

STUDIES ON APATITE FORMATION
ON ORGANIC POLYMERS
BY A BIOMIMETIC PROCESS

MASAMI TANAHASHI

1995

CONTENTS

CHAPTER 1.	GENERAL INTRODUCTION	1
CHAPTER 2.	APATITE FORMATION ON ORGANIC POLYMERS BY A BIOMIMETIC PROCESS USING CaO-SiO_2 -BASED GLASS AS NUCLEATING AGENT	11
CHAPTER 3.	IMPROVEMENT IN ADHESION OF APATITE LAYER TO POLYMER SUBSTRATES BY HCl TREATMENT	31
CHAPTER 4.	IMPROVEMENT IN ADHESION OF APATITE LAYER TO POLYMER SUBSTRATES BY NaOH TREATMENT . .	59
CHAPTER 5.	IMPROVEMENT IN ADHESION OF APATITE LAYER TO POLYMER SUBSTRATES BY GLOW DISCHARGE TREATMENT	87
CHAPTER 6.	APATITE FORMATION ON ORGANIC POLYMERS BY A BIOMIMETIC PROCESS USING $\text{Na}_2\text{O-SiO}_2$ GLASSES AS NUCLEATING AGENTS	127
CHAPTER 7.	ULTRASTRUCTURAL STUDY OF APATITE LAYER FORMED BY A BIOMIMETIC PROCESS AND ITS BONDING TO BONE	157
CHAPTER 8.	GENERAL SUMMARY	169
LIST OF PUBLICATIONS		173
ACKNOWLEDGEMENTS		177

CHAPTER 1.

GENERAL INTRODUCTION

Generally, artificial materials implanted into bone defects are encapsulated by a fibrous tissue to be isolated from the surrounding bone. In early 1970s, however, Hench et al. discovered that some glasses in the system $\text{Na}_2\text{O}-\text{CaO}-\text{SiO}_2-\text{P}_2\text{O}_5$ spontaneously bond to the living bone without forming the fibrous tissue around them, and named them Bioglass® [1]. Since then, various kinds of ceramics such as glass-ceramic Ceravital® containing crystalline apatite [2], sintered hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) [3], glass-ceramic A-W [4] containing crystalline apatite and wollastonite ($\text{CaO} \cdot \text{SiO}_2$), glass-ceramic Bioverit® containing crystalline apatite and phlogopite [5], $\text{MgO}-\text{CaO}-\text{SiO}_2-\text{P}_2\text{O}_5$ glass [6], $\text{CaO}-\text{SiO}_2$ glasses [7], sintered β - $3\text{CaO} \cdot \text{P}_2\text{O}_5$ [8] and natural calcite [9] were also found to bond to the living bone. They are now called bioactive ceramics and some of them are already clinically used as artificial middle ear bones, maxillofacial implants, bone fillers, artificial iliac crests, artificial vertebrae, artificial intervertebral discs etc. Among them, glass-ceramic A-W shows a fairly high mechanical strength as high as 200 MPa [10, 11] as well as high bioactivity [12], and hence can be used under rather loaded conditions [13–14].

Highly loaded bones such as tibial and femoral bones, however, can not be replaced even with this glass-ceramic, since its fracture toughness is lower and its elastic moduli are higher than those of the human cortical bones. For these purposes, metallic implants such as titanium metal and its alloys coated with plasma-sprayed apatite layer are now used [15]. In this

form, apatite is, however, difficult to control its composition and structure, and may not be stable for a long period in the living body. In addition, high elastic moduli of the metallic implants are liable to induce resorption of the surrounding bone because of their stress shielding. Therefore, highly bioactive materials with mechanical properties analogous to those of the natural bone are desired to be developed.

All the bioactive ceramics described above except for resorbable sintered β - $3\text{CaO}\cdot\text{P}_2\text{O}_5$ [16] and the natural calcite [17] bond to the bone through a layer of an apatite which is formed on their surfaces in the body [6, 7, 18–24]. The same type of the apatite layer is formed on their surfaces even in an acellular simulated body fluid with ion concentrations nearly equal to those of the human blood plasma [18, 19, 20, 25–30] and is characterized with carbonate-containing hydroxyapatite with small crystallites and defective structure, similar to the apatite in the bone. This type of the apatite layer is not observed on the surfaces of non-bioactive ceramics *in vivo* [6, 31] as well as *in vitro* [26, 29]. Therefore, it is believed that the essential requirement for the artificial material to bond to the living bone is the formation of the bone-like apatite layer on their surfaces *in vivo* or *in vitro* [32, 33].

All the bioactive glasses and glass-ceramics described above are based on CaO-SiO_2 compositions. According to the analysis of their surfaces structural changes due to exposure to the simulated body fluid, the bone-like apatite layer is speculated to be formed on their surfaces by the following mechanism [34–37]: the calcium ion dissolved from the glasses and glass-ceramics increases the ionic activity product of the apatite in the surrounding body fluid, and the hydrated silica formed on their surfaces provides favorable sites for the apatite nucleation. As a result, a large number of the apatite nuclei are formed rapidly on the surfaces of the

glasses and glass-ceramics. Once the apatite nuclei are formed, they spontaneously grow consuming the calcium and phosphate ions from the surrounding fluid, since the body fluid is already supersaturated with respect to the apatite even under the normal condition.

On the basis of these findings, it is expected that a dense and uniform bone-like apatite layer could be formed on various kinds of substrates at the normal temperature and pressure by the following process: A substrate is faced to a CaO-SiO_2 -based glass taking a short distance between them and soaked in the simulated body fluid. Actually it was confirmed by Abe et al. that a dense and uniform bone-like apatite layer is formed on various kinds of substrates including metals, ceramics and organic polymers by this method, when a MgO 4.6, CaO 44.7, SiO_2 34.0, P_2O_5 16.2 and CaF_2 0.5 wt% glass G in a plate form is used as the CaO-SiO_2 -based glass and the distance between the substrates and the glass plates is less than 0.5 mm [38]. Later, it was also confirmed by T. Kokubo et al. that the same type of the apatite layer is formed uniformly not only on the flat surfaces but also on curved surfaces of small particles, long fibers and woven fabrics of various materials including metals, ceramics and organic polymers by the following modified process [39]: First a substrate is set in contact with particles of glass G soaked in the simulated body fluid so that a number of apatite nuclei are formed on the substrate. Second the substrate is soaked in another solution highly supersaturated with respect to the apatite so that the apatite nuclei grow on the substrate *in situ* to form a continuous layer. This process can be called a biomimetic process, since it mimics a process by which the biologically active bone-like apatite layer is formed on the surfaces of bioactive ceramics in the living body.

Among the composites thus obtained, the apatite-organic polymer composites are especially interesting, since they are expected to be useful

not only as bone-repairing materials, because they could exhibit their mechanical properties analogous to those of natural bone as well as high bioactivity. In addition, they might be useful even as soft tissue-repairing materials because they could exhibit ductility as well as high compatibility even with the soft tissues.

In the present study, the biomimetic process described above are applied to various kinds of organic polymers with different chemical structures and surface structures. The induction period for the apatite nucleation on their surfaces as well as the adhesive strength of the apatite layer to the substrates are investigated in terms of the kind of the polymers, their surface treatments and the kind of the glasses used as the nucleating agent. The microstructure of the apatite layer formed by the present biomimetic method and that of its interface with living bone are also investigated. All these are fundamentally important factors for obtaining practically useful composites.

In Chapter 2, the effects of the kind of organic polymers on the induction periods for the apatite nucleation as well as the adhesive strengths of the apatite layer to the substrate are investigated in terms of the chemical structure of the organic polymers.

In Chapter 3, the effects of the surface treatment of the organic polymers with the 1M-HCl aqueous solution on the induction periods for the apatite nucleation and the adhesive strengths of the apatite layer are investigated in terms of the structural change of the polymer surface with the HCl treatment.

In Chapter 4, the effects of the treatment of the organic polymers with the 5M-NaOH aqueous solution on the induction periods for the apatite nucleation and the adhesive strengths of the apatite layer are investigated in terms of the structural change of the polymer surface with

the NaOH treatment.

In Chapter 5, the effects of the treatment of the organic polymers with glow-discharge in O₂ gas atmosphere on the induction periods for the apatite nucleation and the adhesive strengths of the apatite layer are investigated in terms of the structure change of the polymer surface with the glow-discharge treatment.

In Chapter 6, the effects of the kind of the glass used as the nucleating agent on the induction periods for the apatite nucleation and the adhesive strengths of the apatite layer are investigated in terms of the dissolution of the ions from the glasses.

In Chapter 7, the microstructure of the apatite layer formed on the polymer surface by the biomimetic process, and that of its interface to living bone after implantation into rat are investigated by means of scanning and transmission electron microscopies.

Chapter 8 summarizes the whole results and discussions of this thesis and points out the future perspectives.

References

- [1] L. L. Hench, R. J. Splinter, W. C. Allen and T. K. Greenlee Jr., "Bonding mechanisms at the interface of ceramic prosthetic materials" *J. Biomed. Mater. Res.*, **2**, 117-141 (1972).
- [2] H. Brömer, E. Pfeil and H. H. Käs, "Glass-ceramic material" *German Patent No.* 2,326,100 (1973).
- [3] M. Jarcho, J. L. Kay, R. H. Gumaer and H. P. Drobeck, "Tissue, cellular and subcellular events at bone-ceramic hydroxyapatite interface" *J. Bioeng.* **1**, 79-92 (1977).

- [4] T. Kokubo, M. Shigematsu, Y. Nagashima, M. Tashiro, T. Nakamura, T. Yamamuro and S. Higashi, "Apatite- and wollastonite-containing glass-ceramics for prosthetic application" *Bull. Inst. Chem. Res., Kyoto Univ.*, **60**, 260-268 (1982).
- [5] W. Hölland, K. Naumann, W. Vogel and J. Gummel, "Machinable bioactive glass ceramic" *Wiss. Ztschr. Friedrich-Schiller-Univ. Jena, Math.-Naturwiss. R.* **32**, 571-580 (1983).
- [6] K. Kitsugi, T. Yamamuro, T. Nakamura and T. Kokubo, "Bone bonding behavior of $\text{MgO-CaO-SiO}_2\text{-P}_2\text{O}_5\text{-CaF}_2$ glass (mother glass of A-W-glass-ceramics)", *J. Biomed. Mater. Res.*, **23**, 631-648 (1989).
- [7] K. Ohura, T. Nakamura, T. Yamamuro, T. Kokubo, Y. Ebisawa, Y. Kotoura and M. Oka, "Bone-bonding ability of P_2O_5 -free CaO-SiO_2 glasses" *J. Biomed. Mater. Res.*, **25**, 357-365 (1991).
- [8] B. V. Rejda, J. G. J. Peelen and K. de Groot, "Tricalcium phosphate as a bone substitute" *J. Bioeng.* **1**, 93-97 (1977).
- [9] M. M. Walker and J. L. Katz, "Evaluation of bonding of bone to inorganic crystal surfaces" *Bull. Hospital for Joint Diseases Orthopaedic Inst.*, XLIII 103-108 (1983).
- [10] T. Kokubo, S. Ito, M. Shigematsu, S. Sakka and T. Yamamuro, "Mechanical properties of a new type of apatite-containing glass-ceramic for prosthetic application", *J. Mat. Sci.*, **20**, 2001-2004 (1985).
- [11] T. Kokubo, S. Ito, M. Shigematsu, S. Sakka and T. Yamamuro, "Fatigue and life-time of bioactive glass-ceramic A-W containing apatite and wollastonite" *J. Mat. Sci.*, **22**, 4067-4070 (1987).
- [12] K. Ono, T. Yamamuro, T. Nakamura and T. Kokubo, "Quantitative study on osteoconduction of apatite-wollastonite containing glass ceramic granules, hydroxyapatite granules, and alumina granules" *Biomaterials*, **11**, 265-271 (1990).
- [13] T. Yamamuro, J. Shikata, H. Okumura, T. Kitsugi, Y. Kakutani, T. Matsui and T. Kokubo, "Replacement of the lumbar vertebrae of sheep with ceramic prostheses" *J. Bone and Joint Surg.*, **72-B**, 889-893 (1990).
- [14] T. Yamamuro, J. Shikata, H. Okumura, S. Yoshii, S. Kotani and T. Kokubo, "Pre-clinical and clinical application of A-W glass-ceramic to various orthopaedic conditions" in *Bioceramics*, Vol. 2, ed. by Günther Heimke, German Ceramic Society, Cologne, pp. 361-366, 1990.
- [15] R. M. Pilliar and M. J. Filiaggi, "New calcium phosphate coating methods" in *Bioceramics*, Vol. 6, ed. by P. Ducheyne and D. Christiansen, Butterworth-Heinemann, London, pp. 165-171, 1993.
- [16] C. Ohtsuki, T. Kokubo, M. Neo, S. Kotani, T. Yamamuro, T. Nakamura and Y. Bando, "Bone-bonding mechanism of sintered $\beta\text{-3CaO-P}_2\text{O}_5$ ", *Phosphorus Research Bull.*, **1**, 191-196 (1991).
- [17] Y. Fujita, T. Yamamuro, T. Nakamura, S. Kotani, C. Ohtsuki and T. Kokubo, "The bonding behavior of calcite to bone" *J. Biomed. Mater. Res.*, **25**, 991-1003 (1991).
- [18] L. L. Hench and A. E. Clark, "Adhesion to Bone" in *Biocompatibility of Orthopedic Implants*, Vol. 2, ed. by D. F. Williams, CRC Press, Boca Raton, pp. 129-170, 1982.
- [19] L. L. Hench, "Stability of ceramics in the physiological environment" in *Fundamental aspects of biocompatibility*, Vol. 1, ed. by D. F. Williams, CRC Press, Boca Raton, pp. 67-85, 1981.
- [20] C. Ohtsuki, H. Kushitani, T. Kokubo, S. Kotani and T. Yamamuro, "Apatite formation on the surface of Ceravital-type glass-ceramic in

- the body" *J. Biomed. Mater. Res.*, **25**, 1363–1370 (1991).
- [21] M. Neo, S. Kotani, T. Nakamura, T. Yamamuro, C. Ohtsuki, T. Kokubo, and Y. Bando, "A comparative study of ultrastructure of the interfaces between four kinds of surface-active ceramic and bone" *J. Biomed. Mater. Res.*, **26**, 1419–1432 (1992).
- [22] T. Kitsugi, T. Nakamura, T. Yamamuro, T. Kokubo, T. Shibuya and M. Takagi, "SEM-EPMA observation of three types of apatite-containing glass-ceramics implanted in bone: The variance of a Ca-P-rich layer" *J. Biomed. Mater. Res.*, **21**, 1255–1271 (1987).
- [23] T. Kokubo, C. Ohtsuki, S. Kotani, T. Kitsugi and T. Yamamuro, "Surface structure of bioactive glass-ceramic A-W implanted into sheep and human vertebra" in *Bioceramics*, Vol. 2, ed. by Günther Heimke, Heidelberg, Germany, pp. 113–120, 1989.
- [24] W. Höland, W. Vogel, K. Naumann and J. Gummel, "Interface reactions between machinable bioactive glass-ceramics and bone" *J. Biomed. Mater. Res.*, **19**, 303–312 (1985).
- [25] T. Kokubo, H. Kushitani, Y. Ebisawa, T. Kitsugi, S. Kotani, K. Ohura and T. Yamamuro, "Apatite formation on bioactive ceramics in body environment" in *Bioceramics*, Vol. 1, ed. by H. Oonishi, H. Aoki and K. Sawai, Ishiyaku Euro America, Tokyo, pp. 157–162, 1989.
- [26] T. Kokubo, S. Ito, Z. T. Huang, T. Hayashi, S. Sakka, T. Kitsugi and T. Yamamuro, "Ca, P-rich layer formed on high-strength bioactive glass-ceramic A-W" *J. Biomed. Mater. Res.*, **24**, 331–343 (1990).
- [27] T. Kokubo, H. Kushitani, S. Sakka, T. Kitsugi and T. Yamamuro, "Solutions able to reproduce in vivo surface-structure changes in bioactive glass-ceramic A-W" *J. Biomed. Mater. Res.*, **24**, 721–734 (1990).
- [28] C. Ohtsuki, Y. Aoki, T. Kokubo, Y. Bando, M. Neo, T. Yamamuro and T. Nakamura, "Characterization of apatite layer formed on bioactive glass-ceramic A-W" in *Bioceramics*, Vol. 5, ed. by T. Yamamuro, T. Kokubo and T. Nakamura, Kobunshi Kankokai, Kyoto, pp. 87–94, 1992.
- [29] T. Kokubo, T. Hayashi, S. Sakka, T. Kitsugi, T. Yamamuro, M. Takagi and T. Shibuya, "Surface structure of a load-bearable bioactive glass-ceramic A-W" in *Ceramics in Clinical Applications* ed. by P. Vincenzini, Elsevier, Amsterdam, pp. 175–184, 1987.
- [30] C. Ohtsuki, T. Kokubo, K. Takatsuka and T. Yamamuro, "Compositional dependence of bioactivity of glasses in the system CaO-SiO₂-P₂O₅: Its in vitro evaluation" *J. Ceram. Soc., Japan*, **99**, 1–6 (1991).
- [31] T. Kitsugi, T. Yamamuro, T. Nakamura and T. Kokubo, "The bonding of glass ceramics to bone" *International Orthopaedics (SICOT)*, **13**, 199–206 (1989).
- [32] T. Kokubo, "Recent progress in glass-based materials for biomedical applications" *J. Ceram. Soc. Japan*, **99**, 965–973 (1991).
- [33] L. L. Hench, "Bioceramics: From concept to clinic" *J. Am. Ceram. Soc.*, **74**, 1487–510 (1991).
- [34] T. Kokubo, H. Kushitani, C. Ohtsuki, S. Sakka and T. Yamamuro, "Chemical reaction of bioactive glass and glass-ceramics with a simulated body fluid" *J. Mater. Sci: Materials in Medicine*, **3**, 79–83 (1992).
- [35] T. Kokubo, H. Kushitani, C. Ohtsuki, S. Sakka and T. Yamamuro, "Effects of ions dissolved from bioactive glass-ceramic on surface apatite formation" *J. Mater. Sci: Materials in Medicine*, **4**, 1–4 (1993).

- [36] C. Ohtsuki, T. Kokubo, and T. Yamamuro, "Mechanism of apatite formation on CaO-SiO₂-P₂O₅ glasses in a simulated body fluid" *J. Non.-Crystl. Solids*, **143**, 84-92 (1992).
- [37] P. Li, C. Ohtsuki, T. Kokubo, K. Nakanishi, N. Soga, T. Nakamura and T. Yamamuro, "Apatite formation induced by silica gel in a simulated body fluid" *J. Am. Ceram. Soc.*, **75**, 2094-2097 (1992).
- [38] Y. Abe, T. Kokubo and T. Yamamuro, "Apatite coating on ceramics, metals and polymers utilizing a biological process" *J. Mater. Sci: Materials in Medicine*, **1**, 233-238 (1990).
- [39] T. Kokubo, K. Hata, T. Nakamura and T. Yamamuro, "Apatite formation on ceramics, metals and polymers induced by a CaO-SiO₂-based glass in a simulated body fluid" in *Bioceramics*, Vol. 4, ed. by W. Bonfield, G. W. Hastings, K. E. Tunner, Butterworth-Heinemann, London, pp. 113-120, 1991.

CHAPTER 2.

APATITE FORMATION ON ORGANIC POLYMERS BY A BIOMIMETIC PROCESS USING CaO-SiO₂-BASED GLASS AS NUCLEATING AGENT

1. Introduction

It is believed that the essential requirement for the artificial material to bond to the living bone is the formation of a layer of a biologically active apatite, which is a carbonate-containing hydroxyapatite similar to the bone apatite, on their surfaces *in vivo* or *in vitro* [1, 2]. This type of apatite layer was found to be formed on the surfaces of CaO-SiO₂-based glasses and glass-ceramics by the following mechanism [3, 4]. The calcium ion dissolved from the materials increases the ionic activity product of the apatite in the surrounding fluid, and the hydrated silica formed on their surfaces provides specific favorable sites for the apatite nucleation [5]. As a result, a large number of apatite nuclei are rapidly formed on their surfaces. Once the apatite nuclei are formed, they grow spontaneously by consuming the calcium and phosphate ions from the surrounding fluid, since the body fluid is already supersaturated with respect to the apatite even under the normal condition [6].

On the basis of the mechanism of apatite formation on their surfaces, T. Kokubo et al. have revealed that the bone-like apatite layer can be formed not only on the flat surfaces of plates but also on curved surfaces of small particles, long fibers and woven fabrics of various materials including metals, ceramics and organic polymers by the following biomimetic process [7]: A substrate is set in contact with particles of a

CaO–SiO₂–based glass soaked in the simulated body fluid (SBF) so that a number of apatite nuclei are formed on the substrate. Then, the substrate is soaked in another solution highly supersaturated with respect to the apatite so that the apatite nuclei grow on the substrate in situ to form a continuous layer. Among the composites thus obtained, the apatite–organic polymer composites are expected to be useful for repairing not only hard human tissues but also as soft ones, since they can exhibit not only a high bone–bonding ability but also a high compatibility with even soft tissues, as well as mechanical properties analogous to those of both hard and soft tissues.

In this chapter, the effects of polymers on the induction period for the apatite nucleation and the adhesive strength of the apatite layer to the polymer were investigated.

2. Experimental

2.1. Preparation of CaO–SiO₂–Based Glass

A glass named G of the nominal composition MgO 4.6, CaO 44.7, SiO₂ 34.0, P₂O₅ 16.2 and CaF₂ 0.5 wt%, which is the parent glass of high strength bioactive glass–ceramic A–W [8], was used as the CaO–SiO₂–based glass for nucleating the apatite. A mixture of the reagent grade chemicals of the corresponding composition was put into a 50 ml platinum crucible and melted at 1450 °C for 1 h in a MoSi₂ furnace. The melt was poured onto a stainless steel plate and pressed into a glass plate. It was crushed by a laboratory planetary type zirconia ball mill, and sieved for obtaining only the grains 150 to 300 μm in size.

2.2. Preparation of Solutions

The simulated body fluid (SBF) with ion concentrations nearly equal to those of human blood plasma [9] as the medium for apatite nucleation, and an aqueous solution with ion concentrations 1.5 times those of SBF (1.5SBF) as the medium for apatite growth were prepared by dissolving the reagents of NaCl, NaHCO₃, KCl, K₂HPO₄·3H₂O, MgCl₂·6H₂O, CaCl₂ and Na₂SO₄ into an ion-exchanged and distilled water in a polystyrene bottle to give the concentrations shown in Table 1. These solutions were buffered at pH 7.25 by tris(hydroxymethyl)aminomethane ((CH₂OH)₃CNH₂) and 1M–hydrochloric acid (HCl) at 36.5 °C, respectively.

Table 1. Ion concentrations of SBF and 1.5SBF in comparison with those of human blood plasma.

	Concentration / mM							
	Na ⁺	K ⁺	Ca ²⁺	Mg ²⁺	HCO ₃ [–]	Cl [–]	HPO ₄ ^{2–}	SO ₄ ^{2–}
Blood plasma	142.0	5.0	2.5	1.5	27.0	103.0	1.0	0.5
SBF	142.0	5.0	2.5	1.5	4.2	148.0	1.0	0.5
1.5SBF	213.0	7.5	3.8	2.3	6.3	223.0	1.5	0.75

2.3. Preparation of Substrate

Ten kinds of organic polymers were prepared as the substrates for the apatite coating by the following methods. Cellulose triacetate (CTA) with a degree of substitution (DS) of 2.86 was supplied by Daicel Chem. Ind. Ltd., (Osaka, Japan) and its plates were prepared by casting process on a teflon template from a chloroform solution of 7 wt% concentration. Poly(ethylene terephthalate) (PET) and poly(vinyl chloride) (PVC) were

supplied by Toyobo Co. Ltd., (Osaka, Japan) and their plates were prepared by hot-compression process under a pressure of 1 Pa at 200 °C and 100 °C, respectively. Plates of poly(methyl methacrylate) (PMMA), polyamide 6 (Nylon 6), polyethylene (PE) and poly(tetrafluoroethylene) (PTFE) plates were supplied by Sanplatec. Co., (Osaka, Japan). Poly-ether sulfone (PESF) was supplied by ICI. PLC (England). Poly-ether sulfone plates were prepared by extrusion. Poly(dimethyl siloxane) (PDMS) sheet was supplied by Shin-etsu Chemical Co. Ltd., (Tokyo, Japan). Poly(vinyl alcohol) (PVA) was dissolved in a mixture of water and dimethyl sulfoxide (20/80 wt ratio) at about 140 °C for 2 h in N₂ atmosphere, and quickly quenched at -20 °C to give a transparent gel. The obtained PVA gel was dried in vacuo at room temperature for 24 h, followed by further annealing at 160 °C for 1 h for obtaining the gel contained water up to 20 wt%. After soaking in the distilled water, PVA hydrogel with a water content of 22 wt% was obtained. All the plates were cut into a rectangular piece of 10 mm x 15 mm x 1 mm and abraded with #400 diamond pad on their one side. A cloth (10 mm x 15 mm in area) woven with ultrafine PET fiber 2 μmφ which was supplied by Toray Co. Ltd., (Ohtsu, Japan) was also used as a substrate. Chemical structures of these polymers are shown in Table 2.

2.4. Formation of Apatite Layer

Each substrate described above was first placed on particles of glass G which were soaked in 30 ml of SBF at 36.5 °C, respectively in different polystyrene bottles 35 mmφ and 70 mm high as shown in Fig. 1 for various periods up to 168 h, for forming the apatite nuclei on the surface of the substrate apposed to the glass particles. Then, the substrate was soaked in 30 ml of 1.5SBF at 36.5 °C in the polystyrene bottle for 6 d for making the apatite nuclei grow on the surface of the substrate in situ. The 1.5SBF

Table 2 Chemical structures of polymers examined.

Name	Structural formula
Cellulose triacetate (CTA)	
Poly(ethylene terephthalate) (PET)	
Poly(vinyl chloride) (PVC)	
Poly(methyl methacrylate) (PMMA)	
Polyamide 6 (Nylon 6)	
Polyethylene (PE)	
Poly(tetrafluoroethylene) (PTFE)	
Poly-ether sulfone (PESF)	
Poly(dimethyl siloxane) (PDMS)	
Poly(vinyl alcohol) (PVA)	

solution was renewed every 2 d. After the second treatment, the substrate was washed moderately with distilled water, and dried at room temperature.

2.5. Surface Analysis of Substrate

The surfaces of the substrates after the second treatment were analyzed with a thin-film X-ray diffraction, Fourier-transform infrared (FT-IR) reflection spectroscopy and a scanning electron micrograph (SEM). The former two techniques enable us to detect only thin layer about $1\text{ }\mu\text{m}$ thick at the surface. In the X-ray diffraction, a thin-film attachment, Rigaku 2651 Al (Rigaku Co., Tokyo, Japan) was used and the glancing angle of the specimen was fixed at 1° against the incident beam. The infrared spectroscopy was performed by using a spectrometer, FT-IR 5M (Japan Spectroscopic Co. Ltd., Tokyo, Japan) with the reflection angle of 75° . In the scanning electron micrograph, the specimen was coated with gold-palladium film and observed under an electron microscope, Hitachi-S-2500CX (Hitachi Co. Ltd., Tokyo, Japan).

2.6. Measurement of Adhesive Strength

A couple of brass jigs with a base of $8\text{ mm}\phi$ were attached to the outer surface of the apatite layer of $10\text{ }\mu\text{m}$ thick formed on the substrates, and to the opposite side of the substrate with a cyanoacrylate adhesives, as shown in Fig. 2, and left untouched for 1 d for complete solidification of the adhesives. Then the adhesive strength of the apatite layer to the substrate was measured by applying a tensile stress to their interface by using an Instron-type testing machine (Shimazu Model, Kyoto, Japan) at a cross head speed of 1 mm/min , until the fracture occurred at the interface between the apatite and the substrate. At least five measurements were done for each data point.

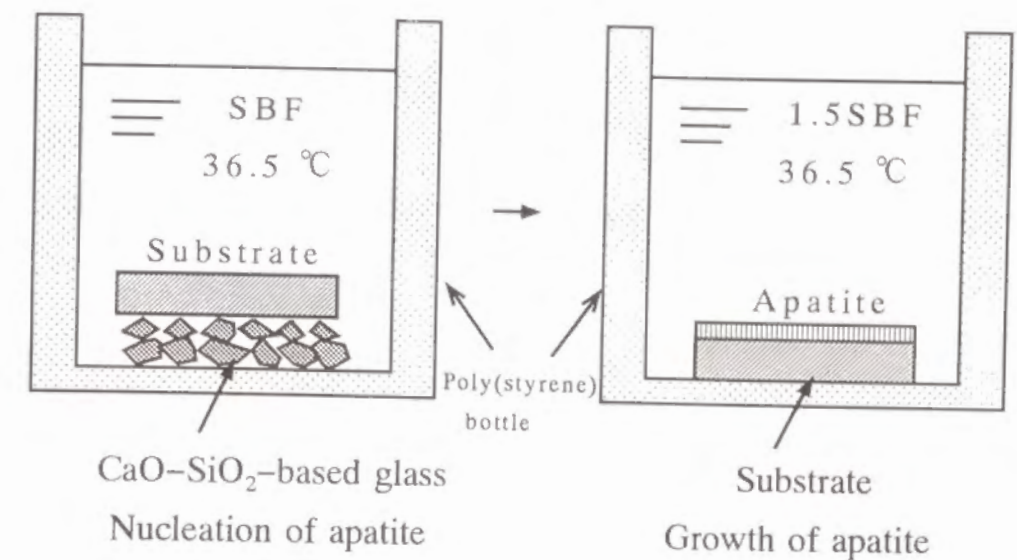


Figure 1. A biomimetic process for forming the bone-like apatite layer on various substrates.

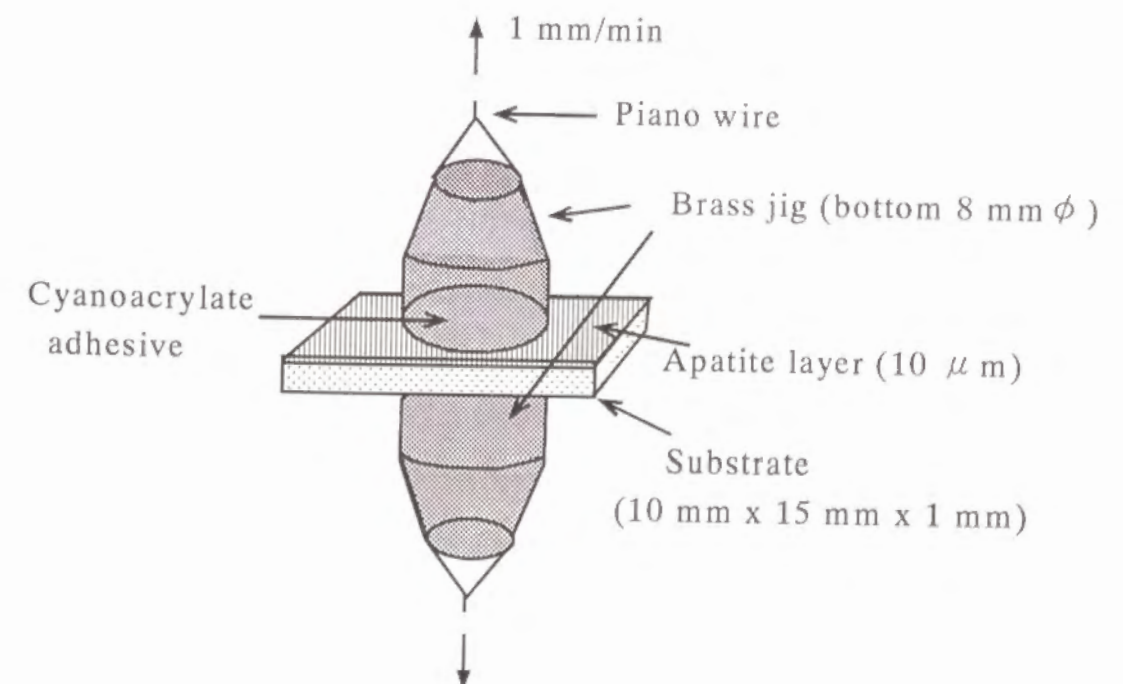


Figure 2. Arrangement for measuring the adhesive strength of the bone-like apatite layer to the substrate.

