker have reported that a PC-containing polyurethane could be prepared using glycerophosphorylcholine as a diol monomer [42]. We have designed the BHPC molecule based on the concepts that BHPC would be more hydrophobic than glycerophosphorylcholine and easier to handle as a monomer for polycondensation or polyaddition. We expected that both of the primary hydroxyl groups of BHPC would make the polymer have a high molecular weight due to its higher reactivity than glycerophosphorylcholine with its secondary hydroxyl group. Recently, Khan *et al*. have reported a potential application of poly(carbonate-urethane) as a long-term biomedical implant material due to its resistance to biodegradation and its biocompatibility [43, 44]. Therefore, we selected polycarbonate diol (PCD) to construct the soft segment of PC-containing segmented polyurethane.

As shown in Scheme 4, the syntheses of segmented polyurethanes containing PC group and polycarbonate segment with different contents were carried out by polyaddition of BHPC and PCD with 4,4'-diphenylmethane diisocyanate (MDI). The compositions and the molecular weights of the obtained polymers are summarized in Table 2. The observed PC contents in mol

% were determined by ^{1}H-NMR and were in good agreement with the molar ratio of BHPC and PCD in the feed of polymerizations.



Sheme 4. Preparation of segmented polyurethane containing PC moiety (SPUPC).

	BHPC/PCD [a]	PC content [b]	*M*n [c]	
Code	(mol %)	(mol %)	(x 10^3)	*M*w/*M*n
SPUPC-1	20/80	20	189	1.38
SPUPC-2	30/70	34	266	1.25
SPUPC-3	40/60	41	44.3	4.25
SPUPC-4	50/50	50	92.5	1.91

[a] The molar ratio of monomers, BHPC and PCD, in the polymerization.

[b] PC contents in the copolymers were estimated by ^{1}H-NMR spectra.

[c] Number-avarage and weight-avarage molecular weights, *M*n and *M*w, were determinated by GPC using DMF as an eluent.

Table 2. Compositions and molecular weights of segmented polyurethanes (SPUPC).

The obtained polyurethanes, SPUPC-1, 2 and 3, exhibited a good solubility in aprotic polar solvents such as DMF, DMSO and NMP at room temperature, whereas SPUPC-4 was insoluble in these solvents. In addition, SPUPC-1 and 2 were soluble in the low boiling solvents, chloroform and THF, but SPUPC-3 and 4 were insoluble in chloroform and THF. Therefore, a trade-off relation was observed between the solubility and the PC content of polyurethanes, which was similar to PIPC series. It would be due to the strong interaction of polar PC group in the side chain and the highly polar urethane bond in the main chain. To obtain a soluble polymer with high PC content, a different concept for the molecular design or the polymerization process should be developed the solubility of these PC-containing polymers. Recently, we have developed PC-containing poly(ester-urethane)s, the solubility of which was improved to some extent by the introduction of ester bond in addition to the highly polar urethane component [22, 23].

The flexible and self-standing films could be prepared from these segmented polyurethanes by a solvent casting method using DMSO as a solvent. Then, the elastic mechanical properties were observed for these segmented polyurethane films, where the Young's modulus increased with increasing PC content. Furthermore, the introduction of such a polar phospholipid group was effective in improving the resistance to protein and platelet adhesions on the polymer film, which was the result of surface properties derived from the PC moiety [21].

3. Fabrication of ultra-thin films (nanosheets)

3.1. Preparation and characterization of nanosheets composed of PIPC and SPUPC

Self-standing nanosheets are easily fabricated by a "sacrificial layer method" as depicted in Fig. 1a [26]. The sacrificial layer can be dissolved with appropriate solvents which do not dissolve nanosheets themselves. In parallel, solvents used for dissolving polymers of nanosheets must not dissolve the sacrificial layer. To this end, we selected poly(vinyl alcohol) (PVA) as a water-soluble sacrificial layer to obtain self-standing nanosheets composed of PIPC-1 and SPUPC-2, because these polymers were soluble in chloroform which did not dissolve PVA. A fabrication procedure of PIPC-1 nanosheet is described as follows. First, an aqueous solution of 10 mg/mL PVA was dropped onto a silicon oxide (SiO_2) substrate, which has an extremely flat surface. The substrate was spin-coated at 4,000 rpm for 20 s and then dried. Next, a chloroform solution of 10 mg/mL PIPC-1 was spin-coated on the PVA-coated substrate under the same conditions. When the substrate was immersed into distilled water, the PIPC-1 nanosheet was detached from the substrate due to dissolution of only the PVA layer with water. The obtained PIPC-1 nanosheet was transparent, amazingly flexible, and maintained the size and shape of the SiO_2 substrate (Fig. 1b, left). In fact, the thickness was 42 ± 2 nm and the roughness was nanometer scale. Furthermore, the thickness was easily controlled by adjusting the concentration of PIPC-1 just before spin-coating as shown in Fig. 1c. As described in section 2.2, the solubility of PIPC series is dependent on the content of the PC unit. For instance, PIPC-4 with high PC content was insoluble in chloroform but soluble in the aprotic polar solvents as DMSO. However, the PVA sacrificial layer is dissolved with DMSO. To this end, we can select other component of the water-soluble sacrificial layer, *e.g.* sodium alginate (Na-Alg), which is insoluble in DMSO. According to this technique, we could prepare the self-standing nanosheets composed of SPUPC series [45]. In the case of a chloroform solution of 10 mg/mL SPUPC-2, the thickness of the nanosheet was 66 ± 4 nm. Intriguingly, these nanosheets composed of SPUPC series, which were elastic polymers, tended to shrink after detaching from the substrate as seen in Fig. 1b, right. This tendency suggested that the nanosheet extended on the substrate by the centrifugal force during the spin-coating, and resulted in shrinking due to its elasticity when they were released from the substrate. This tendency was not observed in the nanosheets composed of non-elastic polymers such as PIPC series. In these years, we have prepared the self-standing nanosheets composed of versatile polymers such as polystyrene and poly(methyl methacrylate), etc., and typical biodegradable polymers such as poly(lactic acid), their copolymers and polycaprolactone, etc. [24, 25].

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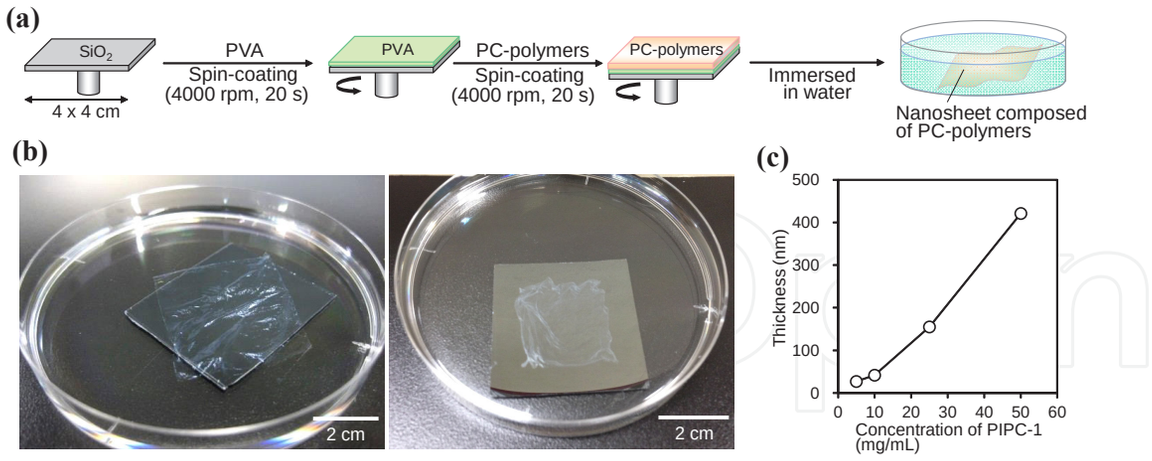

Figure 1. Fabrication of self-standing nanosheets composed of PIPC-1 and SPUPC-2. (a) Fabrication procedure of the nanosheets by spin-coating. (b) Macroscopic images of PIPC-1 (left) and SPUPC-2 (right) nanosheets suspended in water. (c) Relationship between thickness of the PIPC-1 nanosheets and concentration of PIPC-1 solution before spin coating.