3. Results

Figures 3, 4 and 5 show thin-film X-ray diffraction patterns, FT-IR reflection spectra and SEM photographs of the surfaces of the PESF substrates which were subjected to the first treatment for different periods and then to the second treatment for 6 d. It can be seen from these figures that a dense and uniform layer of carbonate-containing hydroxyapatite with a small crystallite and/or defective structure, similar to the apatite in the natural bone, can be formed on an organic polymer substrate by the present biomimetic method. Formation of the apatite layer, however, largely depends on the period of the time for the first treatment. When the first treatment was omitted, the apatite was not formed even after the second treatment for 6 d. This indicates that the first treatment in the presence of the CaO-SiO_2 -based glass is essential for the apatite nucleation on the substrate. The apatite was formed only sparsely on the substrate subjected to the first treatment for the periods shorter than 12 h, and made a continuous layer only on that subjected to the first treatment for the periods longer than 24 h. This indicates that the number of the apatite nuclei formed on the substrate increases with increasing period of time of the first treatment. Here, the time of the first treatment required for forming sufficient amount of the apatite nuclei to make a continuous layer after the second treatment is tentatively defined as the induction period for the apatite nucleation. The induction periods thus obtained for each organic polymer described in Section 2.3. were almost 24 h independent of the kind of polymers, except 12 h for PVA-H22%.

The adhesive strengths of the apatite layer which were measured by the method described in Section 2.6. for some organic polymer substrates described in Section 2.3. are shown in Table 3. It can be seen from Table

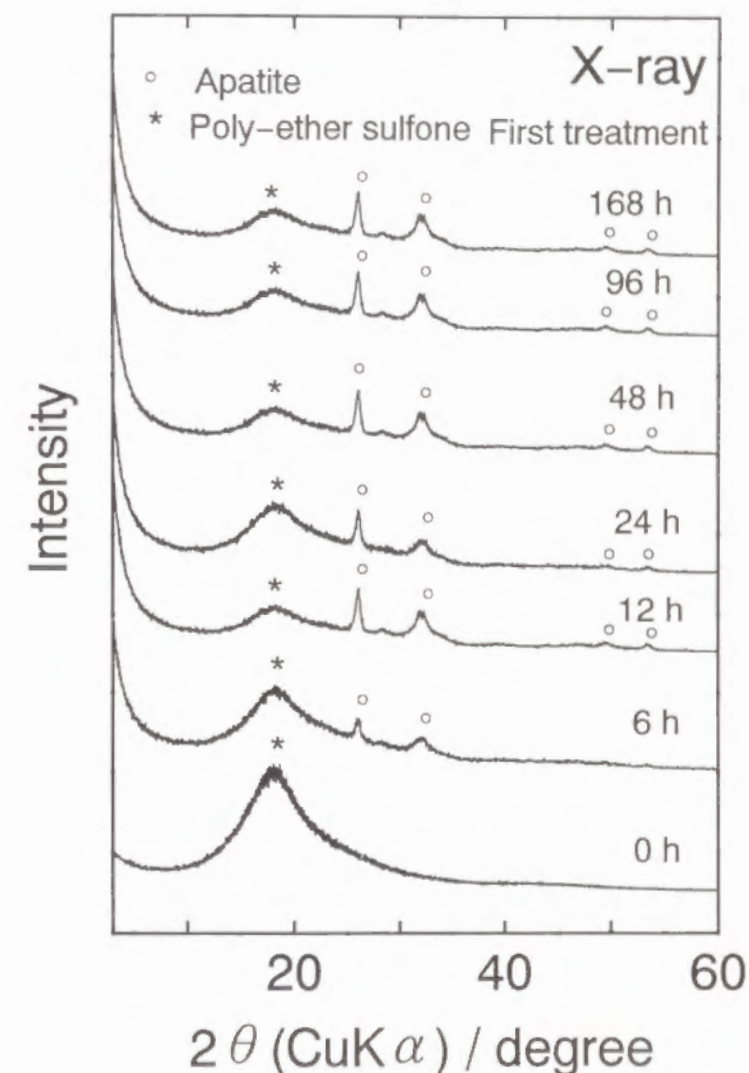


Figure 3. Thin-film X ray diffraction patterns of the surfaces of PESF substrates placed on glass G grains in SBF for different periods and then soaked in 1.5SBF for 6 d.

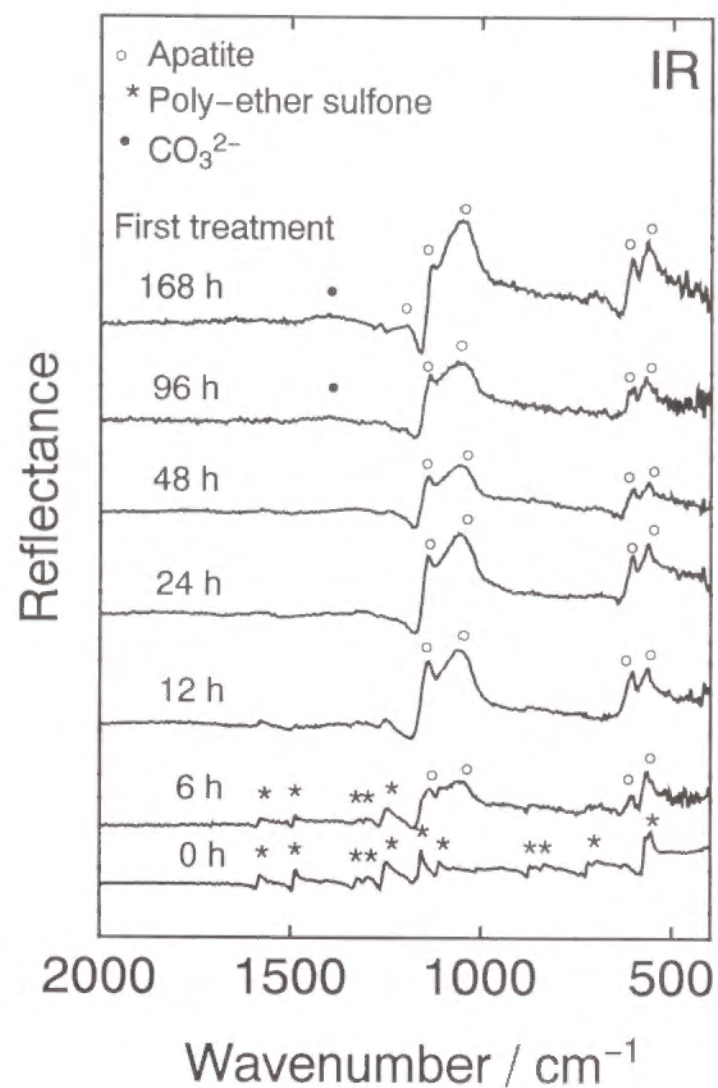


Figure 4. FT-IR reflection spectra of the surfaces of PESF substrates placed on glass G grains in SBF for different periods and then soaked in 1.5SBF for 6 d.

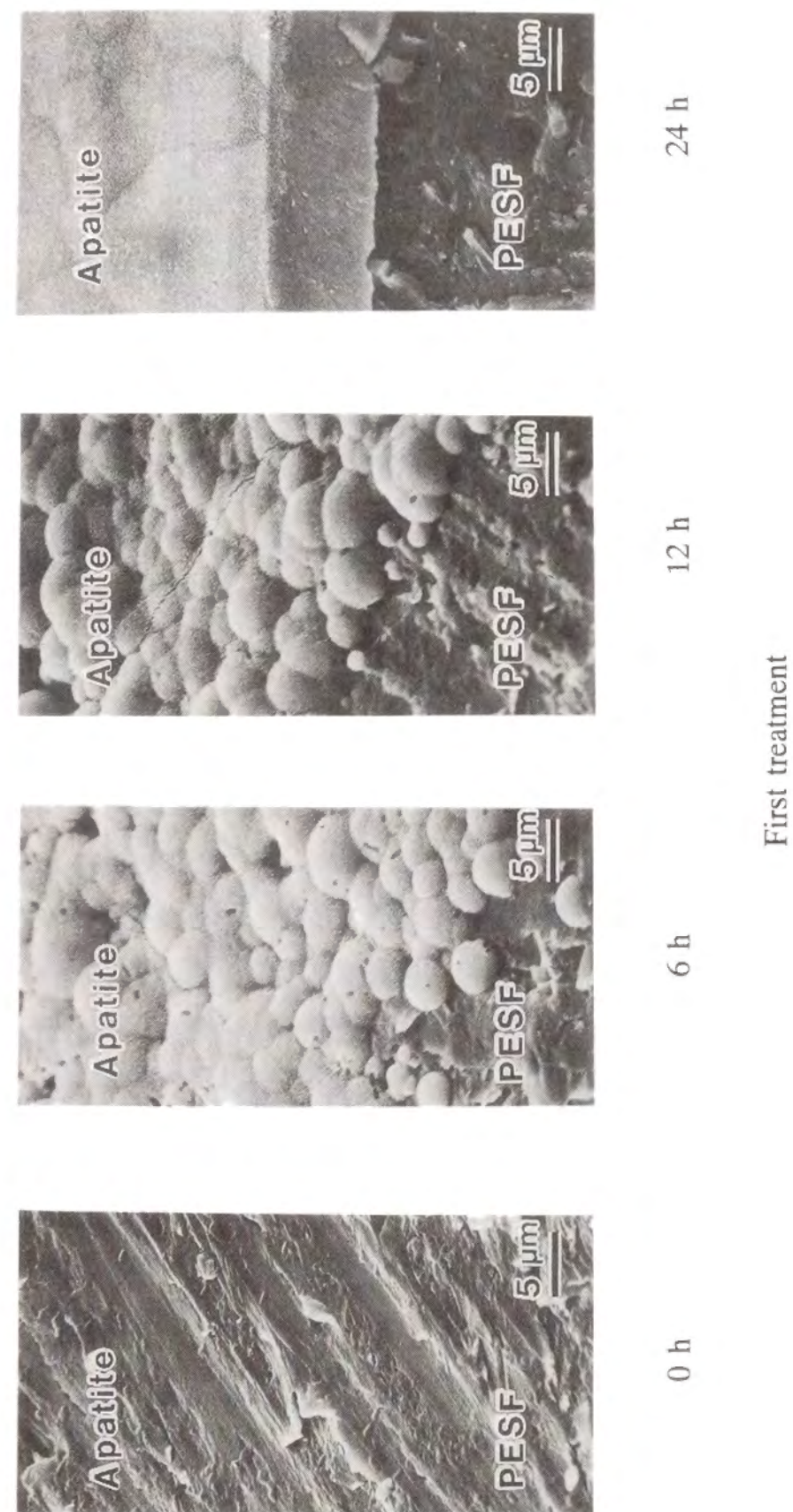


Figure 5. SEM photographs of the surfaces of PESF substrates placed on glass G grains in SBF for different periods and then soaked in 1.5SBF for 6 d.

3 that the adhesive strength largely varies with the kind of the polymers. Among the examined polymers, PET, PESF and PVA-H22% showed relatively high adhesive strengths of about 4 MPa.

Table 3. Adhesive strengths of bone-like apatite layer to various organic polymer substrates.

Substrate	Adhesive strength / MPa
PET	3.48 [0.15]
PMMA	1.06 [0.17]
PESF	4.40 [0.28]
Nylon 6	0.63 [0.02]
PE	1.93 [0.23]
PTFE	$< 1.10 \times 10^{-2}$
PVA-H22%	3.20 [0.12]

[] Standard deviation

Figure 6 shows SEM photographs of PET fabric before and after coating of the bone-like apatite layer by the present biomimetic method. It can be seen from Fig. 6 that the apatite layer is formed uniformly on the individual constituents fine fibers. This fabric can be bent sharply without peeling off the apatite layer.

4. Discussion

It is interesting to know that the induction period for the apatite nucleation on the substrates is almost 24 h, independent of the kind of the organic polymers. Poly(vinyl alcohol) hydrogel is exceptional, since it

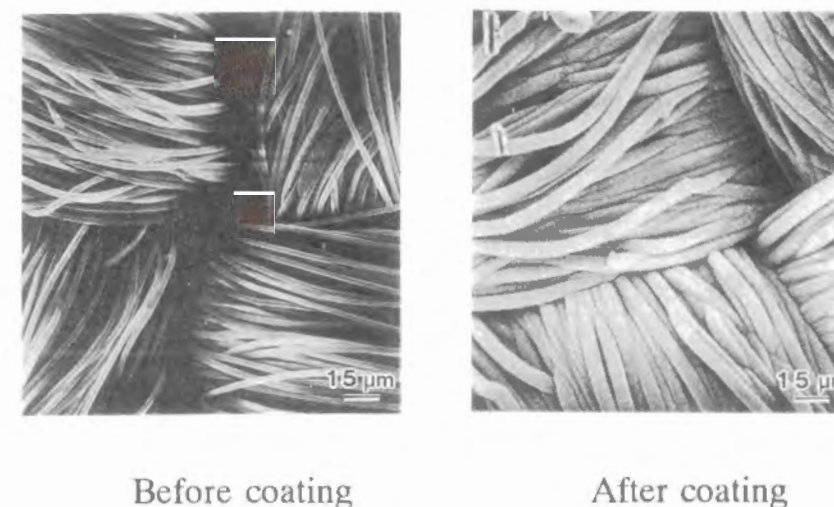


Figure 6. SEM photographs of surfaces of PET fabric before and after coating of the bone-like apatite.

contains a large amount of the water.

Glass G used in the first treatment dissolves an appreciable amount of the calcium as well as the silicate ions [10]. Among them, the calcium ion increases the ionic activity product of the apatite in the SBF in the narrow gap between the substrate and the glass particles, since the calcium is the constituent element of the apatite. The silicate ion is speculated to be attached to the surface of the organic polymer substrates to induce the apatite nucleation on the surface of the substrates similar to the surfaces of the glass particles. Therefore, the induction period for the apatite nucleation on the substrate may be closely related to the affinity of the silicate ion to the substrate. If this assumption is correct, the present result indicates that the affinity of the silicate ion to the organic polymers is almost equal among the examined polymers, independent of their chemical structure.

In spite of this, the adhesive strength of the apatite layer formed on

the polymer substrates varied largely depending upon the kind of the polymers. Among the examined polymers, PET, PESF and PVA-H22% showed a relatively high adhesive strength, whereas PMMA, Nylon 6, PE and PTFE showed a low adhesive strength. PVA-H22% is again exceptional, since it contains a large number of micropores. The apatite layer formed on the surfaces of PET and PESF was not able to be peeled off, even by scratching with a sharp knife edge. Mechanical interlocking of the apatite layer to the substrate could contribute to the adhesive strength at least partly, since the surfaces of the polymers were previously abraded with a #400 diamond pad. This effect, however, might be almost equal for all the examined polymers. Therefore, the relatively high adhesive strength of PET and PESF can be attributed mainly to their chemical and/or electric properties. Both polymers include phenyl groups and polar groups such as ester and sulfonyl groups in the main chains, and have appropriate hydrophile-lipophile balance. It is also known that these polymers exhibit proper electric properties. It is expected that a fairly strong bond could be formed between these polar groups of the polymers and hydroxyl groups or calcium ions of the apatite. Poly(methyl methacrylate) showed a low adhesion, although it has also the ester groups in the molecular chain. This might be attributed to the facts that the chemical structure of PMMA which bears the ester groups not in the main chain but in the side chain and that its electrical properties such as static electricity are very different from those of PET. Polyamide 6 also showed a low adhesion, although it has amide group showing the highest polarity among the functional group of the examined polymers [11]. This might be attributed to the following two factors: The amide group does not exist on its surface, since it is liable to condense within Nylon 6. In addition, the static electricity of Nylon 6 is similar to that of PMMA. Polyethylene and PTFE can bond to the apatite

only by weak van der Waals force.

These results indicate that the adhesive strength of the apatite layer formed by the present biomimetic method to the organic polymer substrates can be improved further with the increase in their surface roughness and/or with the increase in the amount of some proper polar groups on their surfaces by physical or chemical treatments.

The apatite in the layer formed by the present biomimetic process is very similar, in its microstructure and composition, to the biologically active bone-like apatite layer which is formed on the surfaces of bioactive ceramics *in vivo* and is responsible for bonding to the living bone [12-16]. It has been shown that the degree of bioactivity of materials which is represented, for example, by rate of formation of bone on the surface of the materials [17], is very closely related to the rate of formation of the biologically active bone-like apatite layer on their surfaces *in vivo*. Higher bioactivities of Bioglass and glass-ceramic A-W than that of the sintered hydroxyapatite [17] are attributed to higher rate of formation of the bone-like apatite layer on the surfaces of the formers [18, 19]. In view of this fact, it is expected that the apatite layer formed by the present biomimetic process could exhibit very high bioactivity. This means that the present method can give highly bioactive materials with various mechanical properties.

It can be seen from Fig. 6 that all the constituent fine fibers are uniformly coated with the dense and uniform apatite layer. This structure is very similar to that of the natural bone at least partly. If this type of material can be fabricated into three dimensional structure analogous to that of the natural bone, the resultant composite could exhibit analogous mechanical properties to those of the natural bone, as well as high bioactivity, and hence might be useful as bone-repairing materials.

In addition, it is noted that the apatite in the bone is intimately combined with some proteins such as collagen. In view of this fact, it is also expected that the bone-like apatite formed by the present biomimetic process also could exhibit good biocompatibility even with soft tissues consisting of organic substances. Actually, it is already reported that PESF coated with the bone-like apatite layer by the present biomimetic process showed good compatibility with the soft tissues of trachea [20].

5. Conclusion

It is apparent from the above description that a dense and uniform layer of the bone-like apatite can be formed on various kinds of organic polymers with a fairly strong bond to the substrate by the present biomimetic method. The induction period for the apatite nucleation, which is defined as the time of the first treatment required for forming sufficient amount of the apatite nuclei to make the continuous layer after the second treatment, were almost 24 h for most of the organic polymers examined. The adhesive strengths of the apatite layer formed to the polymer substrates were as high as 3 to 4 MPa for PET, PESF and PVA-H. Such a high adhesion for PET, PESF and PVA-H is attributed to the chemical bond between the polar groups in PET, PESF and PVA-H and the hydroxyl group or calcium ion in the apatite. In practical uses as materials hard and soft tissues, however, higher adhesive strengths of the apatite layer to the substrates are desirable.

References

- [1] T. Kokubo, "Recent progress in glass-based materials for biomedical applications" *J. Ceram. Soc. Japan*, **99**, 965-973 (1991).
- [2] L. L. Hench, "Bioceramics: From concept to clinic" *J. Am. Ceram. Soc.*, **74**, 1487-510 (1991).
- [3] C. Ohtsuki, T. Kokubo, K. Takatsuka, and T. Yamamuro, "Compositional dependence of bioactivity of glasses in the system $\text{CaO-SiO}_2\text{-P}_2\text{O}_5$: Its *in vitro* evaluation" *J. Ceram. Soc., Japan*, **99**, 1-6 (1991).
- [4] C. Ohtsuki, T. Kokubo and T. Yamamuro, "Mechanism of apatite formation on $\text{CaO-SiO}_2\text{-P}_2\text{O}_5$ glasses in a simulated body fluid" *J. Non. Crystl. Solids.*, **143**, 84-92 (1992).
- [5] P. Li, C. Ohtsuki, T. Kokubo, K. Nakanishi, N. Soga, T. Nakamura and T. Yamamuro, "Apatite formation induced by silica gel in a simulated body fluid" *J. Am. Ceram. Soc.*, **75**, 2094-2097 (1992).
- [6] W. F. Neuman and M. W. Neuman, in *"The Chemical Dynamics of Bone Mineral"* University of Chicago Press, Chicago, p. 3, 1958.
- [7] T. Kokubo, K. Hata, T. Nakamura and T. Yamamuro, "Apatite formation on ceramics, metals and polymers induced by a CaO-SiO_2 -based glass in a simulated body fluid" in *Bioceramics* Vol. 4, ed. by W. Bonfield, G. W. Hastings and K. E. Tunner, Butterworth-Heinemann, Guilford, London, pp. 113-120, 1991.
- [8] T. Kokubo, M. Shigematsu, Y. Nagashima, M. Tashiro, T. Nakamura, T. Yamamuro and S. Higashi, "Apatite and wollastonite-containing glass-ceramics for prosthetic application" *Bull. Inst. Chem. Res., Kyoto Univ.*, **60**, 260-268 (1982).
- [9] J. Gamble, in *"Chemical Anatomy Physiology and Pathology of*

Extracellular Fluid", 6th Ed. Harvard Univ., Cambridge, pp. 1-8, 1967.

- [10] T. Kokubo, H. Kushitani, C. Ohtsuki, S. Sakka and T. Yamamuro, "Chemical reaction of bioactive glass and glass-ceramics with a simulated body fluid" *J. Mater. Sci: Mat. in Medicine*, **3**, 79-83 (1992).
- [11] D. W. Van Krevelen in *"PROPERTIES OF POLYMERS"* 3th Ed. ELSEVIER, New York, p. 216, 1990.
- [12] M. Neo, S. Kotani, T. Nakamura, T. Yamamuro, C. Ohtsuki, T. Kokubo, and Y. Bando "A comparative study of ultrastructures of the interfaces between four kinds of surface-active ceramic and bone" *J. Biomed. Mater. Res.*, **26**, 1419-1432 (1992).
- [13] T. Kokubo, C. Ohtsuki, S. Kotani, T. Kitsugi and T. Yamamuro, "Surface structure of bioactive glass-ceramic A-W implanted into sheep and human vertebra" in *Bioceramics*, Vol. 2, ed. by Günther Heimke, Heidelberg, Germany, pp. 113-120, 1989.
- [14] T. Kokubo, S. Ito, Z. T. Huang, T. Hayashi, S. Sakka, T. Kitsugi and T. Yamamuro, "Ca, P-rich layer formed on high-strength bioactive glass-ceramic A-W", *J. Biomed. Mater. Res.*, **24**, 331-343 (1990).
- [15] C. Ohtsuki, Y. Aoki, T. Kokubo, Y. Bando, M. Neo, T. Yamamuro and T. Nakamura, "Characterization of apatite layer formed on bioactive glass-ceramic A-W", in *Bioceramics*, Vol. 5, ed. by T. Yamamuro, T. Kokubo and T. Nakamura, Kobunshi Kankokai, Inc., Kyoto, pp. 87-94, 1992.
- [16] T. Kokubo, K. Hata, T. Nakamura and T. Yamamuro, "Apatite formation on ceramics, metals and polymers induced by a CaO-SiO₂-based glass in a simulated body fluid", in *Bioceramics* Vol. 4, ed. by W. Bonfield, G. W. Hastings, K. E. Tunner, Butterworth-

Heinemann, Guilford, London, pp. 113-120, 1991.

- [17] K. Ono, T. Yamamuro, T. Nakamura and T. Kokubo, "Quantitative study on osteoconduction of apatite-wollastonite containing glass ceramic granules, hydroxyapatite granules, and alumina granules", *Biomaterials*, **11**, 265-271 (1990).
- [18] L. L. Hench, "Stability of ceramics in the physiological environment" in *Fundamental aspects of biocompatibility*, Vol. 1, ed. by D. F. Williams, CRC Press, Boca Raton, pp. 67-85, 1981.
- [19] T. Kokubo, H. Kushitani, Y. Ebisawa, T. Kitsugi, S. Kotani, K. Ohura and T. Yamamuro, "Apatite formation on bioactive ceramics in body environment", in *Bioceramics*, Vol. 1, ed. by H. Oonishi, H. Aoki and K. Sawai, Ishiyaku Euro America, Tokyo, pp. 157-162, 1989.
- [20] K. Suzuki, R. Kobayashi, Y. Yokoyama, Y. Harada and T. Kokubo, "Experimental study of an artificial trachea made of polymers coated with hydroxyapatite" in *Bioceramics*, Vol. 6, ed. by P. Ducheyne and D. Christiansen, Butterworth-Heinemann, Guilford London pp. 245-250, 1993.

CHAPTER 3.

IMPROVEMENT IN ADHESION OF APATITE LAYER TO POLYMER SUBSTRATES BY HCl TREATMENT

1. Introduction

A dense and uniform layer of bone-like apatite can be formed on the surfaces of various kinds of organic polymers even in a form of woven fine fiber fabric by the biomimetic process described in Chapter 2 [1]. The apatite-organic polymer composites obtained by this biomimetic process are expected to be useful as bone-repairing materials, because they could exhibit mechanical properties analogous to those of natural bone as well as high bioactivity. In addition, they might be also useful even as soft tissue-repairing materials because they could exhibit ductility as well as high compatibility even with soft tissues [2].

In the present biomimetic process, if the first treatment is omitted, the apatite is never formed. Therefore, the period of time necessary in the first treatment for forming apatite nuclei in sufficient amount to make a continuous layer after the second treatment is important and is hereafter referred to the induction period for the apatite nucleation. The induction periods for the organic polymers were all longer than 12 h as shown in the previous chapter.

The adhesive strength of the apatite layer to the substrates was shown in the previous chapter to vary from almost 0 to 4 MPa, depending on the kind of polymers. The apatite adhered with a strength of about 4 MPa to poly(ethylene terephthalate) (PET) and poly-ether sulfone (PESF), but with very low strength to poly(methyl methacrylate) (PMMA), polyamide 6

(Nylon 6), polyethylene (PE) and poly(tetrafluoroethylene) (PTFE) . In the biomedical application of the apatite-organic polymer composites, it is important that the apatite layer fairly strongly adheres to the substrate.

In this chapter, various kinds of polymer substrates were treated with 1M-HCl aqueous solution, and then to the same biomimetic process as that described in Chapter 2. The effects of the HCl treatment on the induction periods and the adhesive strength of the apatite layer to the substrates were investigated.

2. Experimental

2.1. Preparation of polymer substrate

Poly(ethylene terephthalate) (PET), poly(methyl methacrylate) (PMMA), poly-ether sulfone (PESF), polyamide 6 (Nylon 6), polyethylene (PE) and poly(tetrafluoroethylene) (PTFE) were prepared as the substrates by the same method as that described in Section 2.3. of Chapter 2. They were cut into rectangular specimens 10 mm x 15 mm x 1 mm in size and abraded with #400 diamond paste.

2.2. HCl Treatment

The substrates were soaked in 10 ml of 1M-HCl aqueous solution at 20 °C for various periods from 0 to 10 min . After the HCl treatment, the substrates were washed with the distilled water moderately, and dried at the room temperature.

2.3. Surface Characterization

Organic polymer substrates treated with the HCl aqueous solution for 3 min were placed in vacuo at 60 °C for 1 h to evaporate the moisture completely. Binding energies of carbon and oxygen in the HCl-treated and Untreated organic polymer substrates were measured by X-ray photoelectron spectroscopy (XPS) with an ESCA Model MT5500 (ULVAC-PHI Co. Ltd., Chigasaki, Japan). MgK α X-ray was used as the source. The photoelectron take-off angle (the angle between the sample surface and the detector axis) was set at 45°. This geometry permits the detection of photoelectrons escaping from a depth within the range of 5–10 nm [3, 4]. Measured binding energies were corrected by referring the binding energy of the C1s in CH₂ group as 284.6 eV. Overlapped peaks in XPS spectra were separated by the pattern fitting method using the Gaussian profile for the individual peaks.

2.4. Apatite Coating

Glass particles named G of the nominal composition MgO 4.6, CaO 44.7, SiO₂ 34.0, P₂O₅ 16.2 and CaF₂ 0.5 wt%, the simulated body fluid (SBF) with ion concentrations nearly equal to those of human blood plasma and an aqueous solution with ion concentrations 1.5 times those of SBF (1.5SBF) were prepared by same methods as those described in Sections 2.1. and 2.2 of Chapter 2.

The HCl-treated substrates were first set in contact with the particles of glass G soaked in 30 ml of SBF at 36.5 °C for various periods less than 24 h, as shown in Fig. 1 in Chapter 2. Then, the substrates were soaked in 30 ml of 1.5SBF at 36.5 °C for 6 d. The 1.5SBF solution was renewed every 2 d. After these treatments, the substrate was washed moderately with distilled water, and dried at room temperature.

2.5. Analysis of Apatite

After the second treatment, the surfaces of the substrates were analyzed by thin-film X-ray diffraction with RINT-1400 (Rigaku Co., Tokyo, Japan) and Fourier transform infrared (FT-IR) spectroscopy with Model FT-IR 5M (Japan Spectroscopic Co. Ltd., Tokyo, Japan). Gold-palladium film was coated on the surface of the specimens and scanning electron microscopic images were observed with S-2500CX (Hitachi Co. Ltd., Tokyo, Japan).

2.6. Measurement of Adhesive Strength

The organic polymer substrates on which the apatite layer of about 10 μm thick was formed by the first treatment for 24 h and the second treatment for 6 d were used as the test samples. The adhesive strength of apatite layer formed to the substrates was measured by the same method as that described in Section 2.6. of Chapter 2.

3. Results

3.1. XPS analysis

The C1s and O1s XPS spectra of PET and PMMA treated with HCl for 3 min and those untreated are shown in Figs. 1 and 2, respectively. The C1s peaks were resolved into four components with binding energies of 284.6, 286.8, 287.8 and 288.8 eV, respectively, by line-profile analysis. The main peak at 284.6 eV, was in agreement with the binding energy of the carbon in $-(\text{CH}_2)_n-$ group for substrates. The C1s peaks at about 286.8, 287.8 and 288.8 eV are attributed to the carbon in >C-O or >C-OH , >C=O or $\text{O}-\overset{\text{O}}{\underset{\text{O}}{\text{C}}}-\text{O}$ groups and $\text{O}-\overset{\text{O}}{\underset{\text{O}}{\text{C}}}=\text{O}$ or $\text{H}-\text{O}-\overset{\text{O}}{\underset{\text{O}}{\text{C}}}=\text{O}$ groups, respectively [5, 6].

These three peaks increased by the HCl treatment. Similarly, the O1s peaks were resolved into two components with binding energies of about 531 eV attributed to the oxygen in >C=O group, and of about 533 eV attributed to the oxygen in $\text{H}-\text{O}-\overset{\text{O}}{\underset{\text{O}}{\text{C}}}=\text{O}$ group. The latter increased by the HCl treatment. These results indicate that the oxidized carbon groups increased in the surface region by the HCl treatment. For Nylon 6 similar changes in C1s and O1s peaks were observed as shown in Fig. 3, and it seems that the oxidized carbon groups also increased by the HCl treatment. Figure 4 shows the C1s and O1s XPS spectra of HCl-treated and untreated PESF. In this case, no change was observed in C1s spectra by the HCl treatment, however, the intensity of O1s peaks about 531 eV increased. It seems that the carbon skeleton did not change but the S-O bond changed and SO_2-OH , sulfonic acid groups were formed in the surface region. For PE and PTFE no change in XPS spectra was observed by the HCl treatment. Table 1 lists the area ratio of the peaks of various polar groups separated by line-profile analysis in the XPS spectra of various kinds of HCl-treated and untreated substrates. It is apparent from Table 1 that the ratios of >C-O or >C-OH , >C=O or $\text{O}-\overset{\text{O}}{\underset{\text{O}}{\text{C}}}-\text{O}$ and $\text{O}=\overset{\text{O}}{\underset{\text{O}}{\text{C}}}-\text{O}$ or $\text{O}=\overset{\text{O}}{\underset{\text{O}}{\text{C}}}-\text{O}-\text{H}$ groups in PET, PMMA and Nylon 6 increased by the HCl treatment. This means that the ester groups in PET and PMMA and the amide groups in Nylon 6 were hydrolyzed to the carboxyl groups by the HCl treatment.

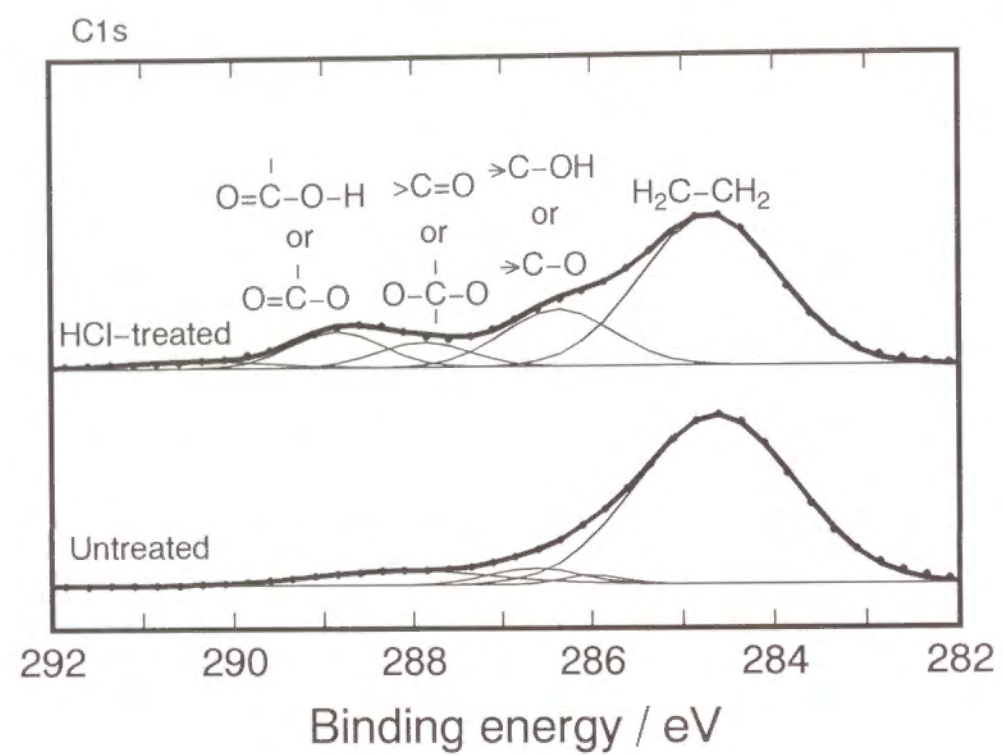


Figure 1. C1s and O1s XPS spectra of PET substrate treated and untreated with 1M-HCl aqueous solution for 3 min.