We analyzed the mechanical properties of the nanosheets by using a bulging test developed for nanosheets [46]. In fact, the PIPC-1 nanosheet with a thickness of approximately 40 nm was physically adhered to a steel plate with a hole as shown in Fig. 2a. The plate was fixed to a custom-made chamber and air was supplied with a syringe pump until bursting the nanosheets. During the analysis, pressure applied to the nanosheets and its deflection was monitored in real time by a differential pressure gauge and a stereomicroscope, respectively. Based on the equations as shown in Fig. 2a, we obtained a strain-stress curve as shown in Fig. 2b. From the slope of the elastic region of the curve, the Young's modulus of the PIPC-1 nanosheet was calculated to be 196 ± 9 MPa. This value was 10-folds lower compared to that of the bulk polyimide films (3-7 GPa), indicating that the PIPC-1 nanosheet was softer than the bulk polyimide film. We have demonstrated that the poly(lactic acid) nanosheets with a thicnkess less than 100 nm represent the same tenency [24]. Mattsson *et al*. have explored the relationship between the glass transition temperature (T_g) and thickness of the ultra-thin films of polystyrene using a Brillouin light scattering method. In fact, T_g of polystyrene films with a thickness of approximately 20 nm was decreased to 37°C compared to that of bulk polystyrene (T_g: 109°C), explaining that the interactions between polymer chains decreased in the ultra-thin films [47]. This may be one of the reasons why the T_g of the PIPC-1 nanosheet would be lower than that of bulk polyimide.

Next, we analyzed the relationship between adhesive strength of the PIPC-1 nanosheets and their thickness with a scratch tester for thin films [48]. As depicted in Fig. 3a, the nanosheets were physically adhered on the SiO_2 substrate, and the surface of the nanosheets were horizontally scratched with a diamond tip under the following conditions; radius of curvature of a diamond tip: 25 μm, scratch length: 100 μm, and scratch rate: 10 μm/s. Critical loads just after detaching the nanosheet from the substrate were monitored. Then, the adhesive strength of the nanosheets was difined as the critical loads divided by the thickness of the nanosheets.

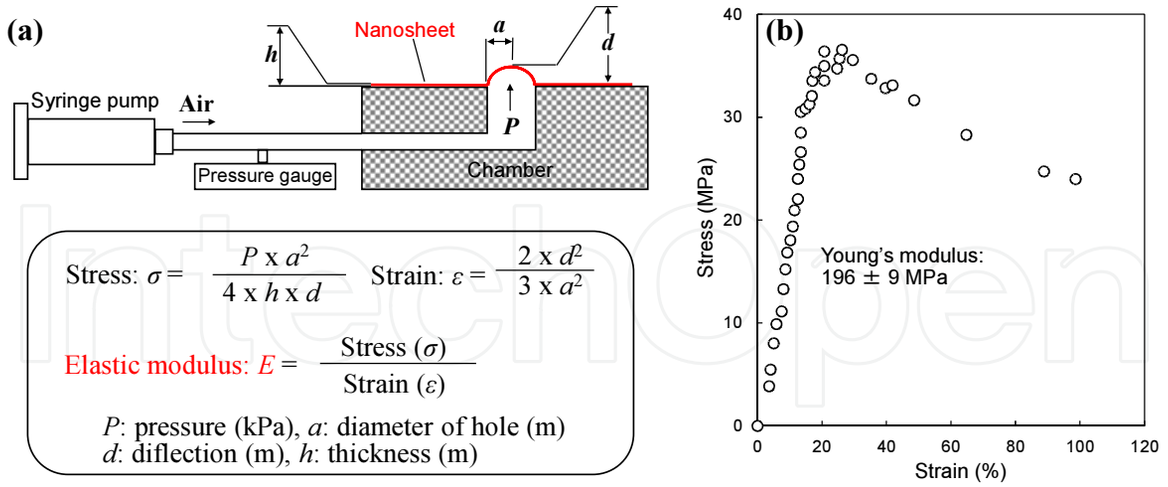

Figure 2. Mechanical properties of the PIPC-1 nanosheet analyzed by a bulging test. (a) Schematic image of the bulging test. (b) Represetative stress-strain curve of the PIPC-1 nanosheet with a thickness of 42 ± 2 nm.