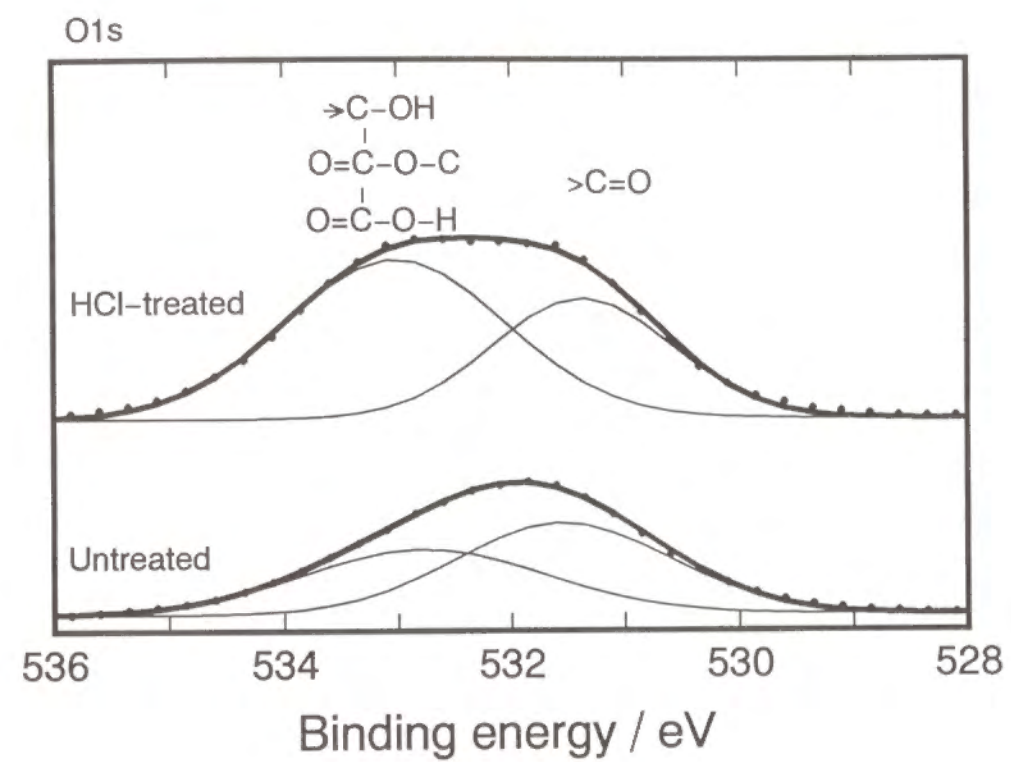


Figure 1. (Continued).

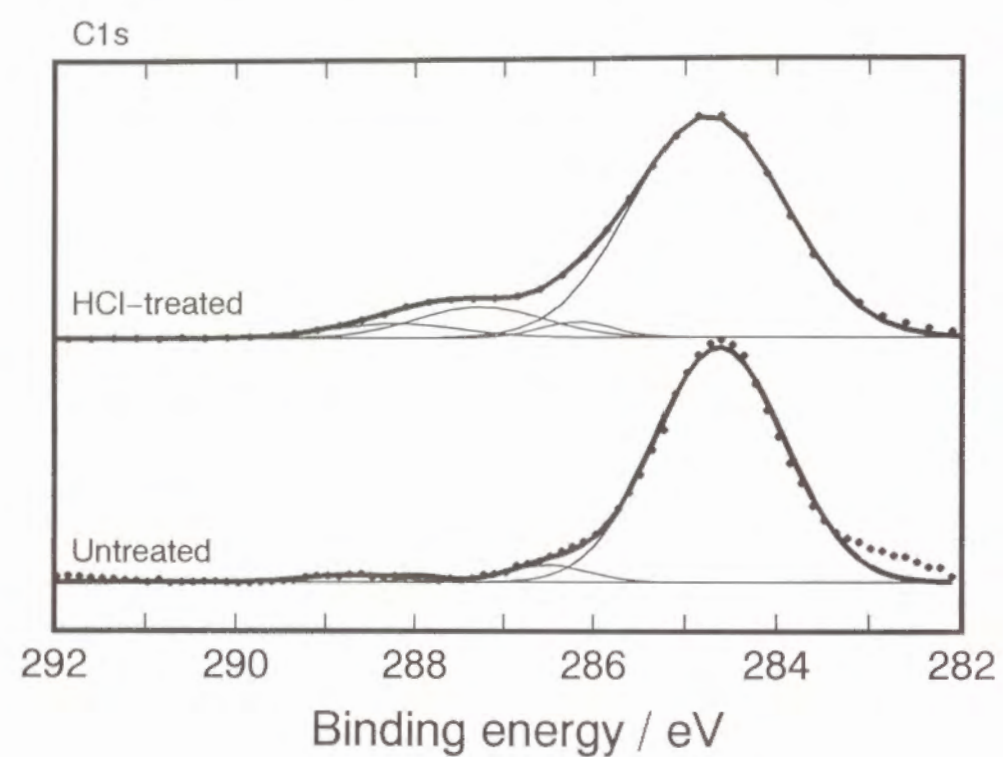


Figure 2. C1s and O1s XPS spectra of PMMA substrate treated and untreated with 1M-HCl aqueous solution for 3 min.

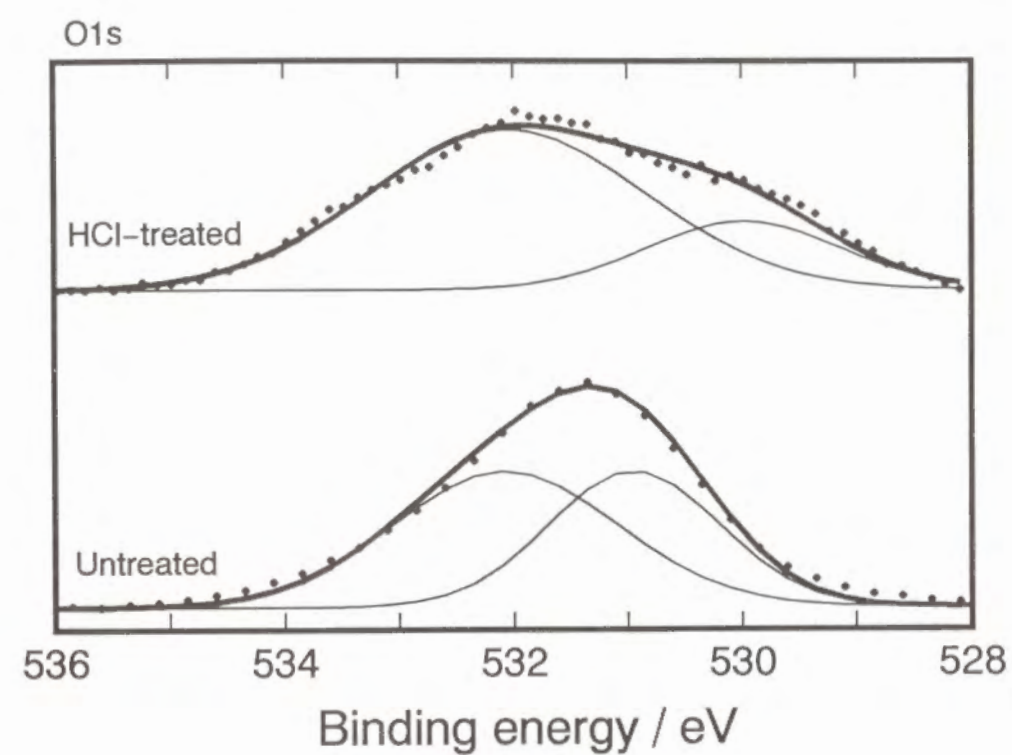


Figure 2. (Continued).

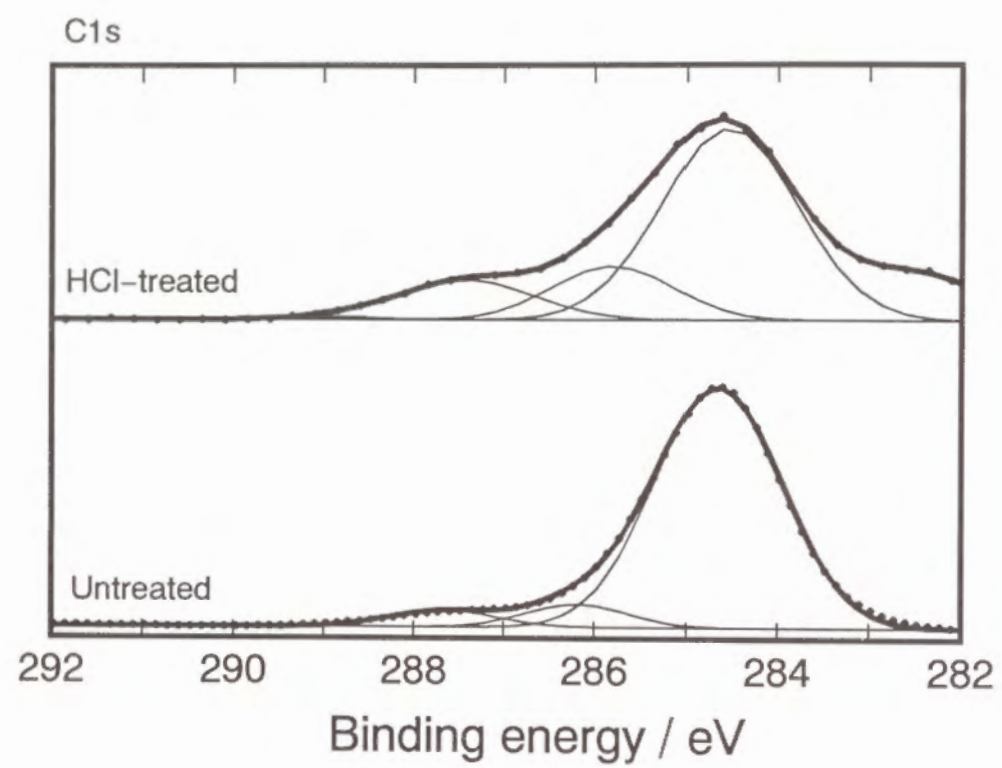


Figure 3. C1s and O1s XPS spectra of Nylon 6 substrate treated and untreated with 1M-HCl aqueous solution for 3 min.

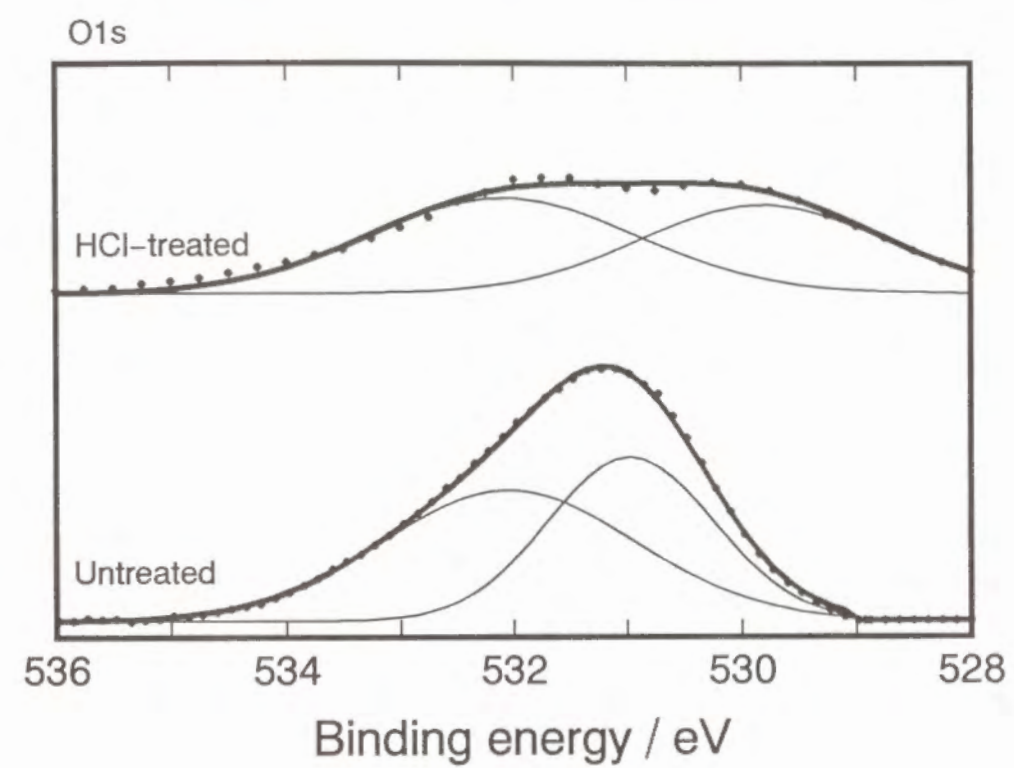


Figure 3. (Continued).

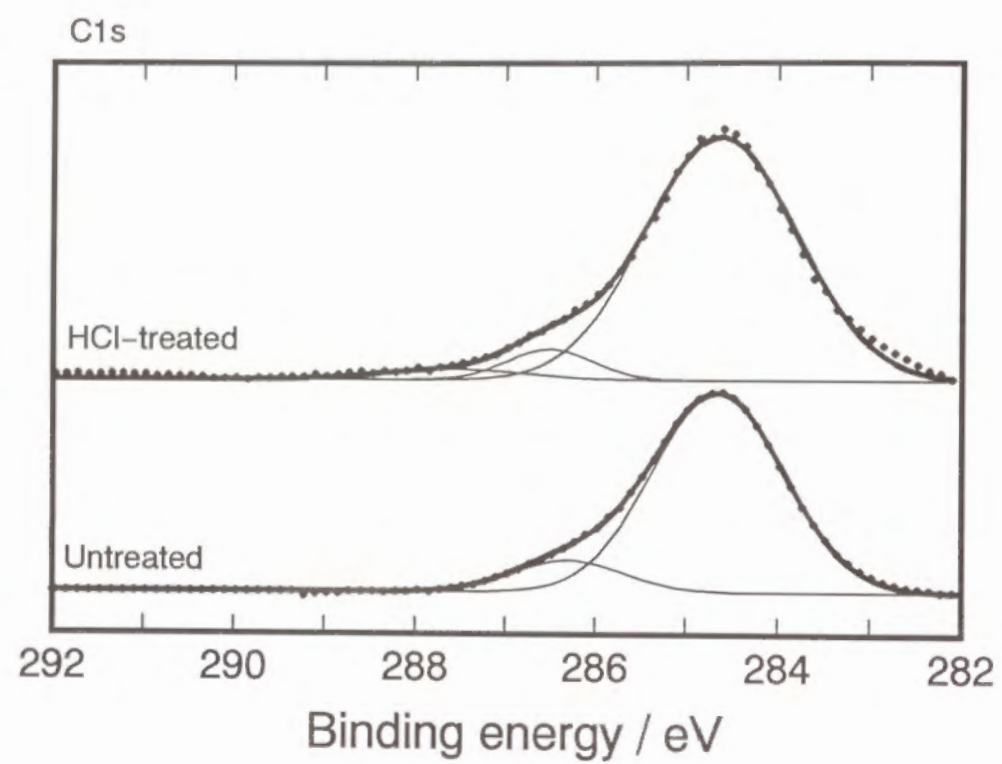


Figure 4. C1s and O1s XPS spectra of PESF substrate treated and untreated with 1M-HCl aqueous solution for 3 min.

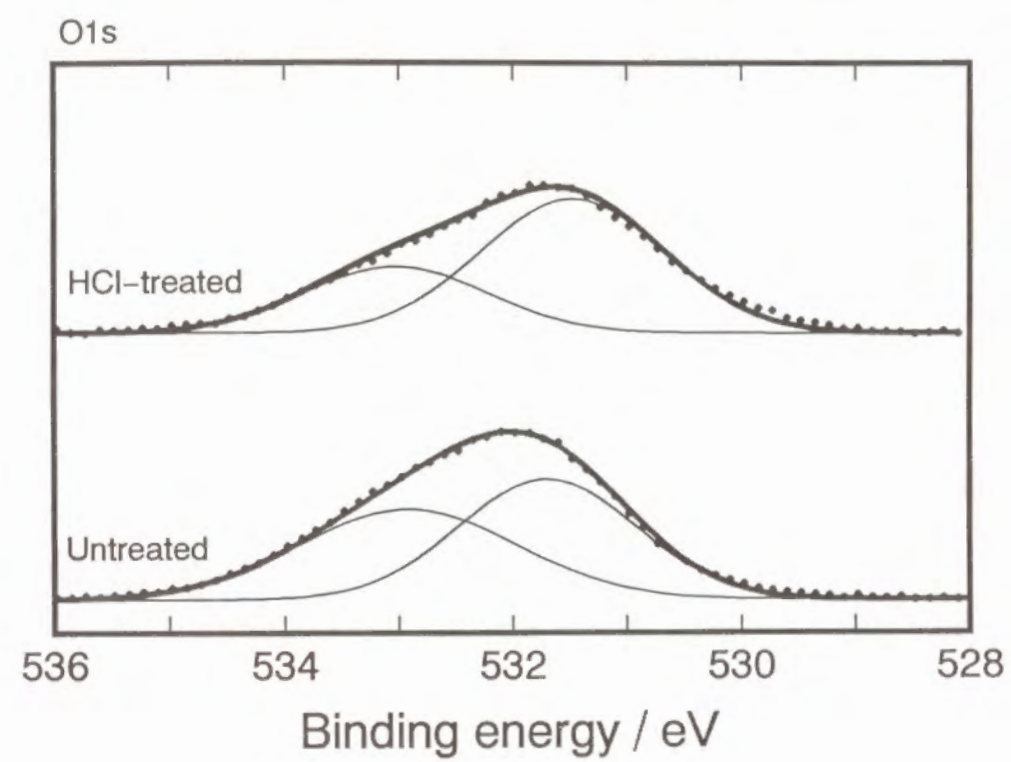


Figure 4. (Continued).

Table 1. Area ratio of functional groups formed on the surface of various organic polymers treated and untreated with 1M-HCl aqueous solution for 3 min.

C1s

Substrate	Untreated							HCl-treated						
	$\text{H}_2\text{C}-\text{CH}_2$	$\text{>C}-\text{O}$	$\text{>C}=\text{O}$	$\text{O}=\text{C}-\text{O}$	O	$\text{F}_2\text{C}-\text{CF}_2$	$\text{F}_2\text{C}-\text{CH}_2$	$\text{H}_2\text{C}-\text{CH}_2$	$\text{>C}-\text{O}$	$\text{>C}=\text{O}$	$\text{O}=\text{C}-\text{O}$	O	$\text{F}_2\text{C}-\text{CF}_2$	$\text{F}_2\text{C}-\text{CH}_2$
		or $\text{>C}-\text{OH}$	or $\text{O}-\text{C}-\text{O}$	or $\text{O}=\text{C}-\text{O}-\text{H}$	 $\text{O}-\text{C}-\text{O}$	or $\text{F}_2\text{C}-\text{CHF}$			or $\text{>C}-\text{OH}$	or $\text{O}-\text{C}-\text{O}$	or $\text{O}=\text{C}-\text{O}-\text{H}$	 $\text{O}-\text{C}-\text{O}$	or $\text{F}_2\text{C}-\text{CHF}$	
PET	84	2	5	9	-	-	-	80	6	7	7	-	-	-
PMMA	82	3	10	15	-	-	-	78	5	11	6	-	-	-
PESF	88	12	-	-	-	-	-	89	7	4	-	-	-	-
Nylon 6	88	7	5	-	-	-	-	88	5	7	-	-	-	-
PE	100	-	-	-	-	-	-	100	-	-	-	-	-	-
PTFE	25	-	-	-	-	69	6	11	-	-	-	-	82	7

- Undetected

Table 1. (Continued)

O1s

Substrate	Untreated				HCl-treated			
	$\text{>C}=\text{O}$	$\text{>C}-\text{OH}$, $\text{O}=\text{C}-\text{O}-\text{C}$	$\text{>SO}-\text{OH}$	$\text{S}-\text{OH}$	$\text{>C}=\text{O}$	$\text{>C}-\text{OH}$, $\text{O}=\text{C}-\text{O}-\text{C}$	$\text{>SO}-\text{OH}$	$\text{S}-\text{OH}$
		or $\text{O}=\text{C}-\text{O}-\text{H}$	or $\text{>SO}_2-\text{OH}$			or $\text{O}=\text{C}-\text{O}-\text{H}$	or $\text{>SO}_2-\text{OH}$	
PET	61	39	-	-	47	53	-	-
PMMA	75	25	-	-	23	77	-	-
PESF	-	-	52	48	-	-	67	33
Nylon 6	60	40	-	-	40	60	-	-
PE	-	-	-	-	-	-	-	-
PTFE	-	-	-	-	-	-	-	-

- Undetected

3.2. Apatite formation

Figures 5 and 6 show thin-film X-ray diffraction patterns and FT-IR reflection spectra, respectively, of the surfaces of the PESF substrates treated with 1M-HCl aqueous solution for 3 min, then subjected to the first treatment for various periods and to the second treatment for 6 d. It can be seen from Figs. 5 and 6 that the apatite formation on the substrate after the second treatment largely depends on the period of the first treatment. When the first treatment was less than 6 h, apatite was not detected on the surface of the substrate.

Figure 7 shows scanning electron microscope photographs of the surfaces of PESF substrates subjected to the same treatments described above. It can be seen from Fig. 7 that the apatite is not formed on the substrates even after the second treatment without the first treatment. The apatite grew only sparsely on the substrate with first treatment shorter than 6 h. With the first treatment longer than 12 h, a dense and uniform apatite layer was formed on the HCl-treated PESF substrates. So the induction period for the apatite nucleation, that is, the period required for the first treatment to form the apatite nuclei in enough number to make the continuous layer after the second treatment was determined as 12 h. The induction periods thus measured for various organic polymers are listed in Table 2. The induction periods were reduced from 24 to 12 h for PET, PMMA, PESF or Nylon 6 by the HCl treatment, but were not changed for PE and PTFE.

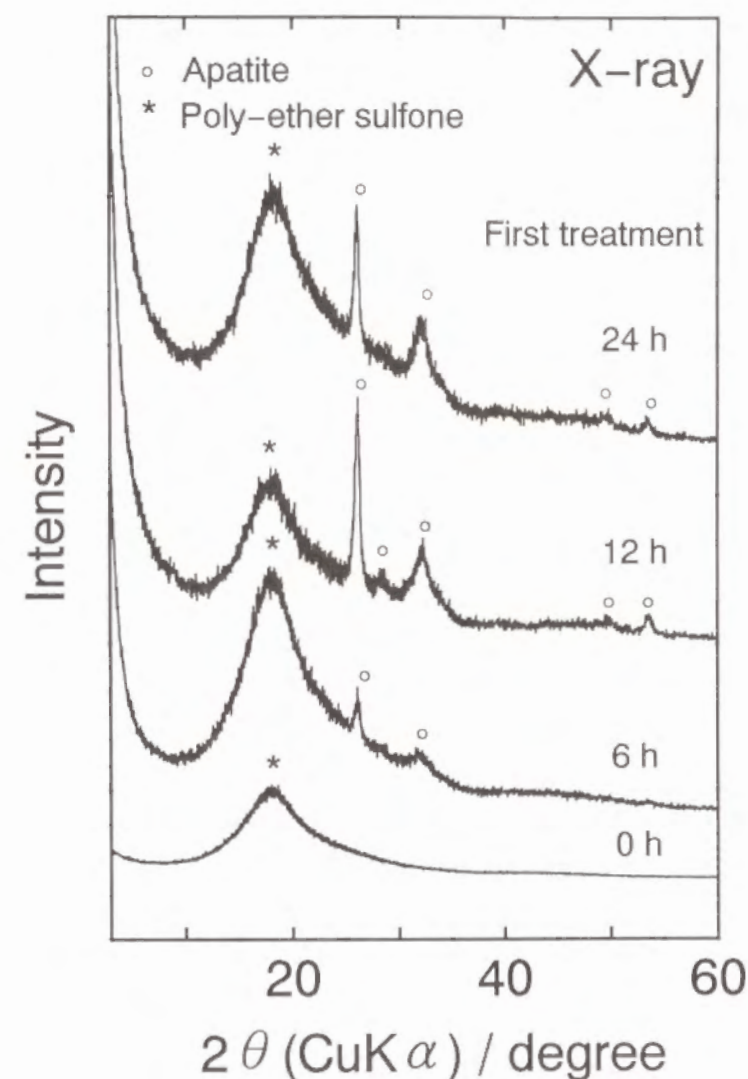


Figure 5. Thin-film XRD patterns of surfaces of HCl-treated PESF substrates soaked in 1.5SBF for 6 d after soaking in SBF with glass G for various periods.

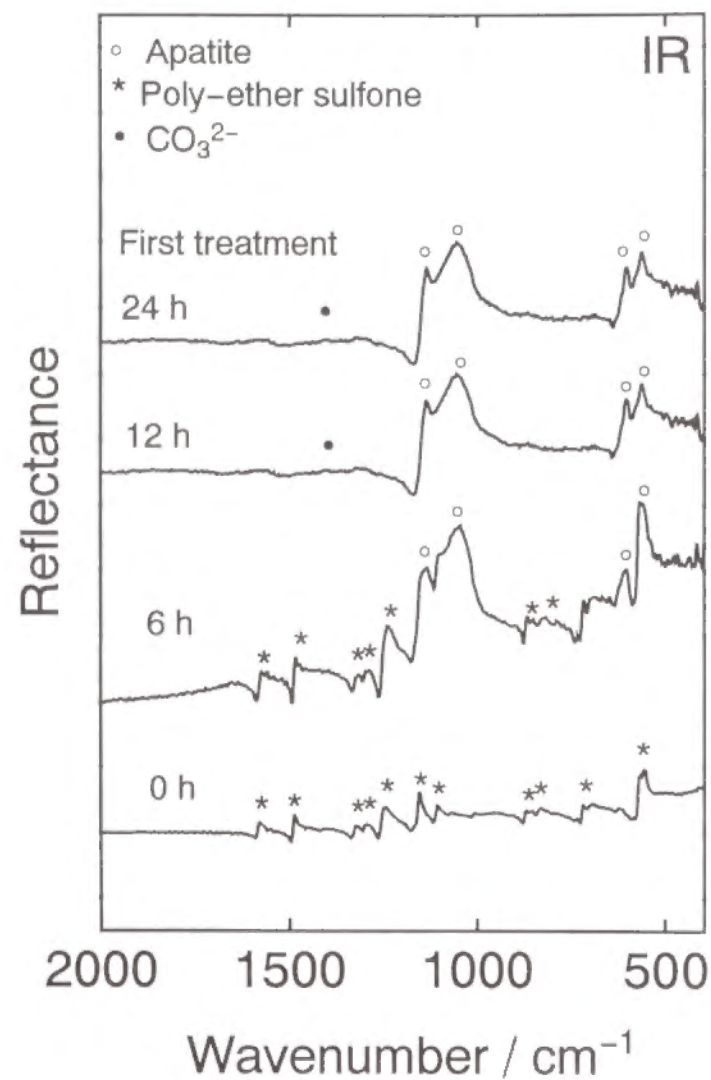


Figure 6. Fourier transform IR reflection spectra of surfaces of HCl-treated PESF substrates soaked in 1.5SBF for 6 d after soaking in SBF with glass G for various periods.

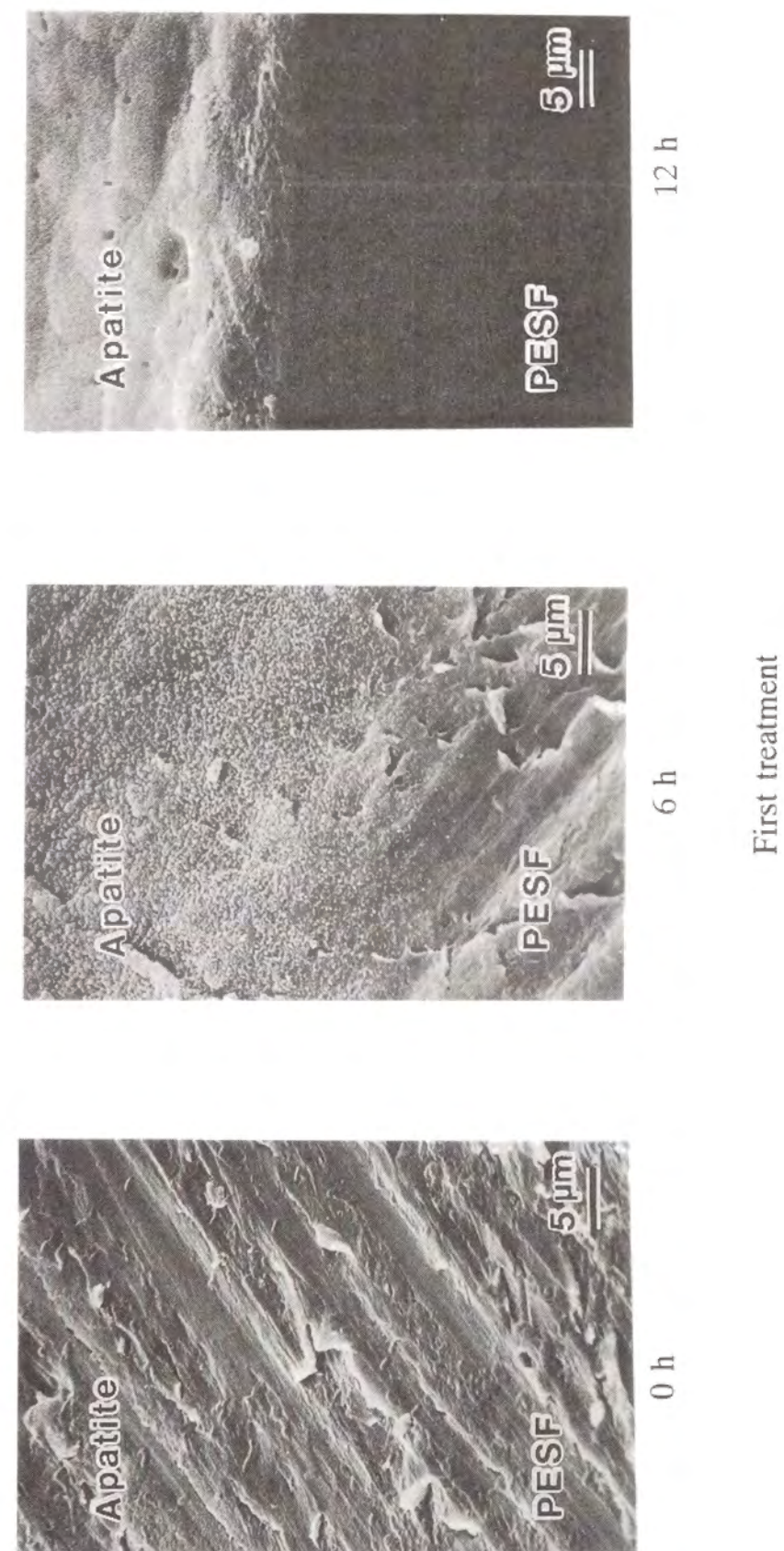


Figure 7. SEM photographs of the surfaces of HCl-treated PESF substrates soaked in 1.5SBF for 6 d after soaking in SBF with glass G for various periods.

Table 2. Induction periods for apatite nucleation on various polymers treated with 1M-HCl aqueous solution in comparison with those untreated.

Substrate	Induction period / h	
	Untreated	HCl-treated
PET	24	12
PMMA	24	12
PESF	24	12
Nylon 6	24	12
PE	24	24
PTFE	24	24

3.3. Adhesive strength

Figure 8 shows the adhesive strength of the apatite layer to organic polymer substrates as a function of HCl treatment time. For PET, PMMA and Nylon 6, the adhesive strength increased from 3.5 to 7.0 MPa, from 1.1 to 2.8 MPa and from 0.6 to 3.1 MPa, respectively, with the HCl treatment for 3 min. They all decreased by the HCl treatment for longer than 3 min. The adhesive strengths decreased with increasing HCl treatment time for PESF and PE from 4.4 to 0.3 and from 1.9 to 0.2 MPa, respectively. For PTFE, the adhesive strength less than 1.1×10^{-2} MPa, was not changed.