The critical loads of the PIPC-1 nanosheet with thicknesses of 27 and 42 nm were calculated to be (1.6 ± 0.3) × 10^5 and (1.4 ± 0.4) × 10^5 N/m, respectively as shown in Fig. 3b. However, in the region of the thickness over 100 nm, the critical roads were obviously decreased to (0.8 ± 0.2) × 10^5 N/m (thickness: 155 nm) and (0.4 ± 0.2) × 10^5 N/m (thickness: 421 nm). This would be the reason that the nanosheets could conform to the roughness of the substrate due to its flat surface and amazingly flexibility. Actually, these nanosheets can be adhered to various surfaces such as plastics, glasses, steels, and tissues without the utilization of adhesive agents. Once the nanosheets were dried on these surfaces, it was often hard to detach with even washing with water. Consequently, we have demonstrated that the greatest benefit of the nano-thickness is high potential to adhere. This phenomenon has been also observed with the poly(lactic acid) nanosheets with the thicnkesses less than 100 nm [24].

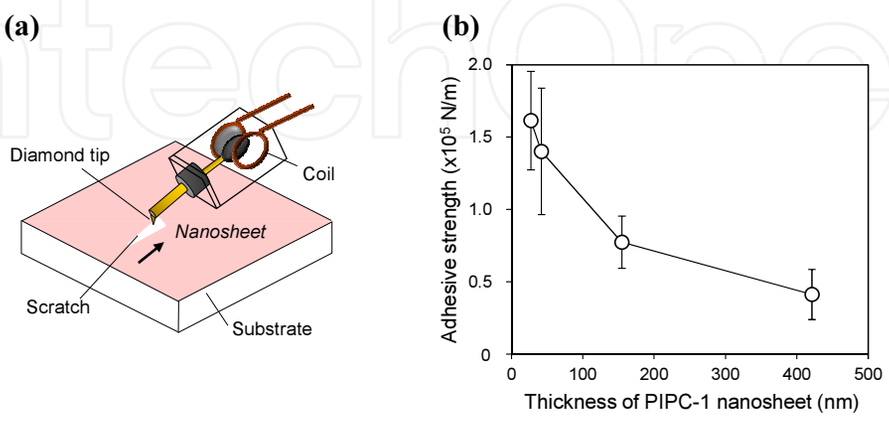

Figure 3. Adhesive strength of the PIPC-1 nanosheet. (a) Schematic image of scratch tester for thin films. (b) Correlation of adhesive strength of the PIPC-1 nanosheet with its thickness.

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3.2. Biocompatibility of nanosheet surface

Platelets are one of blood cells and involved in both normal hemostasis and pathological thrombosis [49]. In development of biocompatible materials with the possibility to contact with blood, what the most critical point is to inhibit non-specific interactions between platelets and the surface of the materials. To this end, we evaluated the blood compatibility of the surface of the nanosheets composed of PC polymers. Poly(ethylene terephtalate) (PET) plates were used as model surfaces, to which the nanosheets were adhered. The nanosheet-coated PET plates were immersed into 0.5 mL of platelet-rich plasma (PRP) obtained from healthy volunteers and incubated at physiological temperature for 2 h. Finally, PRP was removed and the substrates were washed out with phosphate buffered saline. The surface of the plates was observed with a scanning electron microscope. As shown in Fig. 4, platelets with filopodial extensions were non-specifically adhered to the bared PET plate and the nanosheet-coated PET plate without PC units (PI and SPU). PI is a polyimide obtained by the polycondensation of BAPB with 6FDA followed by the chemical imidization, and SPU is a segmented polyurethane obtained by the polyaddition of 3,5-bis(2-hydroxyethoxy)benzene and PCD (molar ratio: 70/30) with MDI. In the case of the PET plates coated with PIPC-1 and SPUPC-2 nanoheets, reduction of platelet adhesion was clearly observed as compared with PET plate and PI/SPU coated plates. Therefore, it was confirmed that the surface of PC-polymer nanosheets exhibited the good blood compatibility. In other words, these results indicate that sealing of the nanosheets could act as a surface modifier to convert the surface property of the PET plates.