4. Discussion

In the first soaking, glass G dissolves calcium and silicate ions in the SBF [7]. It is thought that the calcium ion increases the ionic activity product of the apatite in the SBF, and the silicate ion diffuses and attaches

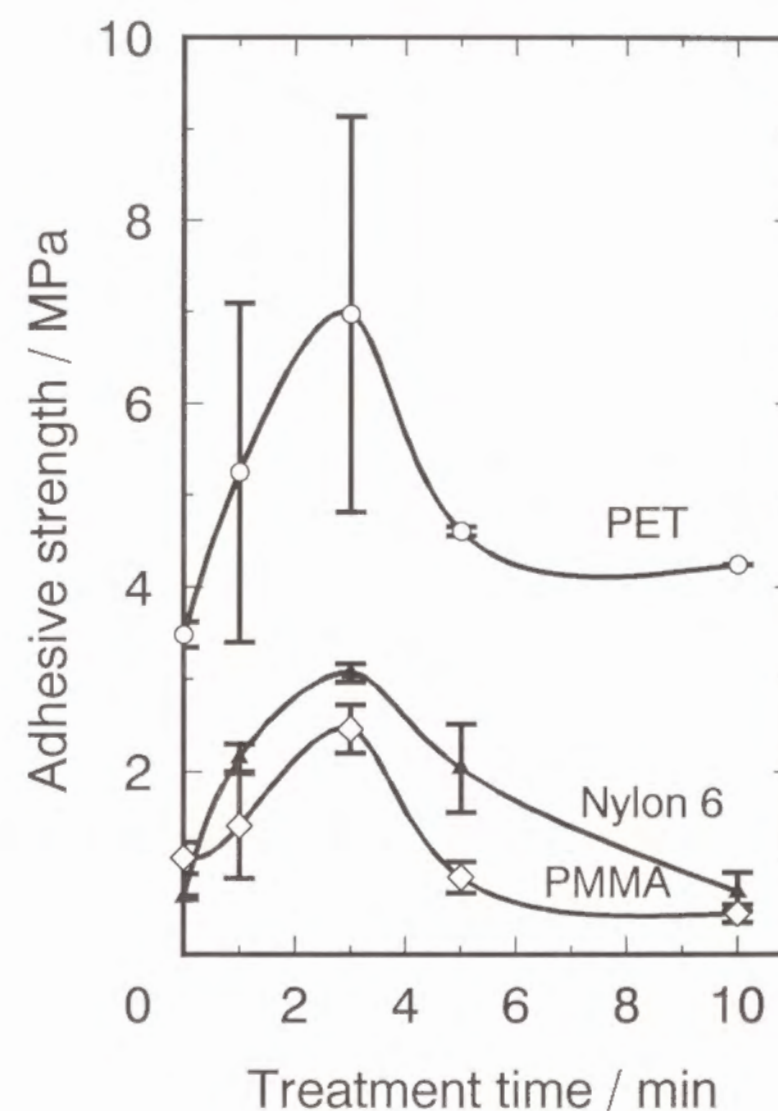


Figure 8. Adhesive strengths of bone-like apatite layer to various substrates as a function of treatment time of 1M-HCl aqueous solution.

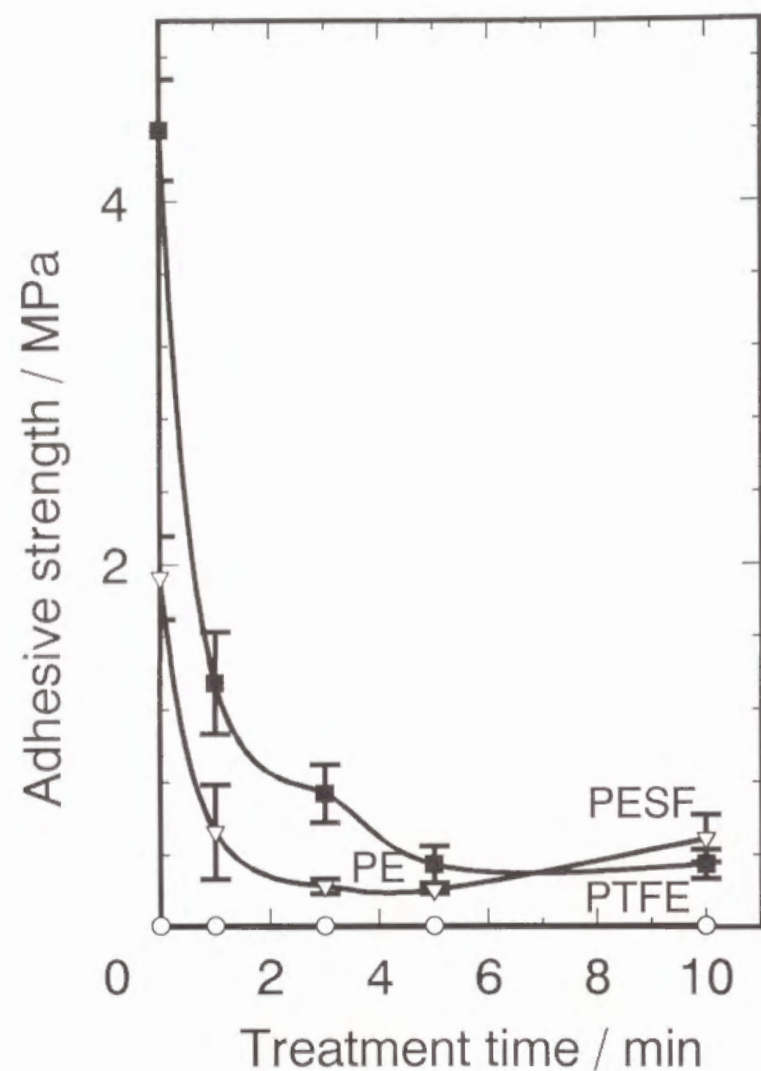
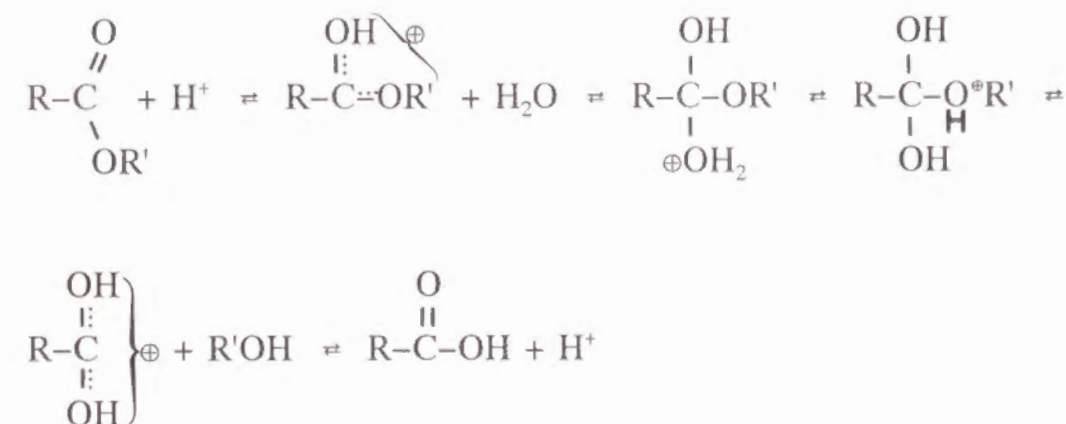


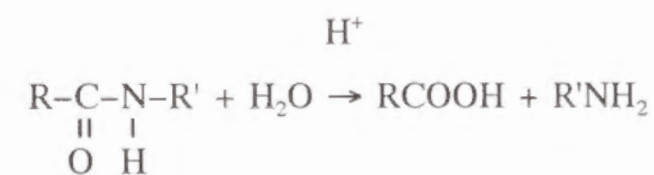
Figure 8. (Continued)

on the surface of the organic polymer substrates, where the apatite nuclei are formed by the similar reaction occurred on the surfaces of the bioactive CaO-SiO₂-based glass [8-10]. Therefore, it is considered that the induction period for the apatite nucleation is closely related with the affinity of the silicate ions to the substrate. The induction period for the apatite nucleation on PET, PMMA, PESF and Nylon 6 was reduced from 24 to 12 h by the HCl treatment. The ester groups in PET and PMMA were confirmed to be hydrolyzed to the carboxylic acid group by the HCl treatment by the following reactions, and the sulfonyl group in PESF was hydrolyzed to the sulfonic acid group (See Table 1).

The hydrolysis reaction of the ester group



The hydrolysis reaction of the amide group



It is considered that these polar groups provide the favorable sites for the silicate ions to bond to the substrates, and hence accelerate the apatite nucleation. On the contrary, the induction periods on PE and PTFE were not reduced. Both PE and PTFE have no ester, amide or other groups to be hydrolyzed to some polar group by the HCl treatment, and hence it is considered that the HCl treatment had no effect on the substrate to increase the affinity with silicate ions.

The adhesive strength of the apatite layer to PET, PMMA and Nylon 6 substrates increased by the HCl treatment. The increase is greatly correlated with the induction period reduction and is considered to be due to the increase of both the number of apatite nuclei and their bonding strength with the substrate caused by the polar group formation. It is also consistent with the fact that no increase of the adhesive strength was observed for PE and PTFE substrates for which the induction periods were not reduced by the HCl treatment. For all these substrates, the adhesive strength decreased with increasing time of HCl treatment for period longer than 3 min. This might be attributed to the decrease in the molecular weight of polymer substrate by the longer HCl treatment and the release of intertwinements among them during the measurement of the adhesive strength. This is fairly consistent with the observation that the fracture was occurred not at the interface between the apatite layer and the substrate but in the surface region of the substrate subjected to longer HCl treatment. For PESF, the increase in the number of the apatite nuclei by the HCl treatment might be cancelled and dominated by the competing reaction that releases the polymer chains. Consequently, decrease in the adhesive strength with the HCl treatment was observed.

5. Conclusion

By the HCl treatment, ester groups in PET and PMMA, amide groups in Nylon 6, and sulfonyl groups in PESF were protonated and then attacked with water, to be hydrolyzed to carboxylic and sulfonic acid groups. These polar groups accelerated the apatite nucleation on the polymer substrates by increasing their affinity to the silicate ions. The adhesive strengths of the apatite layer to PET, PMMA and Nylon 6 increased by the HCl treatment due to the increases in both the number of the apatite nuclei and their bonding strength to the substrates. On the other hand, PE and PTFE, which have no functional groups to be hydrolyzed to some polar groups, showed neither the reduction of the induction period for the apatite nucleation nor the increase of the adhesive strength of the apatite layer to the substrate by the HCl treatment.

The present method can give highly bioactive materials with various mechanical properties, which can be applied to soft tissue-repairing materials as well as bone-repairing materials.

References

- [1] M. Tanahashi, T. Yao, T. Kokubo, M. Minoda, T. Miyamoto, T. Nakamura and T. Yamamuro "Apatite coating on organic polymers by a biomimetic process", *J. Am. Ceram. Soc.*, **77**, 2805-2808 (1994).
- [2] K. Suzuki, R. Kobayashi, Y. Yokoyama, Y. Harada and T. Kokubo, "Experimental study of an artificial trachea made of polymers coated with hydroxyapatite" in *Bioceramics*, Vol. 6, ed. by P. Ducheyne and

- D. Christiansen, Butterworth-Heinemann, Guilford London pp. 245–250, 1993.
- [3] H. Yasuda, H. C. Marsh, S. Brandt and C. N. Reilley, "ESCA study of polymer substrates treated by plasma", *J. Poly. Sci. Polym. Chem. Ed.*, **15**, 991–1019 (1977).
- [4] R. F. Roberts, D. L. Allara, C. A. Pryde, D. N. E. Buchanan and N. D. Hobbins, "Mean free path for in elastic scattering of 1.2 keV electrons in thin poly(methyl methacrylate) films", *Surf. & Interface, Anal.*, **2**, 5–10 (1980).
- [5] D. T. Clark, B. J. Cromarty and A. Dilks, "A theoretical investigation of molecular core binding and relaxation energies in a series of oxygen-containing organic molecules of interest in the study of surface oxidation of polymers", *J. Polym. Sci. Polym. Chem. Ed.*, **16**, 3173–3184 (1978).
- [6] A. Dilks, and A. VanLaeken, "Physicochemical aspects of polymer surfaces", ed. by K. L. Mittal, Plenum, New York, p. 749, 1983.
- [7] T. Kokubo, H. Kushitani, C. Ohtsuki, S. Sakka, and T. Yamamuro, "Chemical reaction of bioactive glass and glass-ceramics with a simulated body fluid" *J. Mater. Sci: Mater. Medicine*, **3**, 79–83 (1992).
- [8] C. Ohtsuki, T. Kokubo, K. Takatsuka and T. Yamamuro, "Compositional dependence of bioactivity of glasses in the system $\text{CaO-SiO}_2\text{-P}_2\text{O}_5$: its *in vitro* evaluation" *J. Ceram. Soc. Japan*, **99**, 1–6 (1991).
- [9] C. Ohtsuki, T. Kokubo and T. Yamamuro, "Mechanism of apatite formation on $\text{CaO-SiO}_2\text{-P}_2\text{O}_5$ glasses in a simulated body fluid", *J. Non-Crystl. Solids*, **143**, 84–92 (1992).

- [10] P. Li, C. Ohtsuki, T. Kokubo, K. Nakanishi, N. Soga, T. Nakamura, and T. Yamamuro, "Apatite formation induced by silica gel in a simulated body fluid" *J. Am. Ceram. Soc.*, **75**, 2094–2097 (1992).

CHAPTER 4.

IMPROVEMENT IN ADHESION OF APATITE LAYER TO POLYMER SUBSTRATES BY NaOH TREATMENT

1. Introduction

A dense and uniform layer of bone-like apatite can be formed on the surfaces of various kinds of organic polymers even in a form of woven fine fiber fabric by the biomimetic process described in Chapter 2 [1].

The induction periods for the apatite nucleation that are defined as the times of the first treatment which is required for forming sufficient amount of apatite nuclei to make a continuous layer after the second treatment were shown in Chapter 2 to be 24 h for as-prepared poly(ethylene terephthalate) (PET), poly(methyl methacrylate) (PMMA), poly-ether sulfone (PESF) and polyamide 6 (Nylon 6) substrates, whereas in Chapter 3, 12 h for those treated with HCl aqueous solution.

The adhesive strengths of the formed apatite layer to the polymer substrates were shown in Chapter 2 to vary from almost 0 to 4 MPa, depending on the kind of the polymers, and in Chapter 3 to increase by 2 to 5 times for PET, PMMA and Nylon 6 with the HCl treatment.

In this chapter, various kinds of organic polymers were first subjected to the 5M-NaOH aqueous solution, and then to the same biomimetic process as that described in Chapter 2. The effects of the NaOH treatment on the induction periods for the apatite nucleation and the adhesive strength of the apatite layer to the substrates were investigated.

2. Experimental

2.1. Preparation of polymer substrate

Poly(ethylene terephthalate) (PET), poly(methyl methacrylate) (PMMA), poly-ether sulfone (PESF), polyamide 6 (Nylon 6), polyethylene (PE) and poly(tetrafluoroethylene) (PTFE) were prepared as the substrates by the same method as that described in Chapter 2. They were cut into rectangular specimens 10 mm x 15 mm x 1 mm in size and abraded with #400 diamond paste.

2.2. NaOH treatment

The substrates described above were soaked in 10 ml of 5M-NaOH aqueous solution at 20 °C for various periods from 0 to 60 min. After the NaOH treatment, the substrates were washed under a slow flow of the distilled water, and dried at the room temperature.

2.3. Surface Characterization

Organic polymer substrates treated with the NaOH aqueous solution for 10 min were placed in vacuo at 60 °C for 1 h to evaporate the moisture completely. Binding energies of carbon and oxygen in the NaOH-treated and untreated organic polymer substrates were measured by X-ray photoelectron spectroscopy (XPS) with an ESCA Model MT5500 (ULVAC-PHI Co. Ltd., Chigasaki, Japan) under the same conditions as those described in Chapter 3. Measured binding energies were corrected by referring the binding energy of the C1s in CH₂ group as 284.6 eV. Overlapped peaks in XPS spectra were separated by the pattern fitting method using the Gaussian profile for the individual peaks.

2.4. Apatite Coating

Glass particles named G of the nominal composition MgO 4.6, CaO 44.7, SiO₂ 34.0, P₂O₅ 16.2 and CaF₂ 0.5 wt%, the simulated body fluid (SBF) with ion concentrations nearly equal to those of human blood plasma and an aqueous solution with ion concentrations 1.5 times those of SBF (1.5SBF) were prepared by the same methods as those described in Sections 2.1. and 2.2. of Chapter 2.

The NaOH-treated substrates were set in contact with the particles of glass G soaked in 30 ml of SBF at 36.5 °C for various periods less than 24 h, as shown in Fig. 1 in Chapter 2. Then, the substrates were soaked in 30 ml of 1.5SBF at 36.5 °C for 6 d. The 1.5SBF solution was renewed every 2 d. After these treatments, the substrate was washed under the slow flow from the distilled water, and dried at room temperature.

2.5. Analysis of Apatite

The surfaces of the NaOH-treated substrates after the second treatment were analyzed by thin-film X-ray diffraction with RINT-1400 (Rigaku Co., Tokyo, Japan) and Fourier transform infrared (FT-IR) spectroscopy with Model FT-IR 5M (Japan Spectroscopic Co. Ltd., Tokyo, Japan). Gold-palladium film was coated on the surface of the specimens and scanning electron microscopic images were observed with S-2500CX (Hitachi Co. Ltd., Tokyo, Japan).

2.6. Measurement of Adhesive Strength

The organic polymer substrates on which the apatite layer of about 10 μm thick was formed by the first treatment for 24 h and the second treatment for 6 d were used as the test samples. The adhesive strengths of apatite layer formed to the substrates was measured by the same method as

that described in Section 2.6. of Chapter 2.

2.7. AFM Observation

The PET particles 2 mm x 3 mm x 2 mm in size were put on the platinum plate and soften at 350 °C for a few minutes to form a smooth free surface, then quenched by dropping into distilled water. The PET substrates thus obtained were treated with 5M-NaOH aqueous solution for 10 min at 20 °C, and then washed with the distilled water. The NaOH-treated and untreated PET substrates were subjected to the first treatment of the biomimetic process described above for various periods less than 24 h. After the treatment, the substrate was washed under the slow flow of the distilled water, and dried at room temperature. The surfaces of these PET substrates were observed with atomic force microscope (AFM) with Nanoscope III (Digital Instruments Inc. U. S. A.) in tapping mode.

3. Results

3.1. XPS Analysis

The C1s and O1s XPS spectra of PET and PMMA treated with NaOH for 10 min and those untreated are shown in Figs. 1 and 2, respectively. The C1s peaks were resolved into four components with binding energies of 284.6, 286.8, 287.8 and 288.8 eV, respectively, by line-profile analysis. The main peak at 284.6 eV, was in agreement with the binding energy of the carbon in $-(CH_2)_n-$ group for substrates. The C1s peaks at about 286.8, 287.8 and 288.8 eV are attributed to the carbons in $\rightarrow C-O$ or $\rightarrow C-OH$, $>C=O$ or $O-\overset{\overset{|}{\text{C}}}{\underset{\underset{|}{|}}{\text{C}}}-O$ groups and $O-\overset{\overset{|}{\text{C}}}{\underset{\underset{|}{|}}{\text{C}}}=O$ or $H-O-\overset{\overset{|}{\text{C}}}{\underset{\underset{|}{|}}{\text{C}}}=O$ groups, respectively [3, 4]. These three peaks increased by the NaOH

treatment. Similarly, the O1s peaks were resolved into two components with binding energies of about 531 eV attributed to the oxygen in $>C=O$ group, and of about 533 eV attributed to the oxygen in $H-O-\overset{\overset{|}{\text{C}}}{\underset{\underset{|}{|}}{\text{C}}}=O$ group. The latter increased by the NaOH treatment. These results indicate that the oxidized carbon groups increased in the surface region by the NaOH treatment. For Nylon 6, similar changes in C1s and O1s peaks were observed as shown in Fig. 3, and it seems that the oxidized carbon groups also increased by the NaOH treatment. Figure 4 shows the C1s and O1s XPS spectra of NaOH-treated and untreated PESF. In this case, no change was observed in C1s spectra by the NaOH treatment, however, the intensity of O1s peaks about 531 eV increased. It seems that the carbon skeleton did not change but the S-O bond changed and SO_2-OH , sulfonic acid groups were formed in the surface region. For PE and PTFE, no changes in XPS spectra were observed by the NaOH treatment.

Table 1 lists the area ratio of the peaks of various polar groups separated by line-profile analysis in the XPS spectra of various kinds of NaOH-treated and untreated substrates. It is apparent from Table 1 that the ratios of $\rightarrow C-O$ or $\rightarrow C-OH$, $>C=O$ or $O-\overset{\overset{|}{\text{C}}}{\underset{\underset{|}{|}}{\text{C}}}-O$ and $O=\overset{\overset{|}{\text{C}}}{\underset{\underset{|}{|}}{\text{C}}}-O$ or $O=\overset{\overset{|}{\text{C}}}{\underset{\underset{|}{|}}{\text{C}}}-O-H$ groups in PET, PMMA and Nylon 6 increased by the NaOH treatment. This means that the ester group in PET and PMMA and the amide group in Nylon 6 were hydrolyzed to the carboxylic acid group by the NaOH treatment.

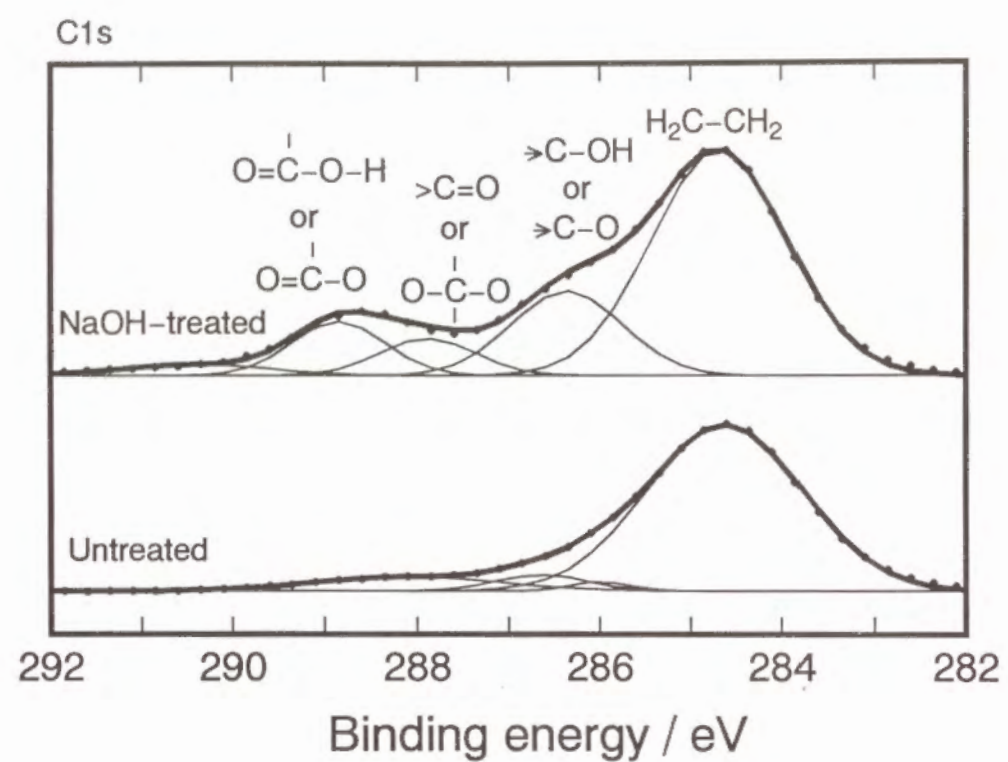


Figure 1. C1s and O1s XPS spectra of PET substrate treated and untreated with 5M-NaOH aqueous solution for 10 min.

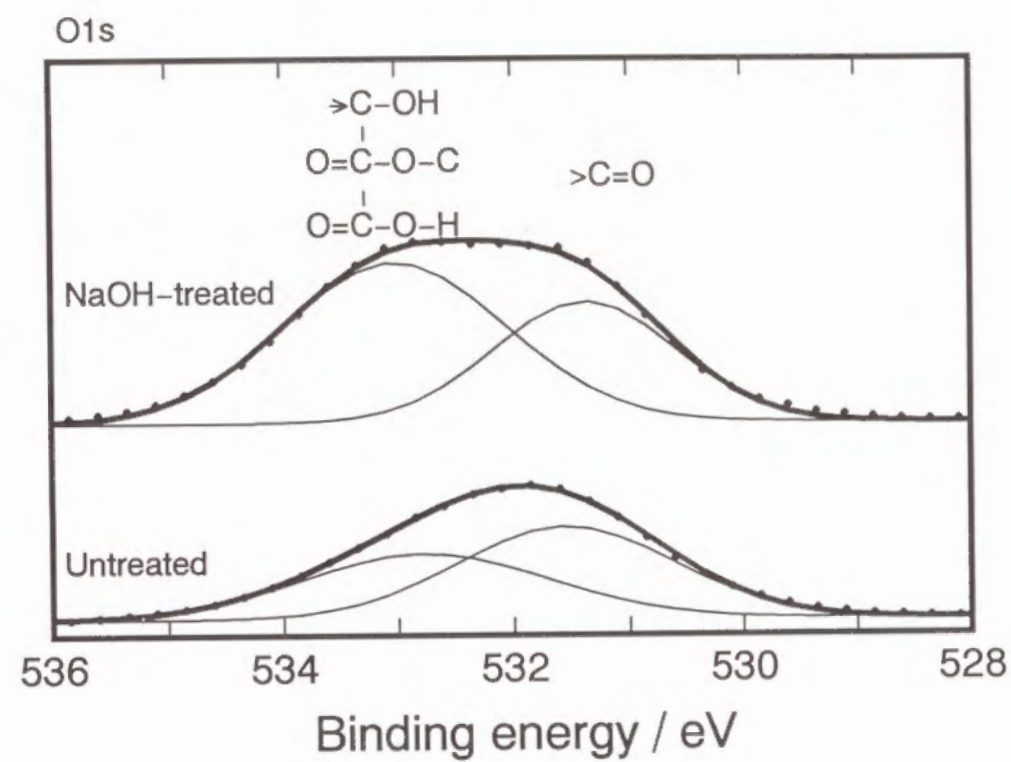


Figure 1. (Continued).

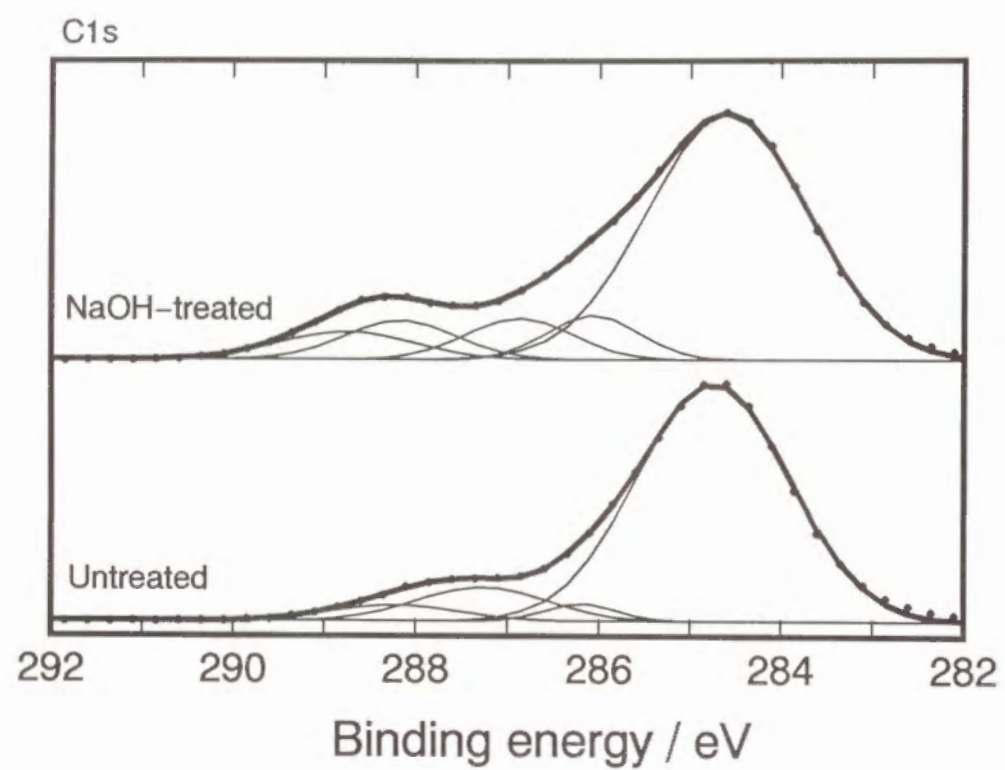


Figure 2. C1s and O1s XPS spectra of PMMA substrate treated and untreated with 5M-NaOH aqueous solution for 10 min.

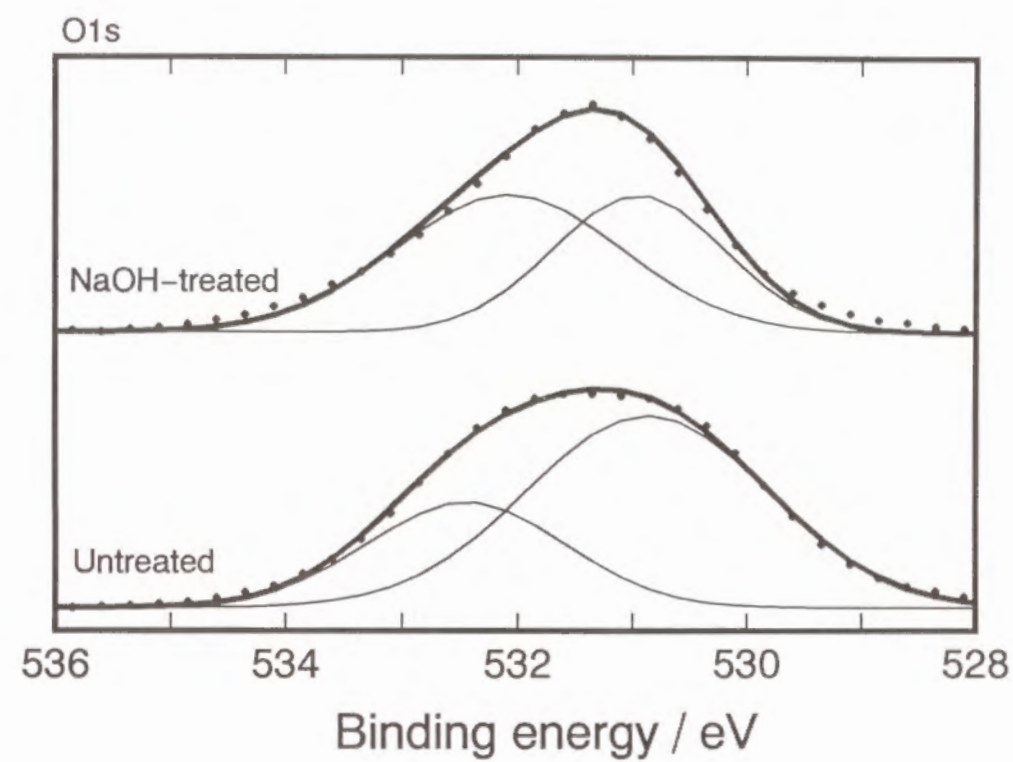


Figure 2. (Continued).

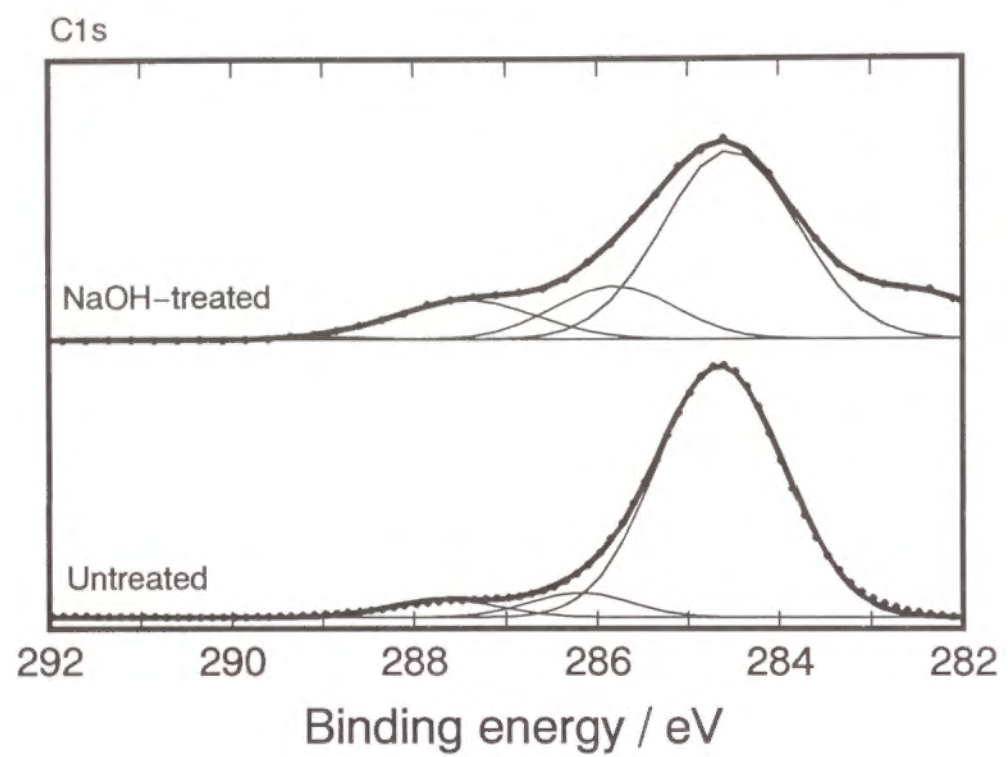


Figure 3. C1s and O1s XPS spectra of Nylon 6 substrate treated and untreated with 5M-NaOH aqueous solution for 10 min.

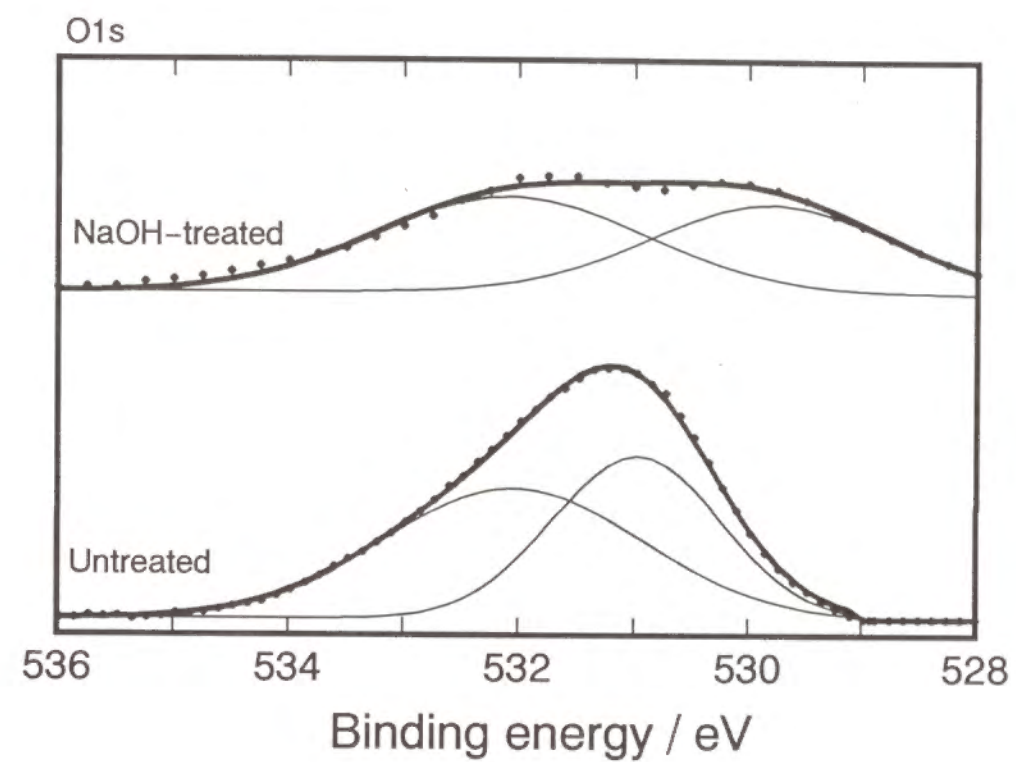


Figure 3. (Continued).

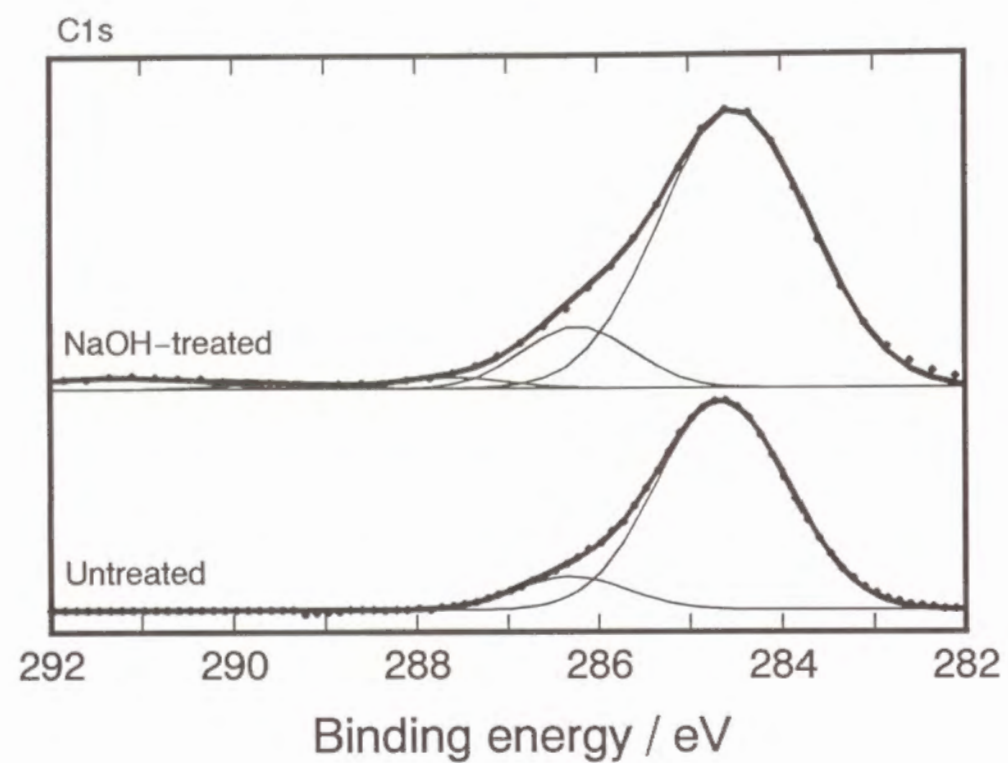


Figure 4. C1s and O1s XPS spectra of PESF substrate treated and untreated with 5M-NaOH aqueous solution for 10 min.

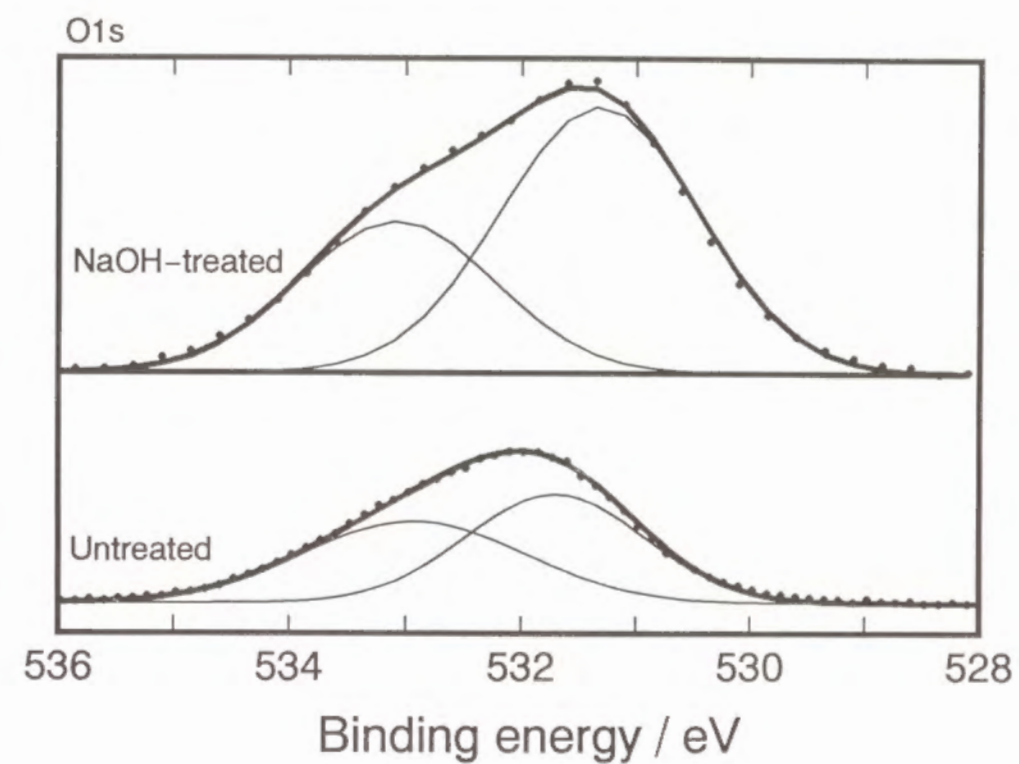


Figure 4. (Continued).

Table 1. Area ratio of functional groups formed on the surface of various organic polymers treated and untreated with 5M-NaOH aqueous solution for 10 min.

C1s

Substrate	Untreated							NaOH-treated						
	H ₂ C-CH ₂	→C-O	>C=O	O=C-O	O	F ₂ C-CF ₂	F ₂ C-CH ₂	H ₂ C-CH ₂	→C-O	>C=O	O=C-O	O	F ₂ C-CF ₂	F ₂ C-CH ₂
		or →C-OH	or O-C-O	or O=C-O-H	 O-C-O	or F ₂ C-CHF			or →C-OH	or O-C-O	or O=C-O-H	 O-C-O	or F ₂ C-CHF	
PET	84	2	5	9	-	-	-	61	19	7	10	3	-	-
PMMA	82	3	10	15	-	-	-	70	7	8	8	7	-	-
PESF	88	12	-	-	-	-	-	80	13	7	-	-	-	-
Nylon 6	88	7	5	-	-	-	-	89	4	7	-	-	-	-
PE	100	-	-	-	-	-	-	100	-	-	-	-	-	-
PTFE	25	-	-	-	-	69	6	11	-	-	-	-	82	7

- Undetected

Table 1. (Continued)

O1s

Substrate	Untreated				NaOH-treated			
	>C=O	→C-OH, O=C-O-C	→SO-OH	S-OH	>C=O	→C-OH, O=C-O-C	→SO-OH	S-OH
		or O=C-O-H	or >SO ₂ -OH			or O=C-O-H	or >SO ₂ -OH	
PET	61	39	-	-	38	62	-	-
PMMA	75	25	-	-	69	31	-	-
PESF	-	-	52	48	-	-	63	37
Nylon 6	60	40	-	-	45	55	-	-
PE	-	-	-	-	-	-	-	-
PTFE	-	-	-	-	-	-	-	-

- Undetected

3.2. Apatite Formation

Figures 5 and 6 show thin-film X-ray diffraction patterns and FT-IR spectra, of the surfaces of the PESF substrates treated with 5M-NaOH aqueous solution for 10 min, then subjected to the first treatment for various periods and to the second treatment for 6 d. Assignment of main peaks on these figures were done by referring the data for sintered hydroxyapatite which was previously published [5]. It can be seen from Figs. 5 and 6 that the apatite formation on the substrate after the second treatment largely depends on the period of the first treatment. When the first treatment was less than 6 h, apatite was not detected on the surface of the substrate.

Figure 7 shows that scanning electron microscope photographs of the surfaces of PESF substrates subjected to the same treatments described above. It can be seen from Fig. 7 that the apatite is not formed on the substrates even after the second treatment without the first treatment. The apatite grew only sparsely on the substrate with first treatment shorter than 6 h. With the first treatment longer than 12 h, a dense and uniform apatite layer about 10 μm thick was formed on the NaOH-treated PESF substrates. So the induction period for the apatite nucleation, that is, the period of the first treatment which is required for forming a sufficient amount of apatite nuclei to make the continuous layer after the second treatment was determined to be 12 h. The induction periods thus measured for various organic polymers in Section 2.1. are listed in Table 2. The induction period decreased to 12 h for PET, PMMA, PESF or Nylon 6 with the NaOH treatment, but did not change for PE and PTFE.

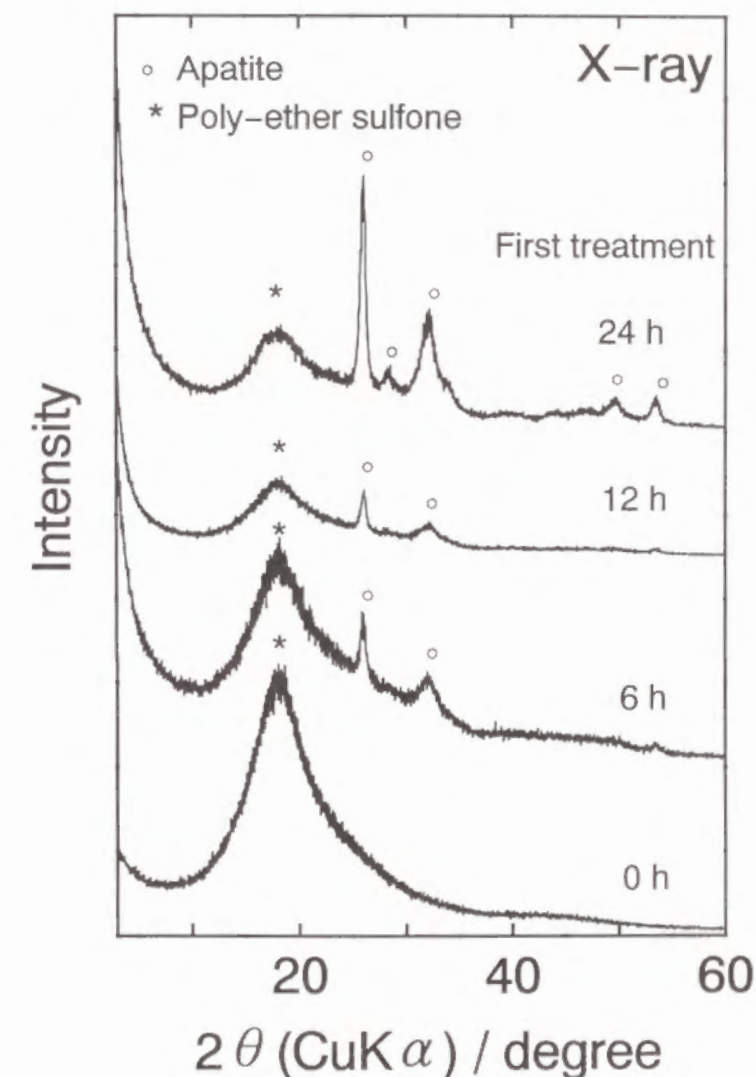


Figure 5. Thin-film XRD patterns of surfaces of NaOH-treated PESF substrates soaked in 1.5SBF for 6 d after soaking in SBF with glass G for various periods.

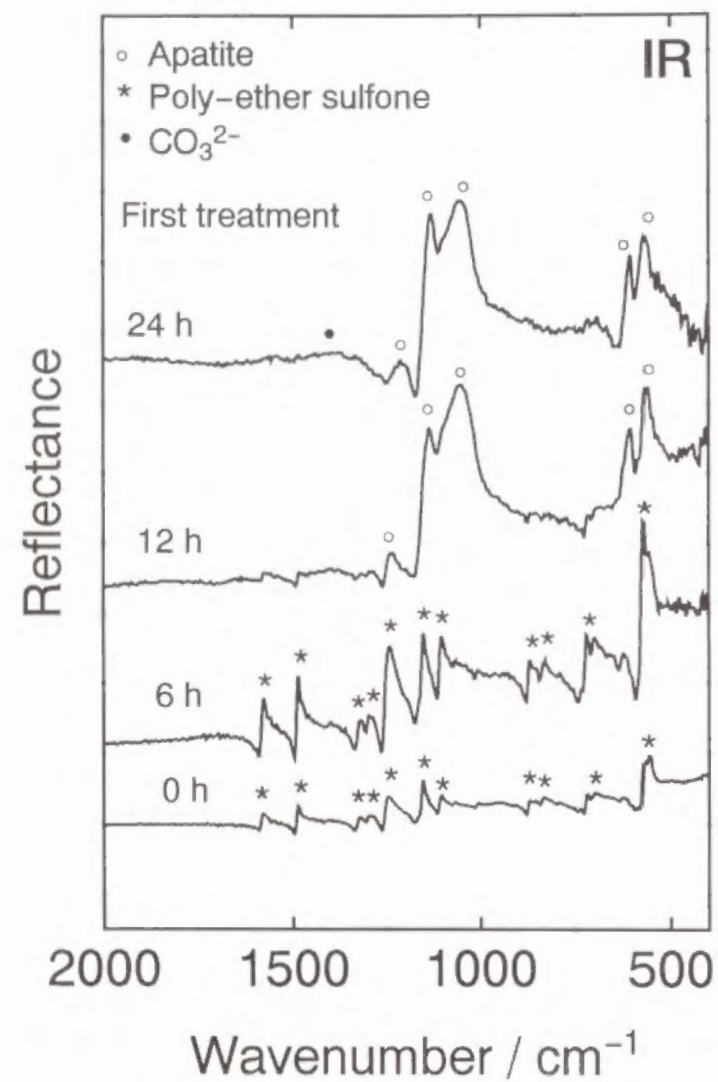


Figure 6. Fourier transform IR reflection spectra of surfaces of NaOH-treated PESF substrates soaked in 1.5SBF for 6 d after soaking in SBF with glass G for various periods.

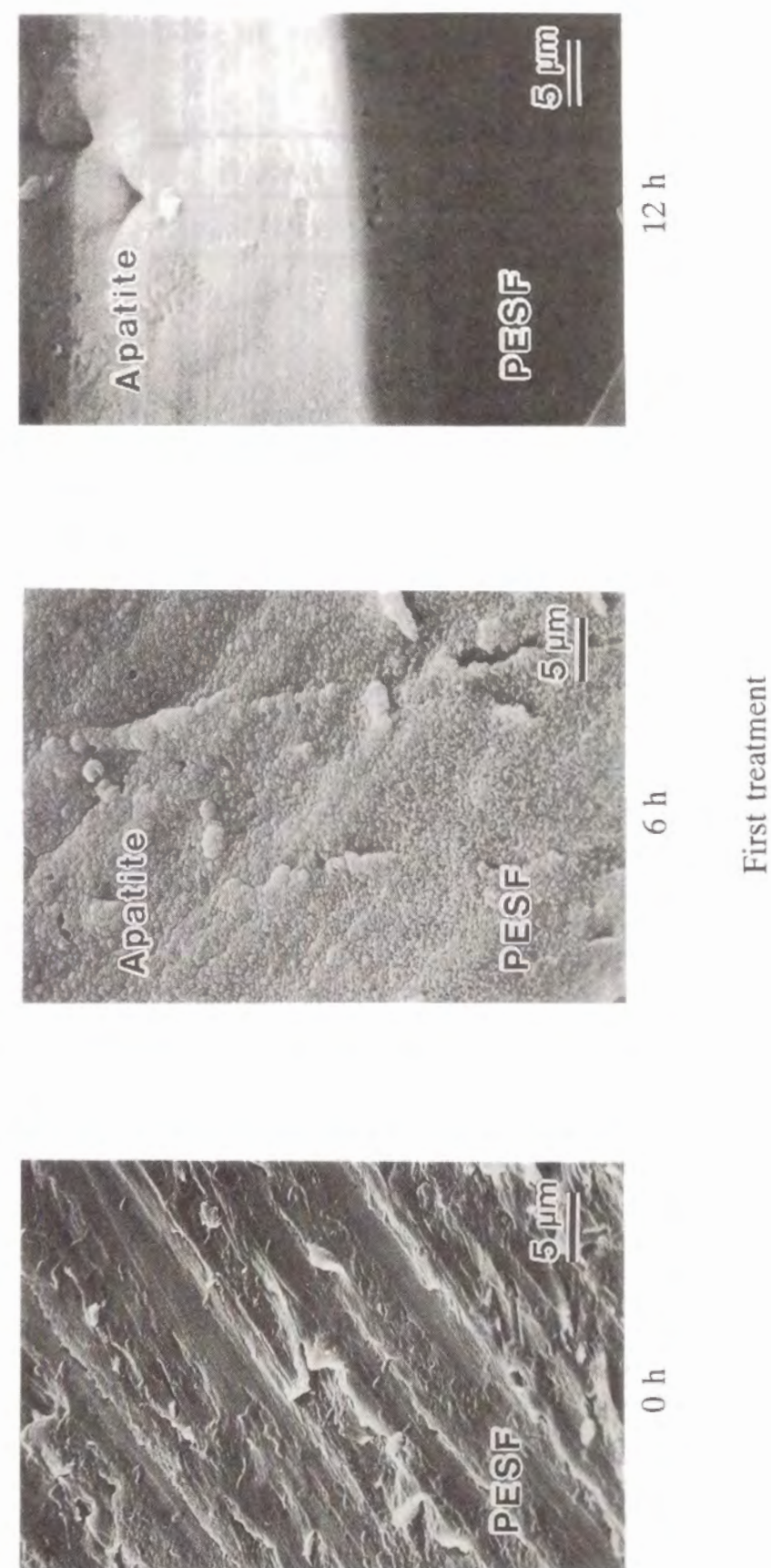


Figure 7. SEM photographs of the surfaces of NaOH-treated PESF substrates soaked in 1.5SBF for 6 d after soaking in SBF with glass G for various periods.

Table 2. Induction periods for apatite nucleation on various polymers treated with 5M-NaOH aqueous solution in comparison with those untreated.

Substrate	Induction period / h	
	Untreated	NaOH-treated
PET	24	12
PMMA	24	12
PESF	24	12
Nylon 6	24	12
PE	24	24
PTFE	24	24

3.3. Adhesive Strength

Figure 8 shows the adhesive strength of the apatite layer to polymer substrates as a function of NaOH-treatment time. For PET, PMMA and Nylon 6, the adhesive strength increased from 3.5 to 8.6 MPa, from 1.1 to 3.4 MPa and from 0.6 to 5.3 MPa, respectively, by 10 min.-treatment with NaOH aqueous solution. All of the substrates examined decreased by the NaOH treatment for longer than 10 min. The adhesive strength decreased with increasing NaOH treatment time for PESF and PE from 4.4 to 1.6 and from 1.9 to 0.8 MPa, respectively. For PTFE, the adhesive strength less than 1.1×10^{-2} MPa, did not change.

3.4. Atomic Force Microscopic Image

Figures 9 (a) and (b) show the AFM images of PET substrates treated and untreated with 5M-NaOH aqueous solution, respectively. As shown in Figs. 9, the surface of the PET was roughed by NaOH treatment. Figures 9 (c) and (d) show the AFM image of PET substrates treated and untreated

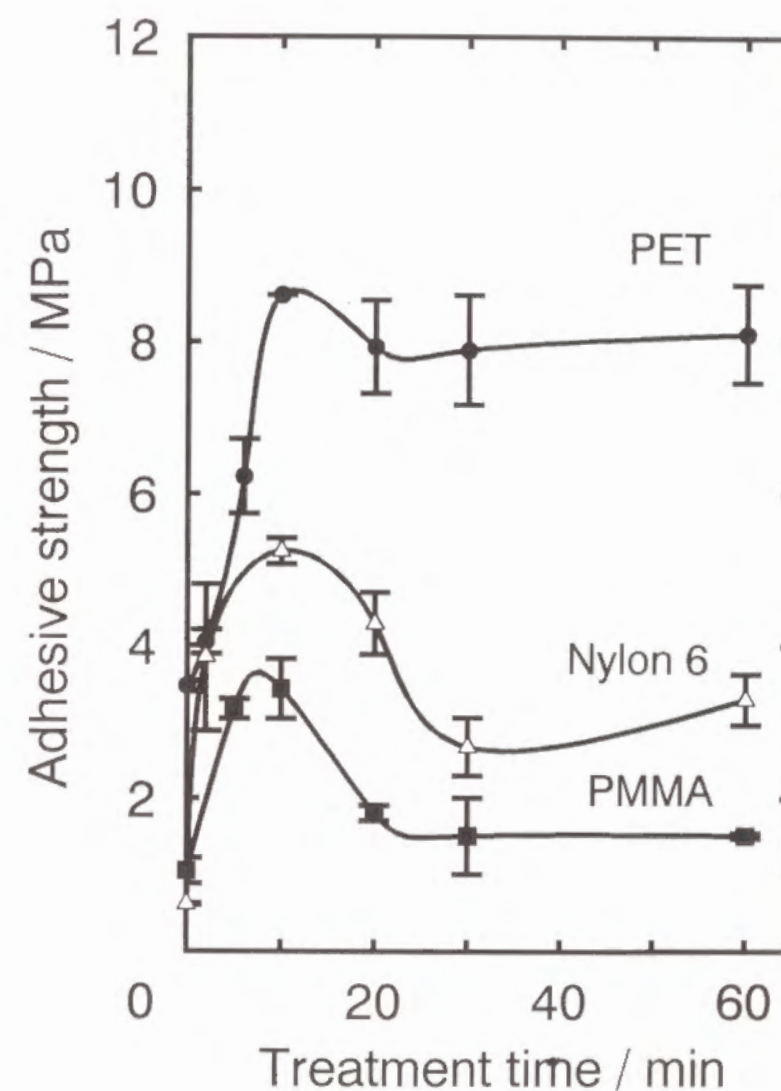


Figure 8. Adhesive strengths of bone-like apatite layer to various substrates as a function of treatment time of 5M-NaOH aqueous solution.

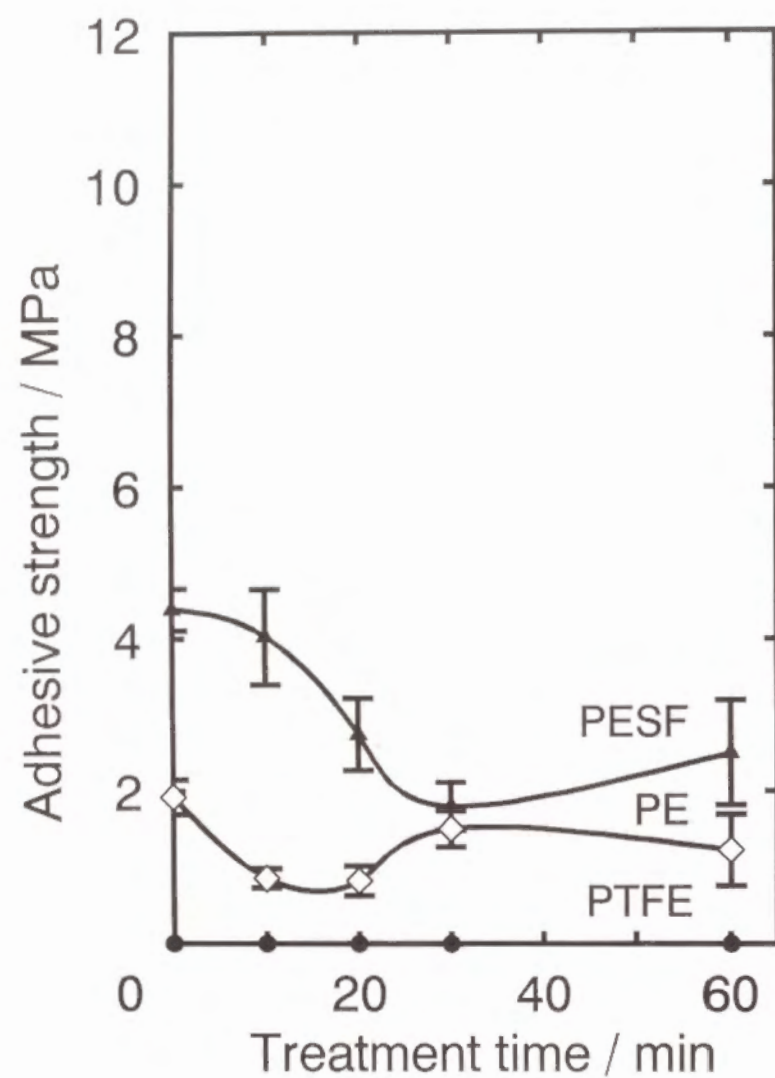


Figure 8. (Continued)

with 5M-NaOH aqueous solution, then subjected to the first soaking for 24 h, respectively. The apatite nuclei were formed sparsely on the untreated PET substrate, but formed closely on the NaOH-treated PET substrate.

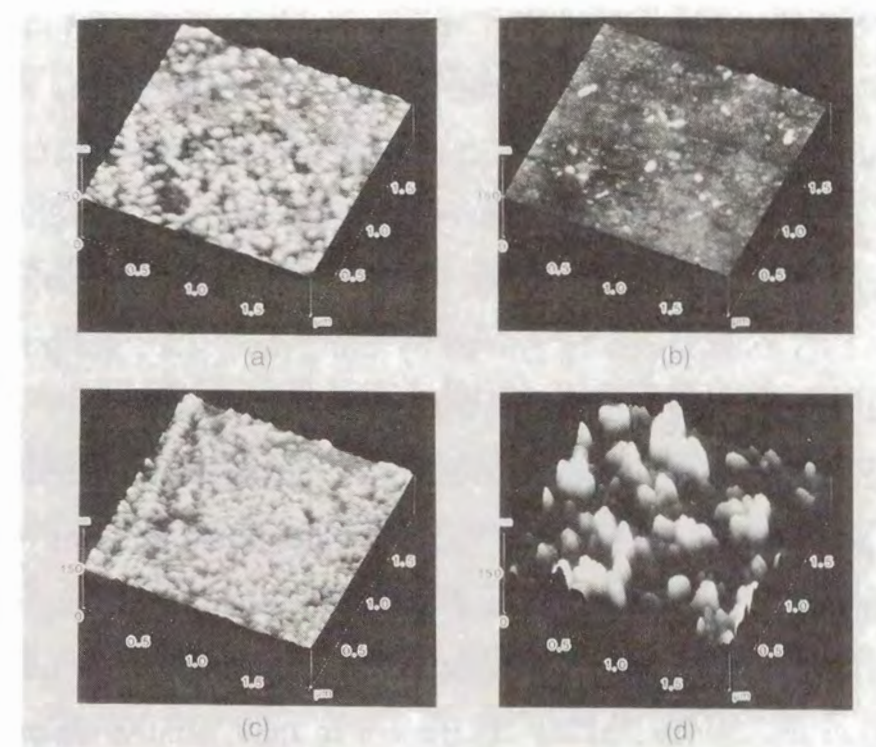


Figure 9. Atomic force microscopic pictures of PET NaOH-treated (a) and untreated (b), and the apatite growing on them (c and d).

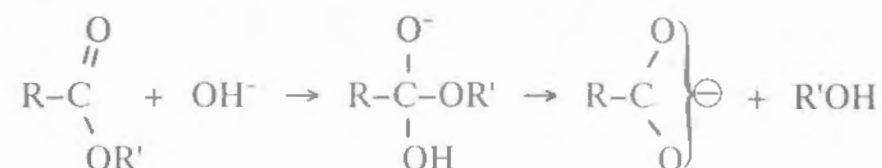
4. Discussion

It was previously shown that glass G dissolves appreciable amounts of the calcium and silicate ions into SBF [6]. These ions might be enriched in SBF at the gap between glass G particles and the substrate during the

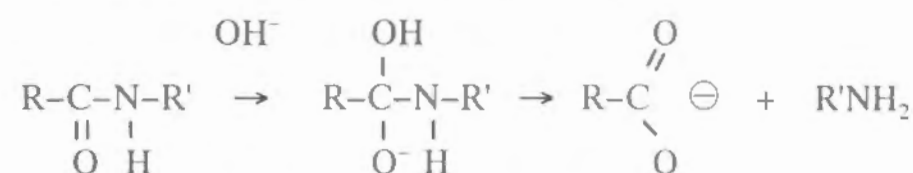
first treatment. Among them, the calcium ion increases the degree of the supersaturation of SBF which is already supersaturated with respect to the apatite even before the dissolution. This provides a favorable condition for the apatite nucleation. For the apatite to be nucleated on the surface of the substrate, however, some kinds of nucleating agent must be formed on the substrate. The dissolved silicate ions are considered to take such forms as Si(OH)_4 , SiO(OH)_3^- or $\text{SiO}_2(\text{OH})_2^{2-}$ and their polymerized ones in SBF [7]. Some of them might attach on the surface of the substrate, and could induce the apatite nucleation there. It was first shown from the kinetic point of view of the apatite nucleation on the surfaces of CaO-SiO_2 -based glasses in SBF that hydrated silica induces the apatite nucleation [8, 9]. This was later experimentally proved by using pure silica gel prepared by a sol-gel method [10].