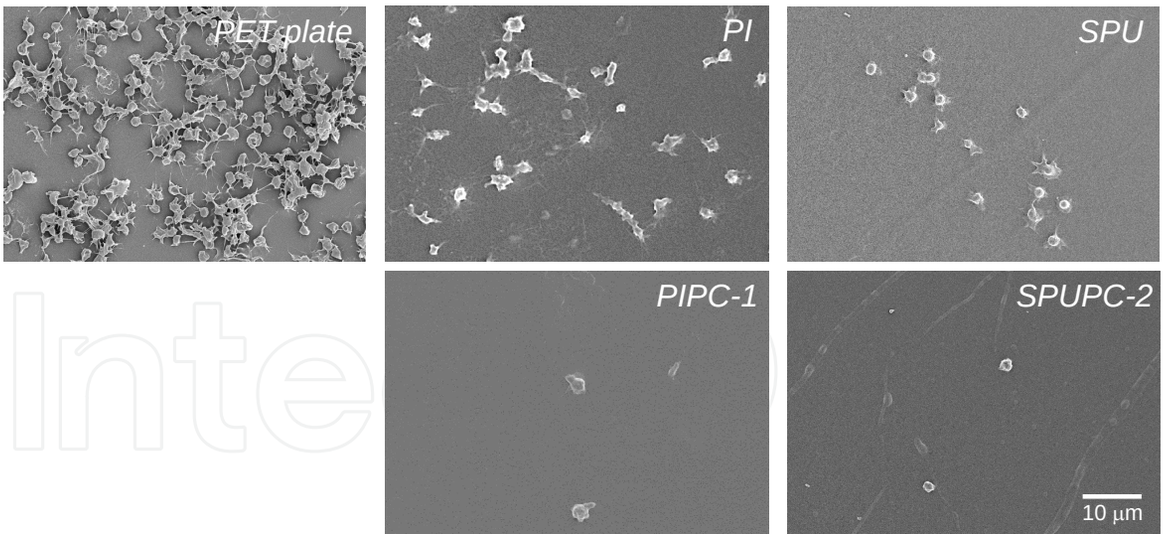

Figure 4. SEM images of nanosheet surfaces with or without PC unit after contact with plateled-rich plasma for 2h at 37°C

3.3. Fragmentation of the nanosheets to coat irregular and uneven surfaces

As described above, we have succeeded in the preparation of the self-standing nanosheets, which represent unique properties such as good adhesiveness, amazingly flexibility and high

transparency. However, such nanosheets possess centimeter size and are only suitable for adhesion to relatively broad surfaces. They are often difficult to adhere to irregular and uneven surfaces because of centimeter size. In our recent study, we have discovered that the fragmented submillimeter-sized nanosheets composed of poly(lactic acid) were adhered to the various surfaces in a spread out configuration that looks like "patchwork" [25, 26]. Once the nanosheets dried on the surface, they were difficult to detach from the surface by even washing with water. Moreover, we have demonstrated that the irregular and uneven surfaces such as a needles and rubbers etc. are effectively coated with the patchwork-like coating of the fragmented nanosheets by just casting or dipping [25, 26]. In this section, we introduce the fragmented nanosheets composed of PIPC and SPUPC series to coat irregular and uneven surfaces and the evaluation of blood compatibility.