On the basis of these consideration, the induction period for the apatite nucleation on the substrate could be interpreted in terms of the rate of attachment of the hydrated silica to the substrate. The long induction period of 24 h for the polymer substrates without the NaOH treatment is attributed to the low rate of the attachment of the hydrated silica to them. On the other hand, shorter induction period of 12 h for PET, PMMA, Nylon 6 and PESF treated with the NaOH treatment is attributed to high rate of the attachment of the hydrated silica to them. The increase of the number of the apatite nuclei on one of these polymers with the NaOH treatment was clearly observed on the AFM picture (Fig. 9). XPS showed that the ester group on PET and PMMA and the amide group on Nylon 6 were hydrolyzed to form the carboxyl groups by the NaOH treatment by the following reaction, and the sulfonyl group on PESF were also considered to be hydrolyzed to form the sulfonic acid group with the NaOH treatment.

The hydrolysis reaction of the ester group



The hydrolysis reaction of the amide group



It is supposed that the polar oxygen of these polar groups could bond easily with the hydrogen of the hydrated silica.

PE and PTFE showed long induction period of 24 h even after the NaOH treatment. This might be attributed to the absence of the groups such as ester and amide groups which can be hydrolyzed to polar groups with the NaOH treatment for these polymers. Consequently, the rate of the attachment of the hydrated silica to them did not change by the NaOH treatment. Similar relation between the decrease in the induction period for the apatite nucleation and the increase in the amount of the polar groups were also observed for PET, PMMA, PESF and Nylon 6 treated with the HCl aqueous solution. But the magnitudes of the increase in the amounts of the polar groups by the NaOH treatment are larger than those by the HCl treatment. It should be noted that both the HCl and NaOH treatments were effective only for PET, PMMA, PESF and Nylon 6 but not for PE and PTFE, in increasing the amounts of the polar groups and hence in decreasing the induction period.

The adhesive strength of the apatite layer to PET, PMMA and Nylon 6 substrates increased by the NaOH treatment. This increase is considered

to be due to the increase in the number of apatite nuclei as well as in their bonding strength with the substrate. The hydroxyapatite might form a stronger bonds with the polar groups such as carboxyl and sulfonic acid groups, which were formed on the surfaces of these polymers by the NaOH treatment. It is consistent with the result that no increase in the adhesive strength was observed for PE and PTFE substrates. Similar relation between the increase in the adhesive strength and the increase in the amounts of the polar groups was also observed for the PET, PMMA and Nylon 6 treated with HCl aqueous solution. But the magnitude of the increase in the adhesive strength by the NaOH treatment is larger than that by the HCl treatment. For all the substrates, the adhesive strength decreased with the increase of the NaOH treatment time for longer than 10 min. This might be attributed to the decrease in the molecular weight of polymer substrate by the longer NaOH treatment and that the intertwinements among them were released. This is fairly consistent with the observation that the fracture occurred not at the interface between the apatite layer and the substrate, but in the surface region of the substrate for the longer NaOH treatment. For PESF, it is considered that the increase in the number of the apatite nuclei by the NaOH treatment was cancelled by the competing reaction that releases the polymer chains, and no increase in the adhesive strength was observed.

5. Conclusion

By the NaOH treatment, ester groups in PET and PMMA, amide groups in Nylon 6, and sulfonyl groups in PESF were attacked with hydroxyl ion to be hydrolyzed to carboxylic and sulfonic acids, which

enhanced the apatite nuclei formation in the biomimetic process. The adhesive strengths of the apatite layer to PET, PMMA and Nylon 6 increased by the NaOH treatment due to the increase of both the number of the apatite nuclei and their bonding strength with the substrates. Similar relation between the increase in the adhesive strength and the increase in the amounts of the polar groups was also observed for the PET, PMMA and Nylon 6 treated with HCl aqueous solution. But the magnitude of the increase in the adhesive strength by the NaOH treatment was larger than that by the HCl treatment. On the other hand, PE and PTFE, which have no functional groups to be hydrolyzed to polar groups, showed neither the reduction of the induction period for the apatite nucleation nor the increase of the adhesive strength by the NaOH treatment, as by the HCl treatment. For these polymers, some other surface treatments are required for improving the adhesive strength of the apatite layer to the substrates.

References

- [1] M. Tanahashi, T. Yao, T. Kokubo, M. Minoda, T. Miyamoto, T. Nakamura and T. Yamamuro, "Apatite coating on organic polymers by a biomimetic process " *J. Am. Ceram. Soc.*, **77**, 2805–2808 (1994).
- [2] K. Suzuki, R. Kobayashi, Y. Yokoyama, Y. Harada and T. Kokubo, "Experimental study of an artificial trachea made of polymers coated with hydroxyapatite" in *Bioceramics*, Vol. 6, ed. by P. Ducheyne and D. Christiansen, Butterworth–Heinemann, Guilford London pp. 245–250, 1993.
- [3] D. T. Clark, B. J. Cromarty and A. Dilks, "A theoretical investigation

of molecular core binding and relaxation energies in a series of oxygen-containing organic molecules of interest in the study of surface oxidation of polymers", *J. Polym. Sci. Polym. Chem. Ed.*, **16**, 3173–3184 (1978).

- [4] A. Dilks, and A. VanLaeken, "Physicochemical aspects of polymer surfaces", ed. by K. L. Mittal, Plenum, New York, p. 749, 1983.
- [5] T. Kokubo, S. Ito, Z. T. Huang, T. Hayashi, S. Sakka, T. Kitsugi and T. Yamamuro, "Ca P-rich layer formed on high-strength bioactive glass-ceramic A-W", *J. Biomed. Mat. Res.*, **24**, 331–343 (1990).
- [6] T. Kokubo, H. Kushitani, C. Ohtsuki, S. Sakka, and T. Yamamuro, "Chemical reaction of bioactive glass and glass-ceramics with a simulated body fluid" *J. Mater. Sci: Mater. Medicine*, **3**, 79–83 (1992).
- [7] R. K. Iler, in "*The Chemistry of Silica*", Wiley, New York, 132, 1979.
- [8] C. Ohtsuki, T. Kokubo, K. Takatsuka and T. Yamamuro, "Compositional dependence of bioactivity of glasses in the system CaO–SiO₂–P₂O₅: its *in vitro* evaluation" *J. Ceram. Soc. Japan*, **99**, 1–6 (1991).
- [9] C. Ohtsuki, T. Kokubo and T. Yamamuro, "Mechanism of apatite formation on CaO–SiO₂–P₂O₅ glasses in a simulated body fluid", *J. Non-Crystl. Solids*, **143**, 84–92 (1992).
- [10] P. Li, C. Ohtsuki, T. Kokubo, K. Nakanishi, N. Soga, T. Nakamura, and T. Yamamuro, "Apatite formation induced by silica gel in a simulated body fluid" *J. Am. Ceram. Soc.*, **75**, 2094–2097 (1992).

CHAPTER 5.

IMPROVEMENT IN ADHESION OF APATITE LAYER TO POLYMER SUBSTRATES BY GLOW DISCHARGE TREATMENT

1. Introduction

A dense and uniform layer of bone-like apatite can be formed on the surfaces of various kinds of organic polymers even in a form of woven fine fiber fabric by the biomimetic process described in Chapter 2 [1]. The induction periods for the apatite nucleation of the polymers were shown in Chapter 2. to be 24 h for as-prepared poly(ethylene terephthalate) (PET), poly(methyl methacrylate) (PMMA), polyamide 6 (Nylon 6) and poly-ether sulfone (PESF) substrates [1] and, in Chapter 3 or 4, to be reduced to 12 h with the HCl [2] or NaOH treatment [3].

It has been also shown that the adhesive strengths of the apatite layer formed to the polymer substrates vary from almost 0 to 4 MPa, depending on the kind of the polymers [1], and increase by 2 to 5 times for PET, PMMA and Nylon 6 with the HCl [2] or NaOH treatment [3]. It should be noted, however, that both the HCl and NaOH treatments were effective only for PET, PMMA and Nylon 6 but little for PESF, while no effect was observed for the induction period and the adhesive strength of polyethylene (PE) and poly(tetrafluoroethylene) (PTFE).

In the present chapter, polymer substrates were first subjected to the glow-discharge treatment in O₂ gas atmosphere, and then to the same biomimetic process as that described in Chapter 2. The effects of the glow discharge treatment on the induction periods for the apatite nucleation and

the adhesive strength of the apatite layer to the substrates were investigated.

2. Experimental

2.1. Preparation of polymer substrate

Poly(ethylene terephthalate) (PET), poly(methyl methacrylate) (PMMA), poly-ether sulfone (PESF), polyamide 6 (Nylon 6), polyethylene (PE) and poly(tetrafluoroethylene) (PTFE) were prepared as the substrates by the same method as that described in Section 2.3. of Chapter 2 [1]. They were cut into rectangular specimens 10 mm × 15 mm × 1 mm in size and abraded with #400 diamond paste.

2.2. Glow-discharge treatment

The substrates were subjected to the glow-discharge (GD) treatment in O₂ gas (99.9999%) atmosphere for 0 to 1800 s. The GD treatment was carried out at a pressure of 30 Pa, under an electric field of 13.56 MHz at 1 W/cm² by using Plasma Polymerization System with Model BP-2 (SAMCO Ltd., Kyoto, Japan).

2.3. Surface Characterization

Binding energies of carbon and oxygen in the GD-treated for 30 s and untreated organic polymer substrates were measured by X-ray photoelectron spectroscopy (XPS) with an ESCA Model MT5500 (ULVAC-PHI Co. Ltd., Chigasaki, Japan) under the same conditions as those described in Section 2.3. of Chapter 3. Measured binding energies were corrected by referring the binding energy of the C1s in CH₂ group as 284.6 eV. Overlapped peaks in XPS spectra were separated by the pattern

fitting method using the Gaussian profile for the individual peaks.

2.4. Apatite Coating

Glass particles named G of the nominal composition MgO 4.6, CaO 44.7, SiO₂ 34.0, P₂O₅ 16.2 and CaF₂ 0.5 wt%, SBF with ion concentrations nearly equal to those of human blood plasma and an aqueous solution with ion concentrations 1.5 times those of SBF (1.5SBF) were prepared by the same method as those described in Section 2.1. and 2.2. of Chapter 2.

The GD-treated substrates were set in contact with the particles of glass G soaked in 30 ml of SBF at 36.5 °C for various periods less than 12 h, as shown in Fig. 1 in Chapter 2. Then, the substrates were soaked in 30 ml of 1.5SBF at 36.5 °C for 6 d. The 1.5SBF solution was renewed every 2 d. After these treatments, the substrate was washed under a slow flow of distilled water, and dried at room temperature.

2.5. Analysis of Apatite

After the second treatment, the surfaces of the GD-treated substrates after the second treatment were analyzed by thin-film X-ray diffraction with RINT-1400 (Rigaku Co., Tokyo, Japan) and Fourier transform infrared (FT-IR) spectroscopy with Model FT-IR 5M (Japan Spectroscopic Co. Ltd., Tokyo, Japan). Gold-palladium film was coated on the surface of the specimens, and scanning electron microscopic images were observed with S-2500CX (Hitachi Co. Ltd., Tokyo, Japan).

2.6. Measurement of Adhesive Strength

The organic polymer substrates on which the apatite layer of about 10 μm thick was formed by the first treatment for 12 h and the second treatment for 6 d were used as the test samples. The adhesive strength of

thus formed apatite layer to the substrates was measured by the same method as that described in Section 2.6. of Chapter 2.

2.7. AFM Observation

The PET particles 2 mm x 3 mm x 2 mm in size were put on the platinum plate and soften at 350 °C for a few minutes to form a smooth free surface, then quenched by dropping into distilled water. The PET substrates thus obtained were treated with GD in O₂ gas atmosphere for 30 s. The GD-treated and untreated PET substrates were subjected to the first treatment of the biomimetic process described above for various periods less than 24 h. After the treatment, the substrate was washed moderately with distilled water, and dried at room temperature. The surfaces of these PET substrates were observed with atomic force microscope with Nanoscope III (Digital Instruments Inc. U. S. A.) in tapping mode.

3. Results

3.1. XPS analysis

The C1s and O1s XPS spectra of PET and PMMA treated with GD for 30 s and those untreated are shown in Figs. 1 and 2, respectively. The C1s peaks were resolved into five components with binding energies of 284.6, 286.8, 287.8, 288.8 and 290.4 eV, which were attributed to carbons in $-(CH_2)_n-$, >C-O or >C-OH , >C=O or O-C(=O)- groups, O-C(=O)-O or H-O-C(=O)- and O-C(=O)-O groups, respectively [4, 5]. The latter four peaks increased with GD treatment. The O1s peaks were resolved into two components with binding energies of about 531 eV attributed to the oxygen in >C=O group, and of about 533 eV attributed to the oxygen in >C-OH ,

C-O-C(=O)- and H-O-C(=O)- group. The latter peak increased with the GD treatment. These results indicate that the oxidized carbon groups increased in the surface region by the GD treatment. For Nylon 6, similar changes in C1s and O1s peaks were observed as shown in Fig. 3, and it seems that the oxidized carbon groups also increased with the GD treatment. Figure 4 shows the C1s and O1s XPS spectra of GD-treated and untreated PESF. In this case, similar change was observed in C1s spectra by the GD treatment. The O1s peak was resolved into two components with binding energies of about 531 eV attributed to the oxygen in >SO-OH , $\text{-SO}_2\text{-OH}$ or >C=O groups, and of about 533 eV attributed to the oxygen in S-OH , >C-OH , C-O-C(=O)- or H-O-C(=O)- groups. The latter peak increased with GD treatment. In view of the result of C1s peak, the increase in the intensity of 533 eV peak is considered to be attributed to the increase in the amounts of the oxidized carbon groups. Even for PE and PTFE, similar changes in C1s and O1s peaks were also observed as shown in Figs. 5 and 6, respectively. It seems that the oxidized carbon groups were formed in the surface region by the GD treatment. Table 1 lists the area ratio of the peaks of various polar groups separated by line-profile analysis in the XPS spectra of various kinds of GD-treated and untreated substrates. It is apparent from Table 1 that various kinds of oxidized carbon groups such as >C-O or >C-OH , >C=O or O-C(=O)- , O-C(=O)-O or O-C(=O)-H and O-C(=O)-O groups increased in the surface region of the examined organic polymers with GD treatment.

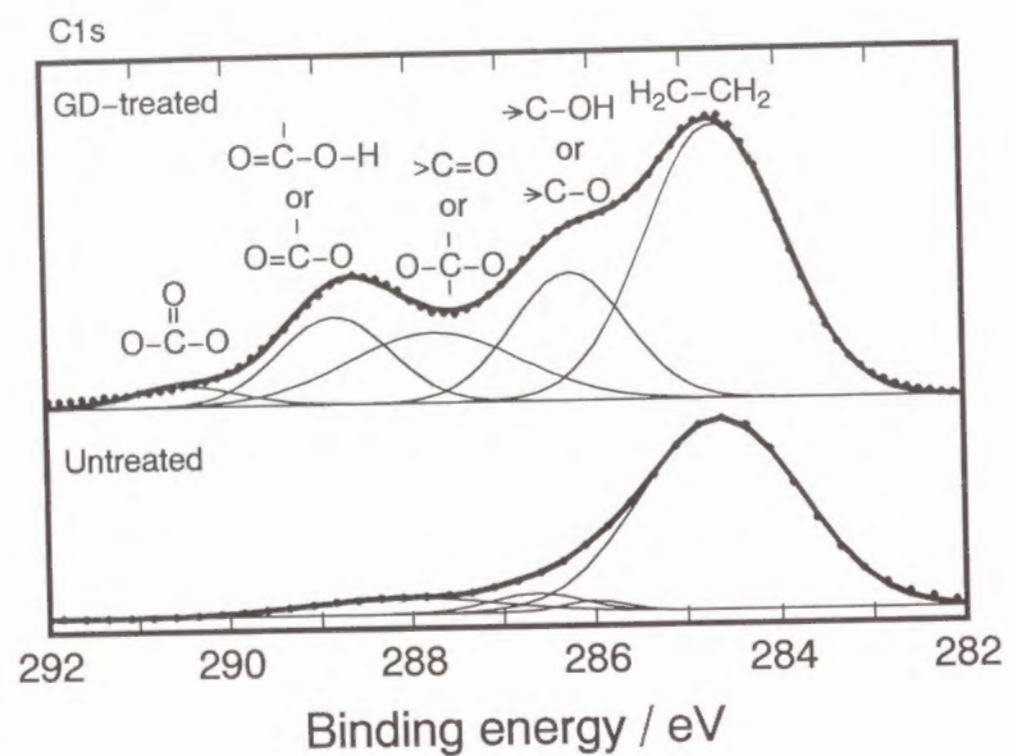


Figure 1. C1s and O1s XPS spectra of PET substrate treated and untreated with GD in O₂ gas atmosphere for 30 s.

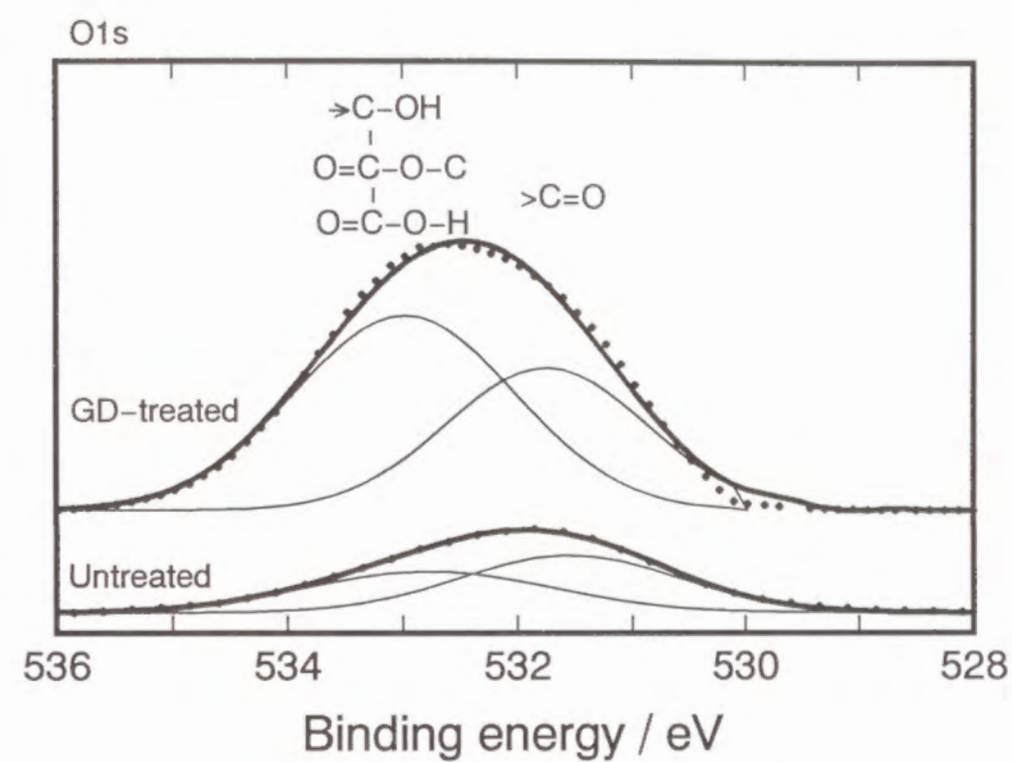


Figure 1. (Continued).

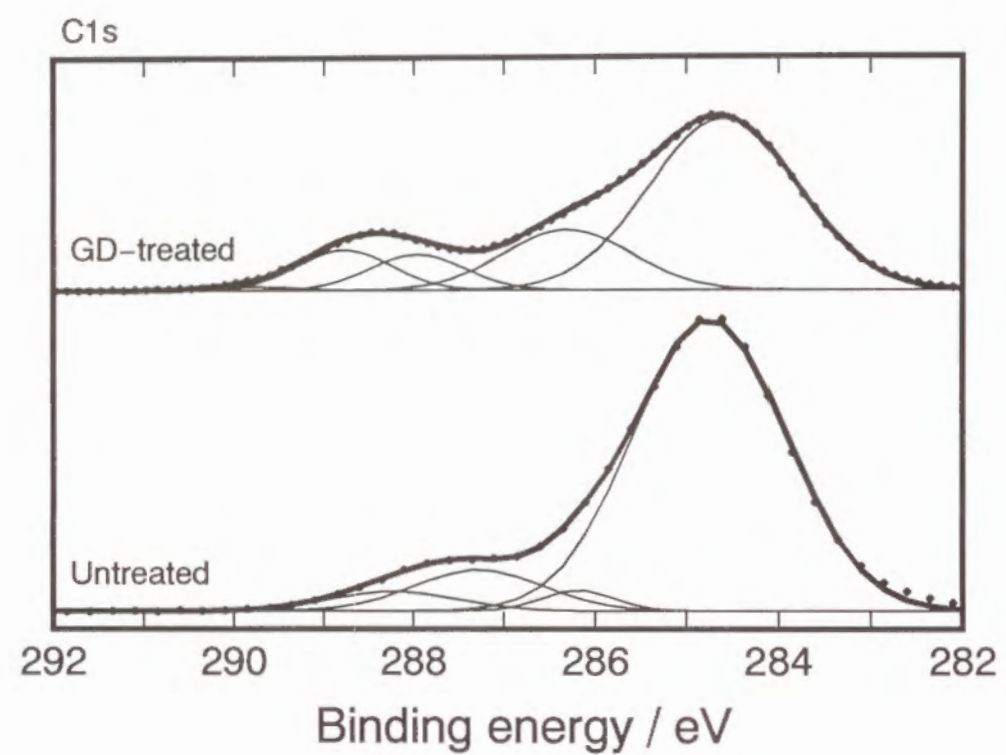


Figure 2. C1s and O1s XPS spectra of PMMA substrate treated and untreated with GD in O₂ gas atmosphere for 30 s.

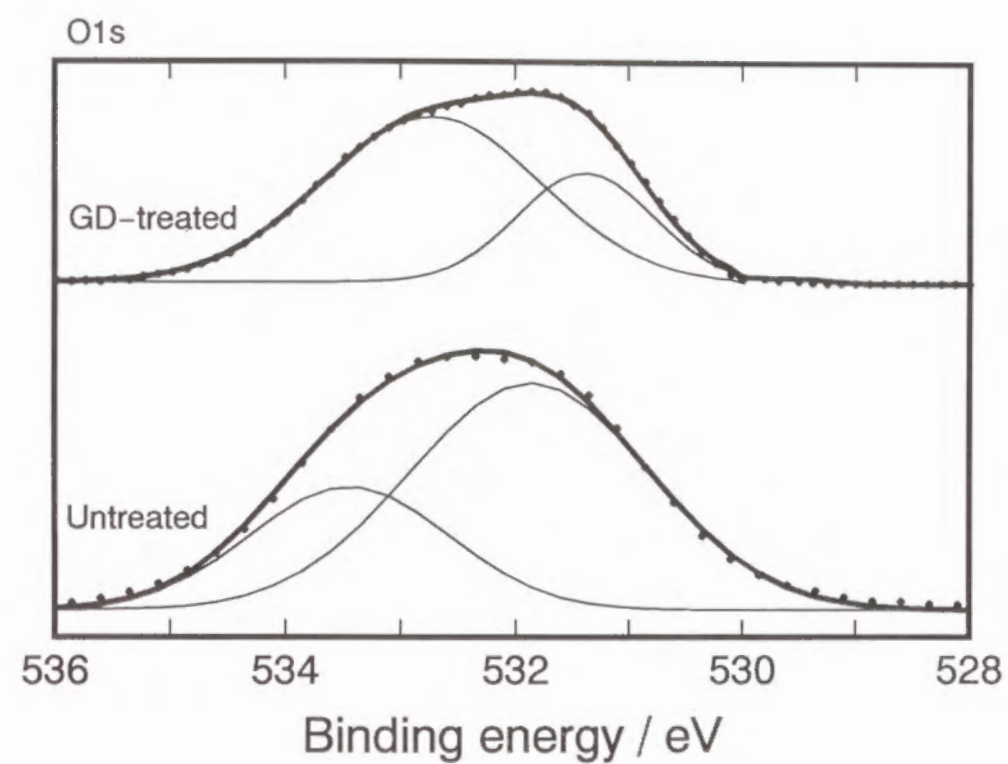


Figure 2. (Continued).

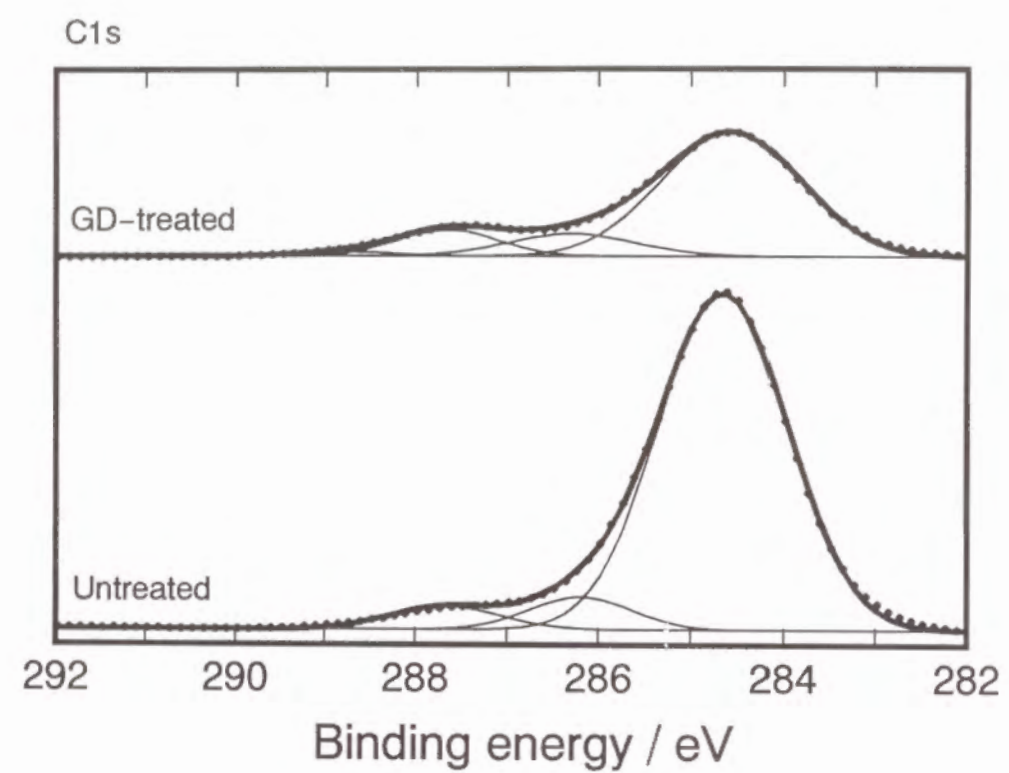


Figure 3. C1s and O1s XPS spectra of Nylon 6 substrate treated and untreated with GD in O₂ gas atmosphere for 30 s.

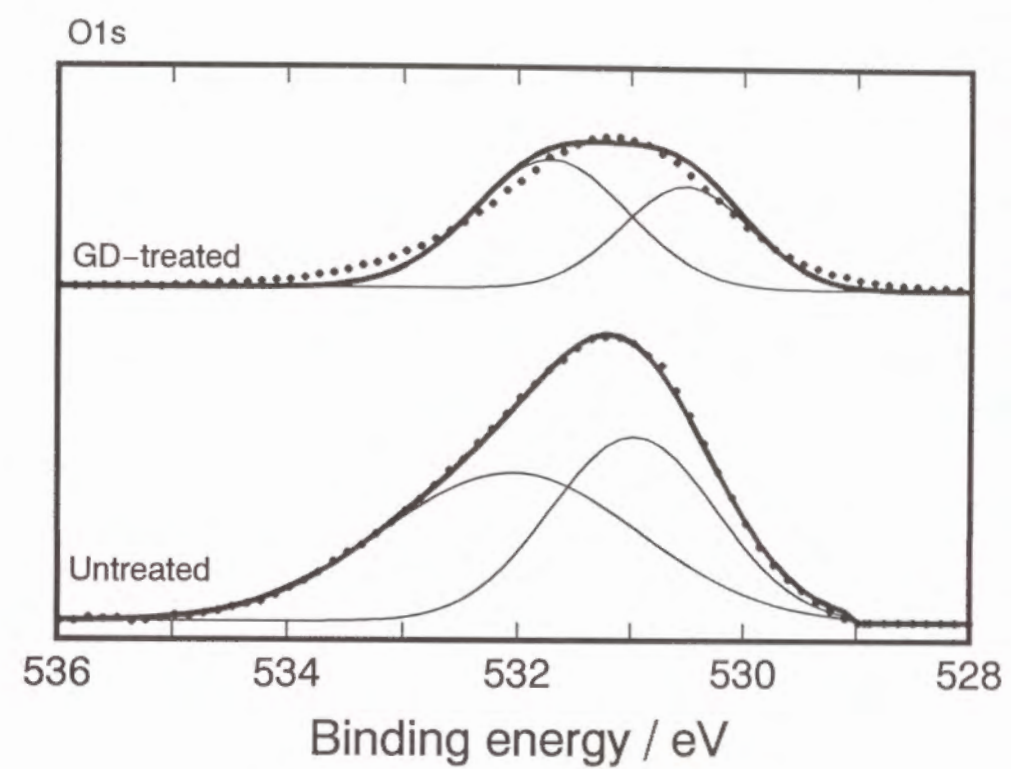


Figure 3. (Continued).

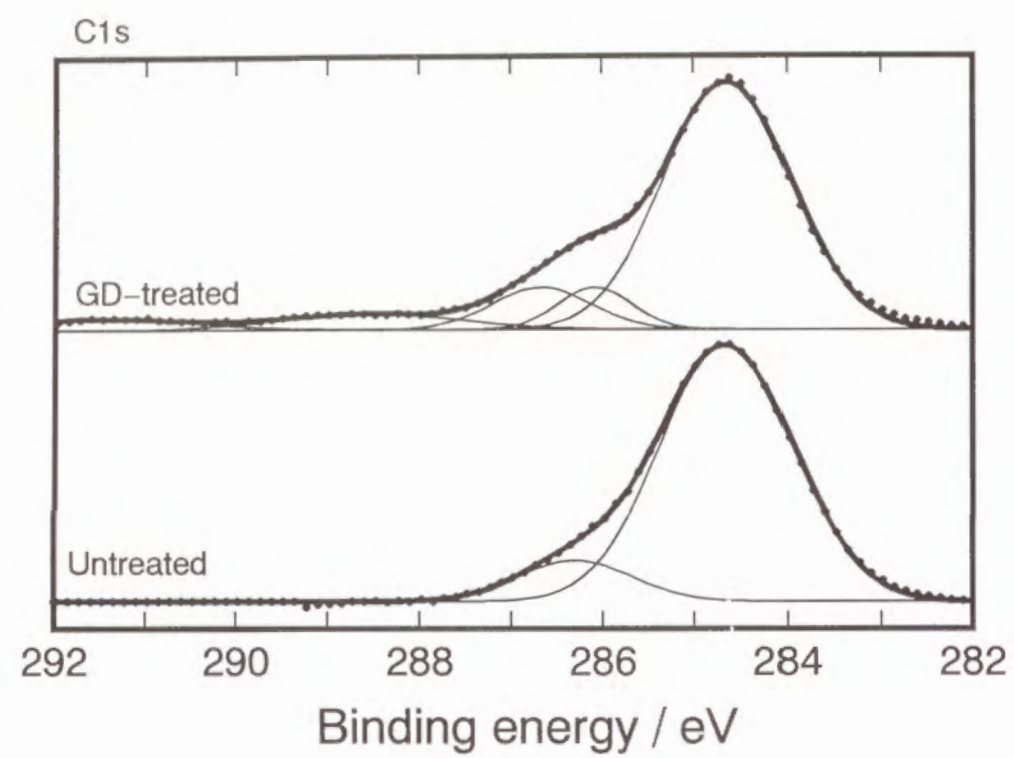


Figure 4. C1s and O1s XPS spectra of PESF substrate treated and untreated with GD in O₂ gas atmosphere for 30 s.

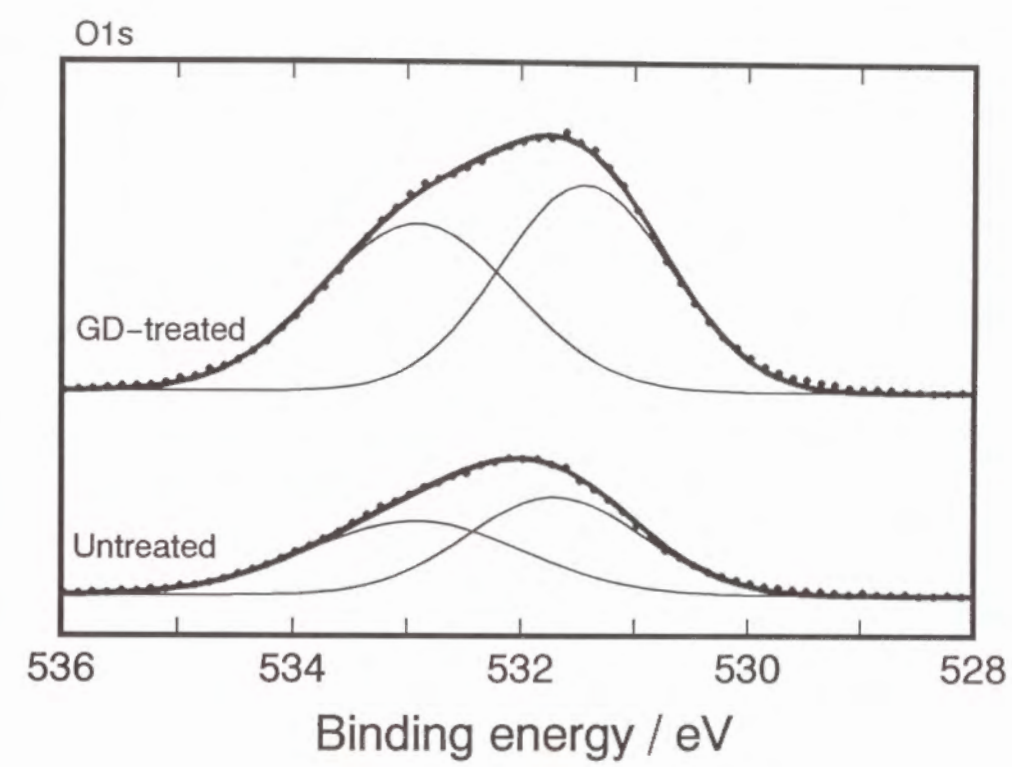


Figure 4. (Continued).

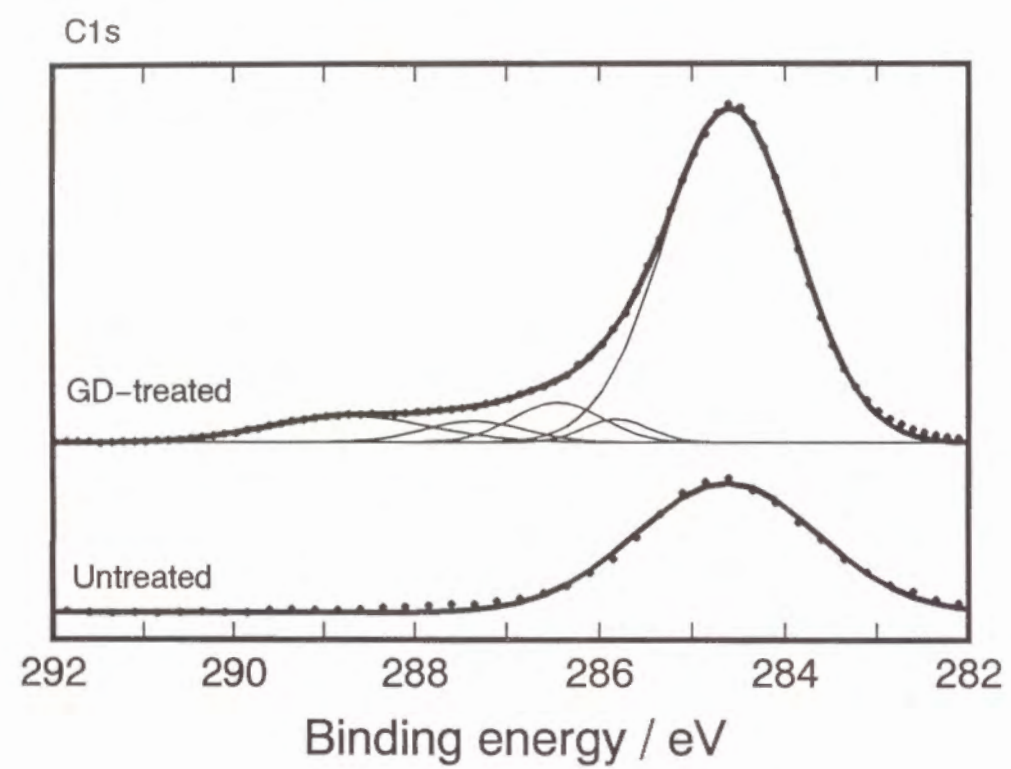


Figure 5. C1s and O1s XPS spectra of PE substrate treated and untreated with GD treatment for 30 s.

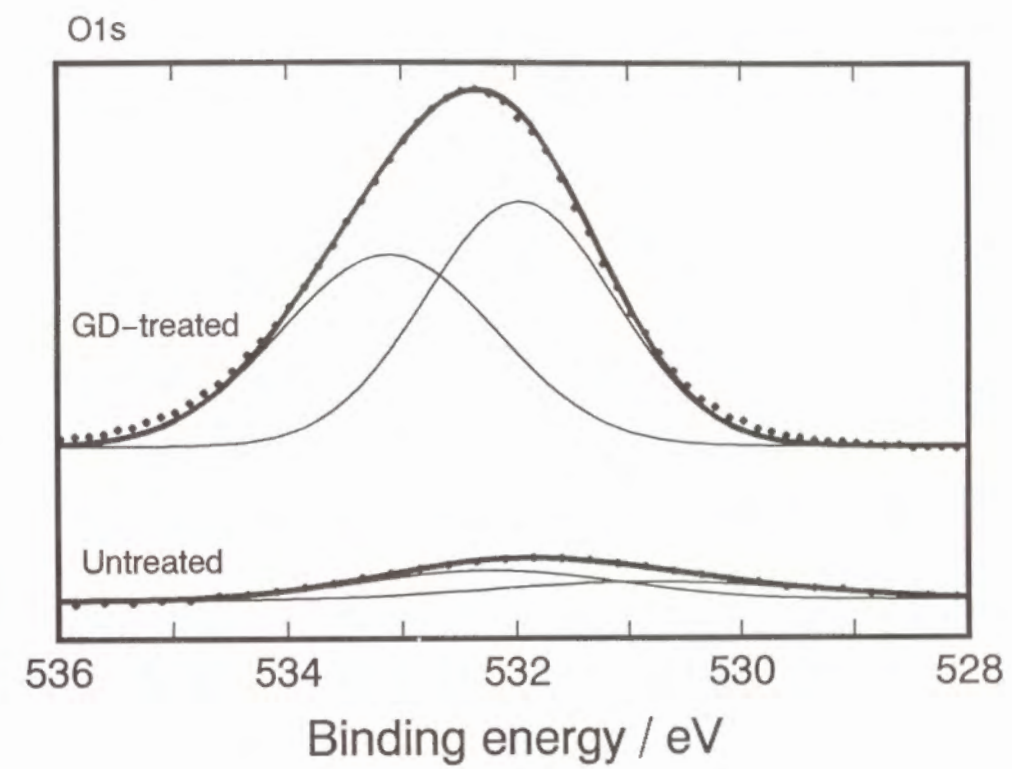


Figure 5. (Continued).

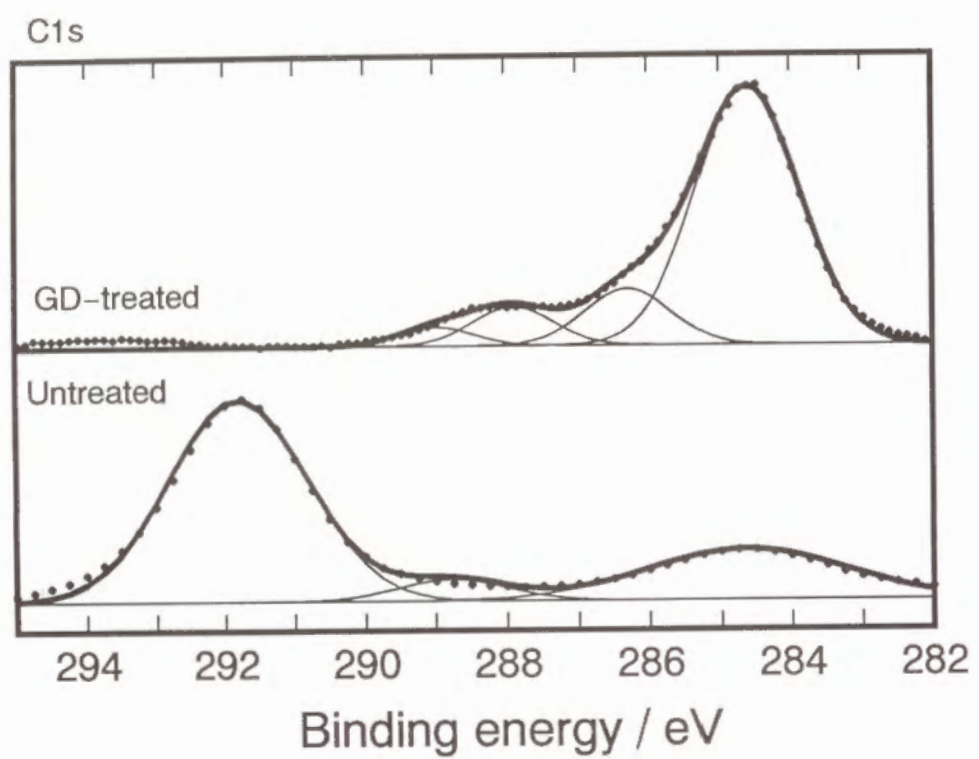


Figure 6. C1s and O1s XPS spectra of PTFE substrate treated and untreated with GD treatment in O₂ gas atmosphere for 30 s.

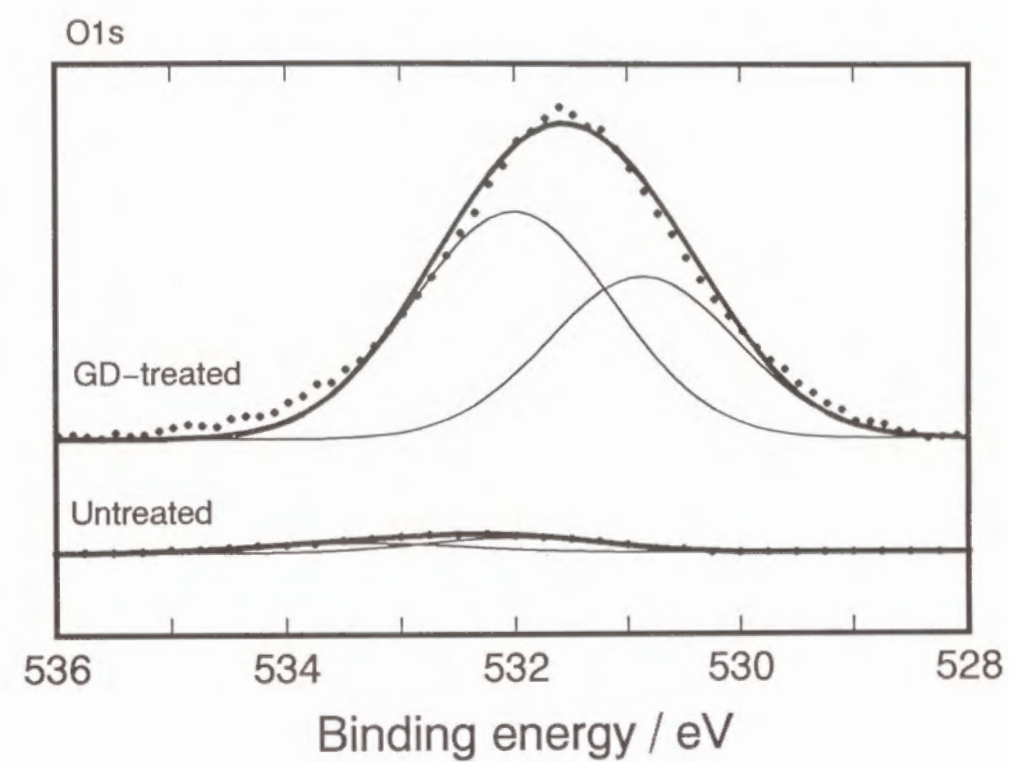


Figure 6. (Continued).

Table 1. Area ratio of functional groups formed on the surface of various organic polymers treated and untreated with GD for 30 s.

C1s

Substrate	Untreated							GD-treated						
	$\text{H}_2\text{C}-\text{CH}_2$	$\text{>C}-\text{O}$	$\text{>C}=\text{O}$	$\text{O}=\overset{\text{I}}{\text{C}}-\text{O}$	O	$\text{F}_2\text{C}-\text{CF}_2$	$\text{F}_2\text{C}-\text{CH}_2$	$\text{H}_2\text{C}-\text{CH}_2$	$\text{>C}-\text{O}$	$\text{>C}=\text{O}$	$\text{O}=\overset{\text{I}}{\text{C}}-\text{O}$	O	$\text{F}_2\text{C}-\text{CF}_2$	$\text{F}_2\text{C}-\text{CH}_2$
		or $\text{>C}-\text{OH}$	or $\text{O}-\overset{\text{I}}{\text{C}}-\text{O}$	or $\text{O}=\overset{\text{I}}{\text{C}}-\text{O}-\text{H}$	 $\text{O}-\text{C}-\text{O}$	or $\text{F}_2\text{C}-\text{CHF}$			or $\text{>C}-\text{OH}$	or $\text{O}-\overset{\text{I}}{\text{C}}-\text{O}$	or $\text{O}=\overset{\text{I}}{\text{C}}-\text{O}-\text{H}$	 $\text{O}-\text{C}-\text{O}$	or $\text{F}_2\text{C}-\text{CHF}$	
PET	84	2	5	9	-	-	-	48	19	16	13	4	-	-
PMMA	82	3	10	15	-	-	-	62	19	9	9	1	-	-
PESF	88	12	-	-	-	-	-	72	7	10	6	5	-	-
Nylon 6	88	7	5	-	-	-	-	75	12	11	2	-	-	-
PE	100	-	-	-	-	-	-	79	3	6	8	4	-	-
PTFE	25	-	-	-	-	69	6	74	13	9	4	-	-	-

- Undetected

Table 1. (Continued)

O1s

Substrate	Untreated				GD-treated			
	$\text{>C}=\text{O}$	$\text{>C}-\text{OH}$, $\text{O}=\overset{\text{I}}{\text{C}}-\text{O}-\text{C}$	$\text{>SO}-\text{OH}$	$\text{S}-\text{OH}$	$\text{>C}=\text{O}$	$\text{>C}-\text{OH}$, $\text{O}=\overset{\text{I}}{\text{C}}-\text{O}-\text{C}$	$\text{>SO}-\text{OH}$	$\text{S}-\text{OH}$
		or $\text{O}=\overset{\text{I}}{\text{C}}-\text{O}-\text{H}$	or $\text{>SO}_2-\text{OH}$			or $\text{O}=\overset{\text{I}}{\text{C}}-\text{O}-\text{H}$	or $\text{>SO}_2-\text{OH}$	
PET	61	39	-	-	41	59	-	-
PMMA	75	25	-	-	37	63	-	-
PESF	-	-	52	48	64	36	-	-
Nylon 6	60	40	-	-	30	70	-	-
PE	-	-	-	-	37	63	-	-
PTFE	-	-	-	-	62	38	-	-

- Undetected

3.2. Apatite formation

Figures 7 and 8 show thin-film X-ray diffraction patterns and FT-IR reflection spectra, respectively, of the surfaces of the PESF substrates treated with GD for 30 s, then subjected to the first treatment for various periods and to the second treatment for 6 d. It can be seen from Figs. 7 and 8 that the a carbonate-containing hydroxyapatite, i. e. bone-like apatite is formed on the substrate after the second treatment when the substrate is subjected to the first treatment for longer than 6 h. For the first treatment was less than 3 h, the apatite was not detected on the surface of the substrate.

Figure 9 shows SEM photographs of the surfaces of the PESF substrates subjected to the same treatments described above. It can be seen from Fig. 9 that the apatite is not formed on the substrates even after the second treatment without the first treatment. With the first treatment longer than 6 h, a dense and uniform apatite layer was formed on the GD-treated PESF substrates. Therefore, the induction period for the apatite nucleation, that is the period of the first treatment required for the apatite nuclei to be formed in enough number to make the continuous layer after the second treatment, is determined as 6 h. The induction periods thus measured were 6 h for all the organic polymer substrates examined subjected to the GD treatment for 30 s.

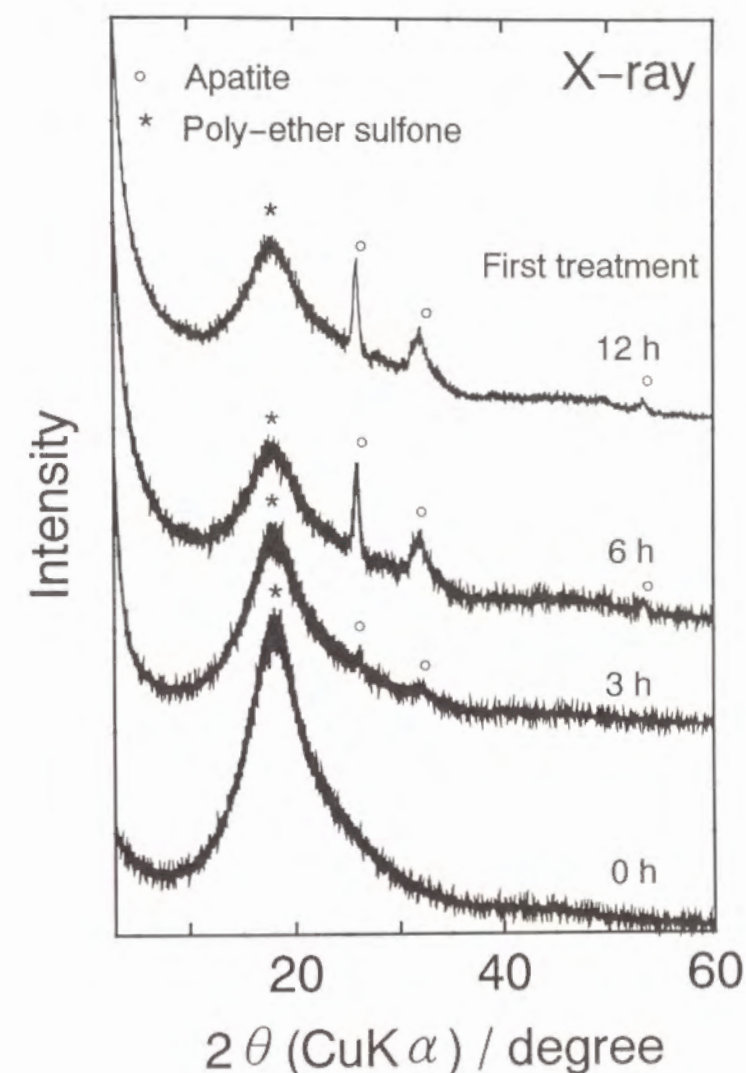


Figure 7. Thin-film XRD patterns of surfaces of GD-treated PESF substrates soaked in 1.5SBF for 6 d after soaking in SBF with glass G for various periods.

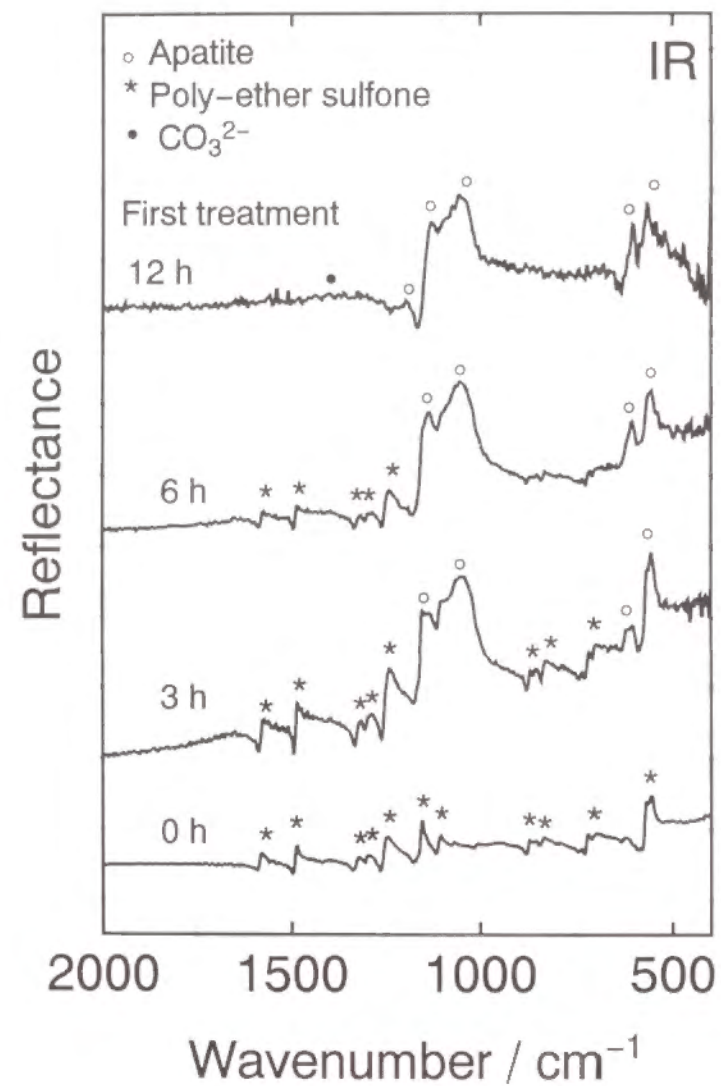


Figure 8. Fourier transform IR reflection spectra of surfaces of GD-treated PESF substrates soaked in 1.5SBF for 6 d after soaking in SBF with glass G for various periods.

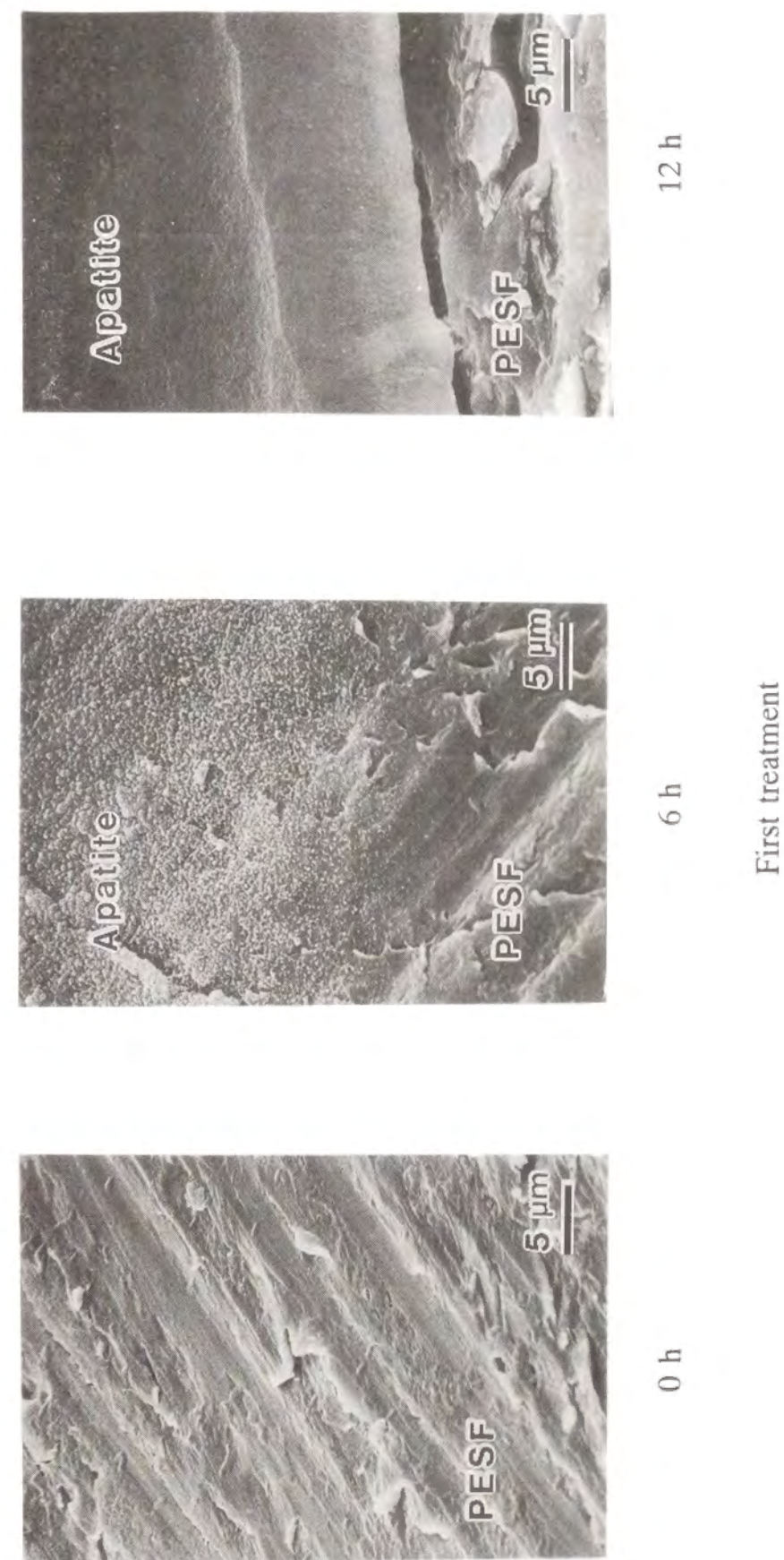


Figure 7. SEM photographs of the surfaces of GD-treated PESF substrates soaked in 1.5SBF for 6 d after soaking in SBF with glass G for various periods.

3.3. Adhesive strength

Figure 10 shows the adhesive strengths of the bone-like apatite layer to various organic polymer substrates as a function of GD treatment time. It can be seen from Fig. 10 that all the polymer substrates examined showed an increase in the adhesive strength of the apatite layer to the substrates by GD treatment for a certain period and then a decrease with longer GD treatment.

The maximum adhesive strength obtained for each of the examined polymer substrates by GD treatment and the time of GD treatment required for obtaining the maximum adhesive strength are summarized in Table 2, in comparison with the adhesive strengths for untreated substrates. It can be seen from Table 2 that the adhesive strengths of PET and PESF increase from about 4 to 10 MPa by GD treatment, and even those of PMMA, Nylon 6 and PE increase from 1~2 to 6~7 MPa.

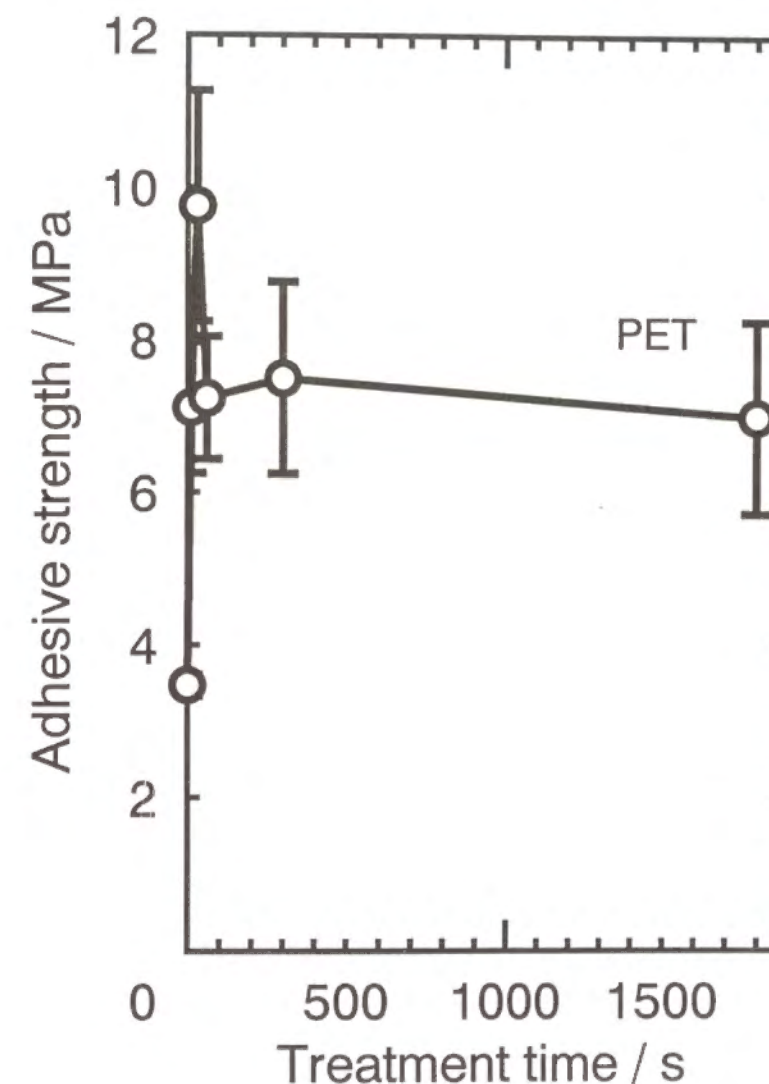


Figure 10. Adhesive strengths of bone-like apatite layer to various substrates as a function of treatment time of GD.