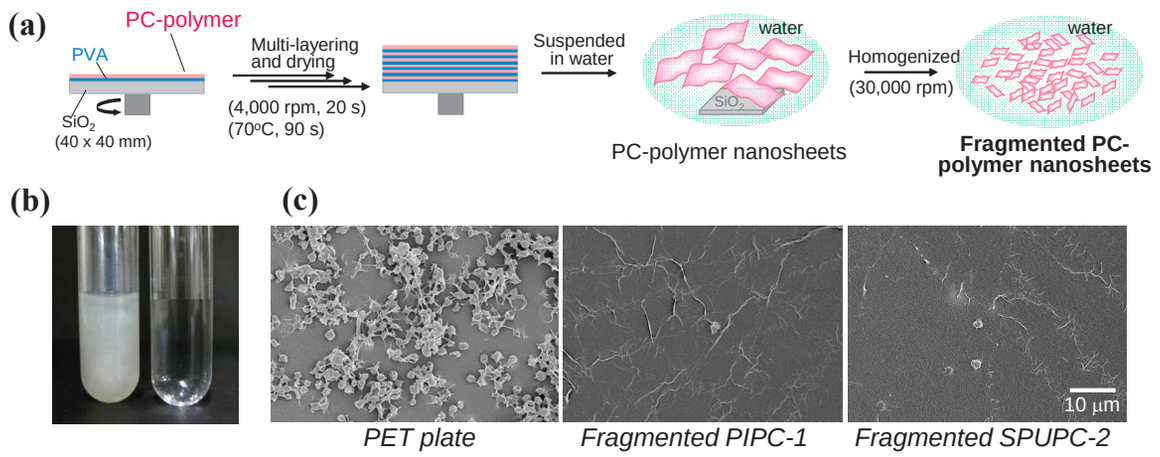

Figure 5. (a) Fabrication of fragmented nanosheets composed od PIPC-1 and SPUPC-2. (b) Macroscopic image of fragmented PIPC-1 nanosheets (left tube)suspended in distilled water. Right tube shows only distilled water. (c) SEM images of fragmented nanosheet surfaces after contact with platelet-rich plasma for 2h at 37°C.

We herein focus on the fragmented PIPC-1 nanosheets as follows. First, we fabricated abundant self-standing nanosheets with centimeter size by a simple multi-layering process of water-soluble PVA and PIPC nanosheets combined with a peeling technique, according to our reports [25, 26]. Concretely, a 100 mg/mL solution of PVA as a water-soluble sacrificial layer was first spin-coated on a SiO_2 substrate at 4000 rpm for 20 s, followed by a drying process as depicted in Fig. 5a. Next, a chloroform solution of 10 mg/mL PIPC-1 was spin-coated on the PVA-coated substrate under the same conditions. Moreover, the multi-layering of PVA and PIPC-1 was repeated twenty times on the substrate. By dissolution of PVA layers in water, tewenty sheets of PIPC-1 nanosheets were obtained. Next, the obtained PIPC-1 nanosheets were fragmented with a homogenzer. When the PIPC-1 nanosheets (size: 40 × 40 mm, thickness: 42 nm) in distilled water were homogenized at 30,000 rpm for 10 min, they were instantly fragmented. The obtained nanosheets were homogeneously suspended in water and the turbidity of the suspension was quite increased as shown in Fig. 5b. In fact, the surface area of one fragmented nanosheet 10 min after homogenizaion was significantly decreased to 6800 ± 208 μm^2, estimating that the average size of the nanosheet was approximately 80 μm. Using the same

prosedure, we also prepared the fragmented nanosheets composed of SPUPC-2 (surface area: 3900 ± 1300 μm^2, thickness: 66 nm).

In order to evaluate the blood compatibility, the fragmented PIPC-1 or SPUPC-2 nanosheets were adhered to a bared PET plate as a model surface. They consisted of a patchwork-like coating in the same manner as the fragmented PLLA nanosheets [25, 26]. The nanosheet-coated PET plates were immersed into PRP and incubated at 37°C for 2 h. As shown in Fig. 5c, very few platelets were adhered to the PIPC-1 and SPUPC-2 coated PET plate. Moreover, some lines were observed on the plates, that correspond to wrinkles (not cracks) formed during drying of patchwork-like coating. In the case of the bared PET plates, abundant platelets were activated and non-specifically adhered. Therefore, we demonstrated that patchwork-like coating with the fragmented nanosheets with PC units acts as an aqueous surface modifier to provide blood compatibility.

4. Conclusions

We have synthesized novel aromatic diamine and diol monomers containing PC group to develop the new biocompatible polycondensation-or polyaddition-type polymers. The obtained polymers exhibited good solubility with aprotic polar solvents and thermostability unlike MPC polymers. Using these polymers, we have succeeded in the fabrication of self-standing nanosheets with a thickness less than 100 nm. The PC-polymer nanosheets exhibited high adhesiveness to the various surfaces, and the surface of adhered nanosheets represented the good blood compatibility based on the platelet adhesion test. Furthermore, we have developed the fragmented nanosheets with submillimeter-size to coat irregular and uneven surfaces by controlling the size of the nanosheets. In fact, fragmented nanosheets were effectively coated with the patchwork-like adhesion behavior by just casting or dipping and provided blood compatibility to the various surfaces. Hence, these nanosheets composed of PC-containing polymers may be great promise as novel coating materials and surface modifiers to provide the biocompatibility to the surface of various medical devices such as catheters, artificial organs, microfluidic devices, etc.

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