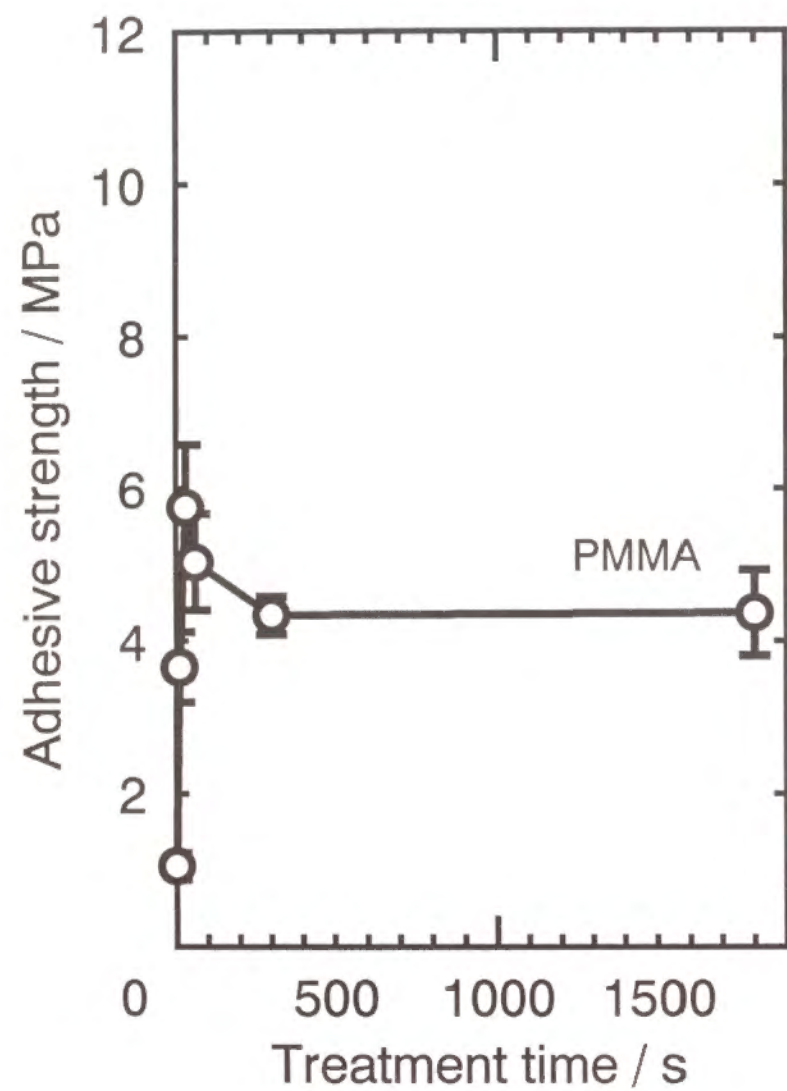


Figure 10. (Continued)

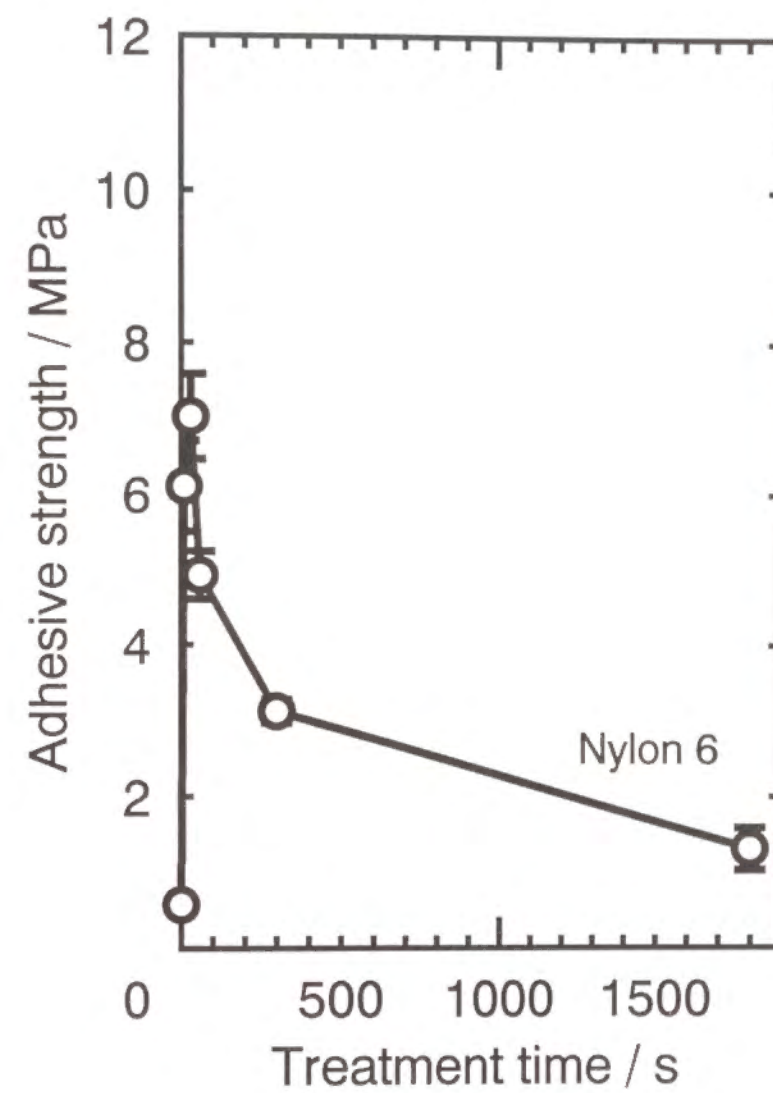


Figure 10. (Continued)

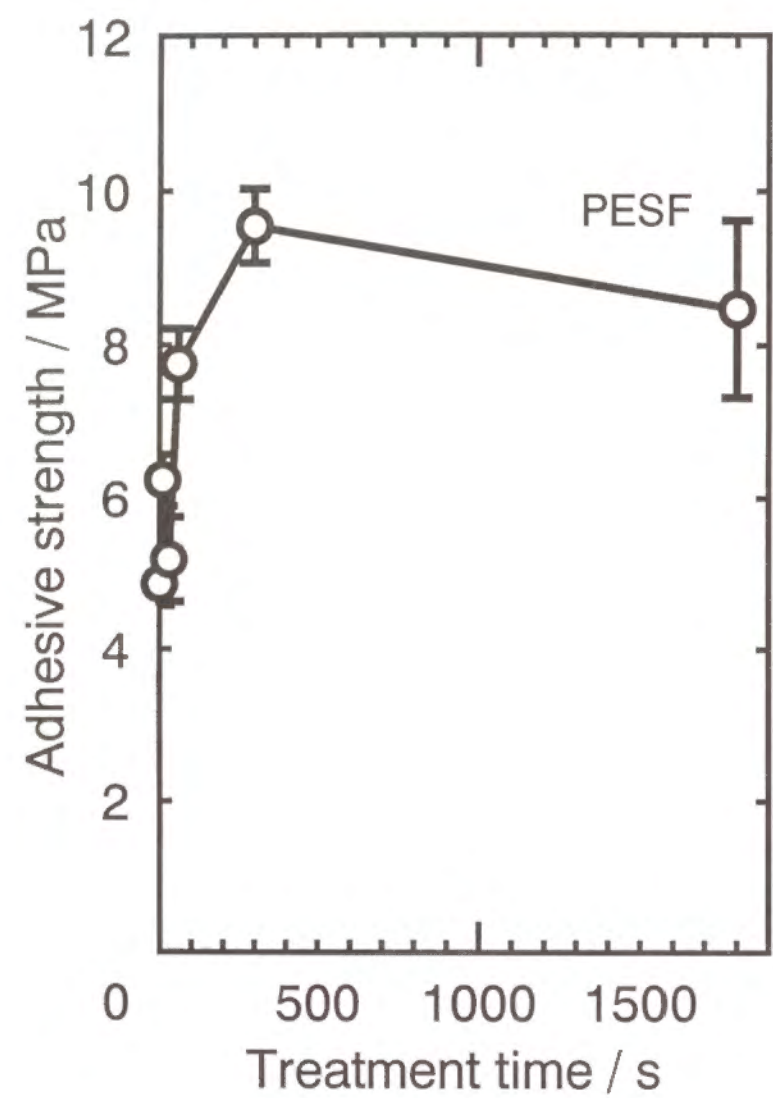


Figure 10. (Continued)

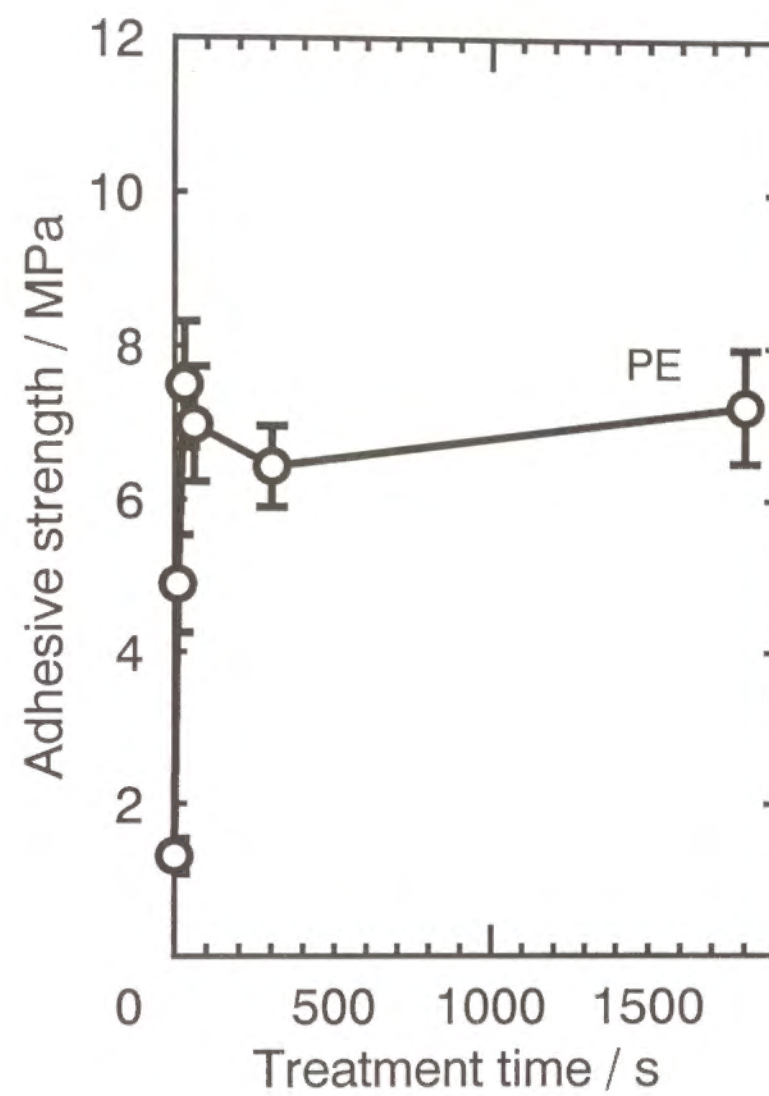


Figure 10. (Continued)

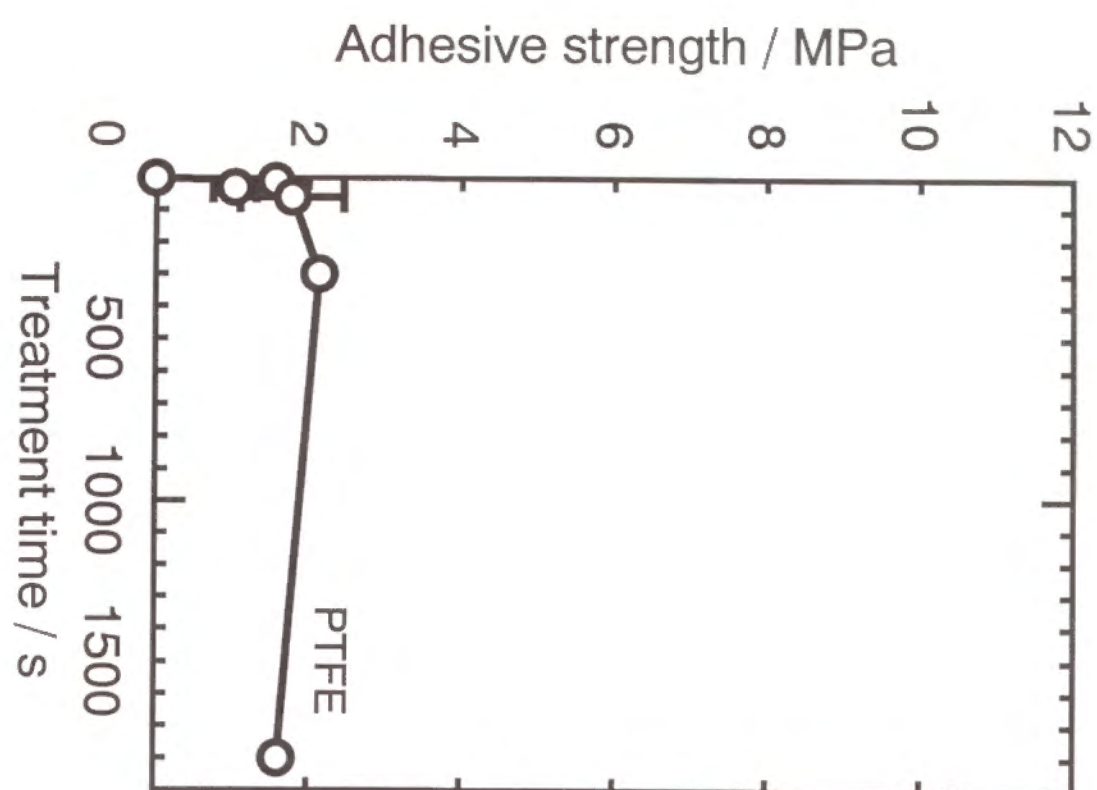


Figure 10. (Continued)

Table 2. Maximum adhesive strengths obtained for each polymer substrates by GD treatment and the times of GD treatment required for obtaining the maximum adhesive strengths, in comparison with the adhesive strength for untreated substrates.

Substrate	Adhesive strength of untreated substrate / MPa	Maximum adhesive strength obtained by GD / MPa	Time of GD / s
PET	3.48	9.77	30
PMMA	1.06	5.76	30
PESF	4.40	9.55	300
Nylon 6	0.63	7.03	30
PE	1.93	7.51	30
PTFE	$< 1.10 \times 10^{-2}$	2.14	30

3.4. Atomic Force Microscopic Image

Figures 11 (a) and (b) show the AFM images of PET substrates treated and untreated with GD in O_2 gas atmosphere for 30 s, respectively. As shown in Figs. 11, the surface of the PET was roughed by GD treatment. Figures 11 (c) and (d) show the AFM image of PET substrates treated and untreated with glow discharge, then subjected to the first soaking for 24 h, respectively. The apatite nuclei were formed sparsely on the untreated PET substrate, but formed much closely on the GD-treated PET substrate.

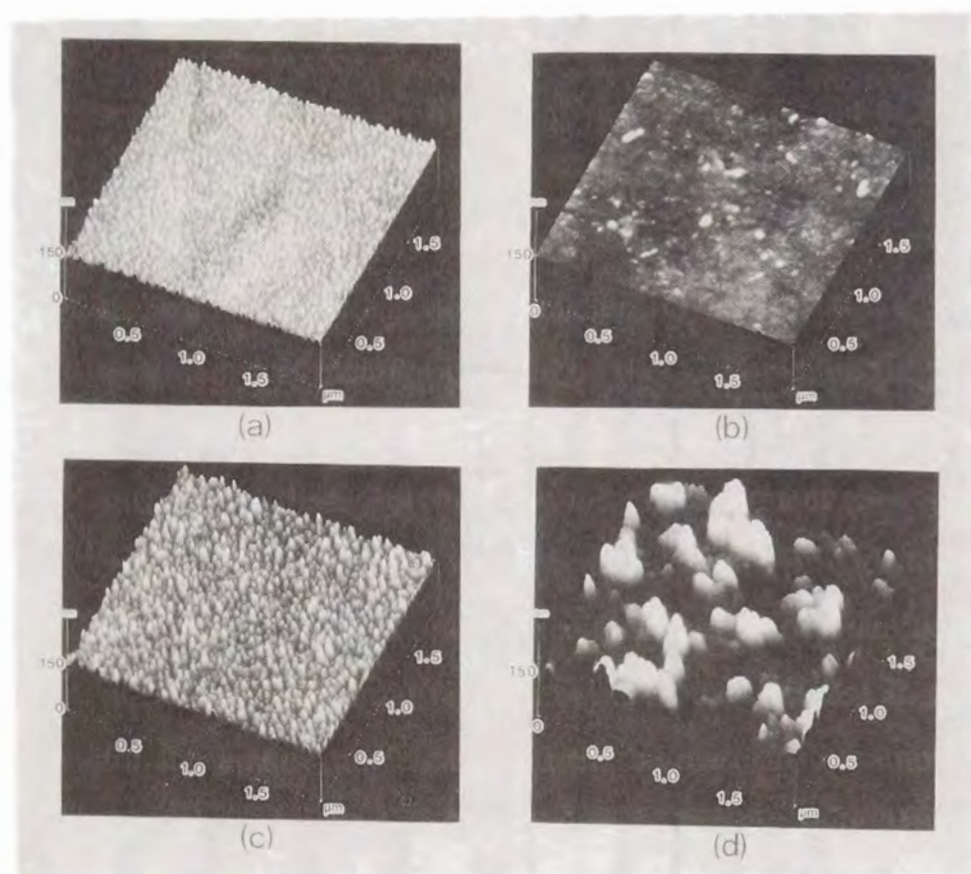


Figure 11. Atomic force microscopic pictures of PET GD-treated (a) and untreated (b), and the apatite growing on them (c and d).

4. Discussion

In the first soaking, glass G dissolves calcium and silicate ions into SBF [6]. It is thought that the calcium ions increase the ionic activity product of the apatite in SBF at the gap between the glass particles and the substrate, and that the silicate ions diffuse and attach on the surface of the organic polymer substrates, where the silicate ion induces the apatite nucleation by a reaction similar to that occurring on the surfaces of bioactive $CaO-SiO_2$ -based glass [7-9]. Therefore, it is considered that the induction period for the apatite nucleation is closely related to the affinity of the silicate ions to the substrate.

The induction periods for apatite nucleation on all the examined organic polymers of PET, PMMA, PESF, Nylon 6, PE and PTFE were reduced from 24 to 6 h by GD treatment. This is attributed to high rate of the attachment of the hydrated silica to them. The appreciable increase in the number of the apatite nuclei on one of these polymers with GD treatment was clearly observed on the AFM picture (Fig. 11). The carbonyl, ester, carboxyl and hydroxyl groups were confirmed to be formed on the surfaces of these polymers by the GD treatment (See Table 1). Therefore, it is considered that these highly polar groups provide the favorable sites for the silicate ions to bond to the substrates, and hence accelerate the apatite nucleation. A similar relation between the decrease in the induction period for the apatite nucleation and the increase in the amount of polar groups was also observed for the polymers treated with HCl [2] or NaOH [3] aqueous solution. It should be noted, however, that both the HCl and NaOH treatments were effective only for PET, PMMA, PESF and Nylon 6, but not for PE and PTFE, in increasing the amounts of the polar groups and hence in decreasing the induction period, whereas the GD treatment was effective

for all the examined polymers. In addition, the magnitudes of the increase in the amounts of the polar groups and the decrease in the induction period for the apatite nucleation by GD treatment were considerably larger than those by HCl or NaOH treatment, even for PET, PMMA, PESF and Nylon 6.

The adhesive strength of the apatite layer to the substrates was significantly increased by the GD treatment for all the examined polymers. This increase is well correlated with the induction period reduction by the GD treatment. Therefore it is attributed to the increases in the number of apatite nuclei as well as to the increase in their bonding strength to the substrate. The polar group formed by the GD-treatment could form a fairly strong bond with OH^- or Ca^{2+} ions in apatite. A similar relation between the increase in the adhesive strength and the increase in the amount of polar groups was also observed for the polymers treated with HCl [2] or NaOH [3] aqueous solution. It should be noted, however, that both the NaOH and HCl treatments were again effective only for PET, PMMA, and Nylon 6 but not effective for PESF, PE and PTFE, in increasing the adhesive strength, whereas the GD treatment is effective for all the examined polymers. In addition, the magnitude of the increase in the adhesive strength by the GD treatment is considerably larger than that by the HCl or NaOH treatment even for PET, PMMA and Nylon 6.

When the polymers were subjected to GD treatment for longer periods, all the polymers examined showed a decrease in the adhesive strength to the apatite layer. This might be attributed to breaking/degradation of the polymer chains by the longer GD treatment and the resultant pull-out of entanglements among them during the adhesive strength measurement. This assumption is consistent with the observation that, in the adhesive strength measurement the fracture occurred not at the

interface between the apatite layer and the substrate, but within the surface layer of the substrate for the polymers treated with GD for longer periods. Especially for Nylon 6, it is considered that the hydrogen bond between the amide groups was broken by the longer GD treatment, and the entanglements among them were easily pulled out.

The apatite layer formed by the present biomimetic process is very similar in its structure and composition, to the biologically active bone-like apatite layer which is formed on the surfaces of bioactive ceramics *in vivo* and is responsible for bonding to the living bone [10–13]. It has been shown that the degree of bioactivity of materials which is represented, for example, by rate of formation of bone on the surface of the materials [14], is very closely related to the rate of formation of the biologically active bone-like apatite layer on their surfaces *in vivo*. Higher bioactivities of Bioglass and glass-ceramic A-W than that of sintered hydroxyapatite are attributed to higher rate of formation of the bone-like apatite layer on the surfaces of the formers [14, 15]. In view of this fact, it is expected that the apatite layer formed by the present biomimetic process could exhibit very high bioactivity. In addition, it is noted that the apatite in the bone is intimately combined with some proteins such as collagen. In view of this fact, it is also expected that the bone-like apatite formed by the present biomimetic process also could exhibit good biocompatibility even with soft tissues consisting of organic substances. Actually, it is already reported that poly-ether sulfone coated with the bone-like apatite layer by the present biomimetic process showed good compatibility with the soft tissues of trachea [16].

On the other hand, inorganic-organic composites can show mechanical properties in wide range from that analogous to the soft tissues to that analogous to the hard tissues, depending on the volume ratio of the

inorganic substance to organic substance and over all three dimensional structure of the composite [17]. This indicates that the apatite-organic polymer composites obtained by the present biomimetic process are expected to be highly useful as bone-repairing materials as well as soft-tissue-repairing materials.

5. Conclusion

A glow discharge treatment formed considerable amounts of new polar groups such as carbonyl, ester, carboxyl and hydroxyl groups on the surface of PET, PMMA, PESF, Nylon 6, PE and PTFE. These polar groups significantly enhanced the apatite nucleation on the polymer substrates and increased the adhesive strength of the apatite layer to the organic polymer substrates. Among the surface treatments attempted in the present study, the GD treatment was most effective for decreasing the induction period and increasing the adhesive strength for all the polymers examined. The present method is believed to give highly bioactive materials with various mechanical properties, which can be applied to soft tissue-repairing materials as well as bone-repairing ones.

References

- [1] M. Tanahashi, T. Yao, T. Kokubo, M. Minoda, T. Miyamoto, T. Nakamura and T. Yamamuro "Apatite coating on organic polymers by a biomimetic process", *J. Am. Ceram. Soc.*, **77**, 2805-2808 (1994).
- [2] M. Tanahashi, T. Yao, T. Kokubo, M. Minoda, T. Miyamoto, T. Nakamura and T. Yamamuro "Apatite coated on organic polymers by biomimetic process: Improvement in its adhesion to substrate by HCl treatment", *J. Mater. Sci. Mater. Medicine*, in press.
- [3] M. Tanahashi, T. Yao, T. Kokubo, M. Minoda, T. Miyamoto, T. Nakamura and T. Yamamuro "Apatite coated on organic polymers by biomimetic process: Improvement in its adhesion to substrate by NaOH treatment", *J. Appl. Biomater.*, **5**, 339-347 (1994).
- [4] D. T. Clark, B. J. Cromarty and A. Dilks, "A theoretical investigation of molecular core binding and relaxation energies in a series of oxygen-containing organic molecules of interest in the study of surface oxidation of polymers", *J. Polym. Sci. Polym. Chem. Ed.*, **16**, 3173-3184 (1978).
- [5] A. Dilks, and A. VanLaeken, "Physicochemical aspects of polymer surfaces", ed. by K. L. Mittal, Plenum, New York, p. 749, 1983.
- [6] T. Kokubo, H. Kushitani, C. Ohtsuki, S. Sakka, T. Yamamuro "Chemical reaction of bioactive glass and glass-ceramics with a simulated body fluid", *J. Mater. Sci. Mater. Medicine*, **3**, 79-83 (1992).
- [7] C. Ohtsuki, T. Kokubo, K. Takatsuka and T. Yamamuro, "Compositional dependence of bioactivity of glasses in the system $\text{CaO-SiO}_2\text{-P}_2\text{O}_5$: Its in vitro evaluation" *J. Ceram. Soc., Japan*, **99**, 1-6 (1991).
- [8] C. Ohtsuki, T. Kokubo, and T. Yamamuro, "Mechanism of apatite formation on $\text{CaO-SiO}_2\text{-P}_2\text{O}_5$ glasses in a simulated body fluid", *J. Non.-Crystl. Solids*, **143**, 84-92 (1992).
- [9] P. Li, C. Ohtsuki, T. Kokubo, K. Nakanishi, N. Soga, T. Nakamura and T. Yamamuro, "Apatite formation induced by silica gel in a simulated body fluid", *J. Am. Ceram. Soc.*, **75**, 2094-2097 (1992).

- [10] M. Neo, S. Kotani, T. Nakamura, T. Yamamuro, C. Ohtsuki, T. Kokubo, and Y. Bando "A comparative study of ultrastructures of the interfaces between four kinds of surface-active ceramic and bone" *J. Biomed. Mater. Res.*, **26**, 1419-1432 (1992).
- [11] T. Kokubo, C. Ohtsuki, S. Kotani, T. Kitsugi and T. Yamamuro, "Surface structure of bioactive glass-ceramic A-W implanted into sheep and human vertebra" in *Bioceramics*, Vol. 2, ed. by Günther Heimke, Heidelberg, Germany, pp. 113-120, 1989.
- [12] T. Kokubo, S. Ito, Z. T. Huang, T. Hayashi, S. Sakka, T. Kitsugi and T. Yamamuro, "Ca, P-rich layer formed on high-strength bioactive glass-ceramic A-W", *J. Biomed. Mater. Res.*, **24**, 331-343 (1990).
- [13] C. Ohtsuki, Y. Aoki, T. Kokubo, Y. Bando, M. Neo, T. Yamamuro and T. Nakamura, "Characterization of apatite layer formed on bioactive glass-ceramic A-W", in *Bioceramics*, Vol. 5, ed. by T. Yamamuro, T. Kokubo and T. Nakamura, Kobunshi Kankokai, Inc., Kyoto, pp. 87-94, 1992.
- [14] K. Ono, T. Yamamuro, T. Nakamura and T. Kokubo, "Quantitative study on osteoconduction of apatite-wollastonite containing glass ceramic granules, hydroxyapatite granules, and alumina granules", *Biomaterials*, **11**, 265-271 (1990).
- [15] T. Kokubo, H. Kushitani, Y. Ebisawa, T. Kitsugi, S. Kotani, K. Ohura and T. Yamamuro, "Apatite formation on bioactive ceramics in body environment", in *Bioceramics*, Vol. 1, ed. by H. Oonishi, H. Aoki and K. Sawai, Ishiyaku Euro America, Tokyo, pp. 157-162, 1989.
- [16] K. Suzuki, R. Kobayashi, Y. Yokoyama, Y. Harada and T. Kokubo, "Experimental study of an artificial trachea made of polymers coated with hydroxyapatite" in *Bioceramics*, Vol. 6, ed. by P. Ducheyne and D. Christiansen, Butterworth-Heinemann, Guilford London pp. 245-250, 1993.
- [17] W. Bonfield "Hydroxyapatite-reinforced polyethylene as an analogous materials for bone replacement" in *Bioceramics: Materials Characteristics vs In Vivo behavior*, ed. by P. Ducheyne and J. E. Lemons, Annals of New York Academy of Sciences, New York, pp. 173-177, 1988.

CHAPTER 6.

APATITE FORMATION ON ORGANIC POLYMERS BY A BIOMIMETIC PROCESS USING $\text{Na}_2\text{O-SiO}_2$ GLASSES AS NUCLEATING AGENTS

1. Introduction

It has been already confirmed for various kinds of bioactive glasses and glass-ceramics that the prerequisite for them to bond to living bone is the formation of a biologically active carbonate-containing hydroxyapatite layer on them in the body [1, 2]. Our recent studies revealed that the mechanism for the formation of bone-like apatite layer on the surfaces of CaO-SiO_2 -based glasses and glass-ceramics is explained as follows [3-5]. The calcium ion dissolved from the glasses and glass-ceramics increases the ionic activity product of the apatite in the surrounding body fluid, which is already supersaturated with respect to the apatite, and the hydrated silica of the surfaces of the glasses and glass-ceramics provides favorable sites for the apatite nucleation. As a result, a large number of the apatite nuclei are formed on the surfaces of the glasses and glass-ceramics. Once the apatite nuclei are formed, they spontaneously grow consuming the calcium and phosphate ions from the surrounding fluid.

On the basis of these findings, we have developed the following biomimetic method for forming bone-like apatite layer in desired thickness on any kind of organic polymer substrate at normal temperature and pressure. First a substrate is set in contact with the particles of bioactive CaO-SiO_2 -based glass soaked in a simulated body fluid (SBF) with ion concentrations nearly equal to those of human blood plasma so that calcium

ion dissolved from the glass increases the ionic activity product of apatite in the surrounding SBF and silicate ion dissolved from the glass adheres on the surface of the substrate to induce the apatite nucleation. Second the substrate is soaked in another solution with ion concentrations 1.5 times those of SBF (1.5SBF) so that the apatite nuclei formed in the first soaking grow in situ on the substrate by taking the phosphate and calcium ions from the solution [6]. Actually, when rectangular substrates 10 mm x 15 mm x 1 mm in size and abraded with #400 diamond paste were subjected to the first treatment for 24 h and then to the second treatment for 6 d, a dense and uniform layer of bone-like apatite were formed on the surfaces of various kinds of organic polymer substrates [7]. According to the explanation described above, it is considered that the other kind of glasses which dissolve the ion able to increase the ionic activity product of apatite as well as silicate ion can also induce the apatite nucleation on the organic polymer substrates.

In the present chapter, the CaO-SiO₂-based glass in the biomimetic method described in Chapter 2 was replaced by Na₂O-SiO₂ glasses, SiO₂ glass and SiO₂ gel, and the induction period for the apatite nucleation on various organic polymer substrates and the adhesive strength of the apatite layer to the substrates were investigated.

2. Experimental

2.1. Preparation of Glass and Gel

The compositional region in the system Na₂O-SiO₂ where glass can be formed by an ordinary melting technique was previously reported by F. C. Kracek [8]. Four kinds of glasses in the glass-forming region, which are

given in Table 1, were chosen for the present experiment. The glasses with Na₂O content higher than 40 mol% were not used, because of their low chemical durabilities. Powder mixtures of compositions in Table 1 were prepared from reagent grade chemicals Na₂CO₃ and SiO₂, put into a 50 ml platinum crucible and melted in a MoSi₂ furnace for 2 h. The temperature for the melting was 1600 °C for Na1Si9 and Na2Si8, and 1400 °C for Na3Si7 and Na4Si6 compositions, respectively. The melts were poured onto a stainless steel plate and pressed into a glass plate.

A high-purity silica glass (metallic impurity < 0.5 ppm, OH concentration < 150 ppm), which had been synthesized by vapor axial deposition (VAD) of SiCl₄, was supplied by Sumitomo Metal Industries, Ltd (Tokyo, Japan). A silica gel was prepared by the hydrolysis and polycondensation of tetraethoxysilane (TEOS) by the following method [5]. First, 0.7 g of poly(ethylene glycol) having an average molecular weight of 10000 was dissolved in 8.0 g of distilled water, and 0.81 g of concentrated nitric acid (62 wt%) was added. Then, 7.0 ml of TEOS was added to the above solution, transferred to a plastic petri dish with its top tightly sealed, and kept at 40 °C in an air-circulating oven for gelation. After it was aged for 18 h, the obtained wet gel was immersed in an equivolume mixture of distilled water and ethanol to remove the organic polymer phase. After 6 h, the wet gel was immersed in 1 mol/dm³ nitric acid for 2 h and dried at 40 °C for 4 d. The dried gel was heated to 400 °C at a rate of 100 °C/h, and held at 400 °C for 2 h, then allowed to cool to room temperature.

Thus prepared glasses and gel were crushed by a laboratory planetary type zirconia ball mill, and sieved for obtaining only the grains 150 to 300 μm in size.

Table 1 Nominal composition of examined Na₂O–SiO₂ glasses.

Name	Composition / mol%	
	Na ₂ O	SiO ₂
Na4Si6	40	60
Na3Si7	30	70
Na2Si8	20	80
Na1Si9	10	90

2.2. Preparation of Solutions

The simulated body fluid (SBF) with ion concentrations nearly equal to those of human blood plasma [9] as the medium for apatite nucleation, and an aqueous solution with ion concentrations 1.5 times those of SBF (1.5SBF) as the medium for apatite growth were prepared by method described in Sections 2.1. and 2.2. of Chapter 2.

2.3. Apatite Coating

A rectangular specimens 10 mm × 15 mm × 1 mm of the poly-ether sulfone (PESF), poly(ethylene terephthalate) (PET), poly(methyl methacrylate) (PMMA), polyamide 6 (Nylon 6), polyethylene (PE) and poly(tetrafluoroethylene) (PTFE) were first placed on 1.5 g of particles of Na₂O–SiO₂ glasses, SiO₂ glass or SiO₂ gel which were soaked in 30 ml of SBF at 36.5 °C, respectively in different polystyrene bottles 35 mmφ and 70 mm high, as shown in Fig. 1 of Chapter 2 for various periods up to 168 h, for forming the apatite nuclei on the surface of the substrate apposed to the glass particles. Then, the substrate was soaked in 30 ml of 1.5SBF at 36.5 °C in the polystyrene bottle for 6 d for making the apatite nuclei grow

on the surface of the substrate in situ. The 1.5SBF solution was renewed every 2 d. After the second treatment, the substrate was washed moderately with distilled water, and dried at room temperature.

2.4. Analysis of Apatite

The surfaces of the substrates after the second treatment were analyzed with a thin-film X-ray diffraction, Fourier-transform infrared (FT-IR) reflection spectroscopy and a scanning electron micrography (SEM). The former two techniques enable us to detect only thin layer about 1 μm thick at the surface. In the X-ray diffraction, a thin-film attachment, Rigaku 2651 Al, (Rigaku Co., Tokyo, Japan) was used and the glancing angle of the specimen was fixed at 1° against the incident beam. The infrared spectroscopy was performed by using a spectrometer, FT-IR 5M (Japan Spectroscopic Co. Ltd., Tokyo, Japan) with the reflection angle of 75°. In the scanning electron micrography, the specimen was coated with gold-palladium film and observed under an electron microscope, Hitachi S-2500CX (Hitachi Co., Ltd., Tokyo, Japan).

2.5. Measurement of Adhesive Strength

The organic polymer substrates on which the apatite layer of about 10 μm thick were used as the test samples. The adhesive strength of thus formed apatite layer to the substrates was measured by the same method as that described in Section 2.6. of Chapter 2.

2.6. Measurement of element concentrations

In order to study the mechanism of the apatite formation, the changes in the concentrations of sodium and silicon in 30 ml of SBF with the immersion of 1.5 g of the Na₂O–SiO₂ glasses, SiO₂ glass or SiO₂ gel

particles 150 to 300 μm in size were measured by inductively coupled plasma (ICP) emission spectroscopy (Seiko Instruments Inc. SPS1500, Osaka, Japan). Change in the pH of the fluid was also measured.

3. Results

3.1. Apatite formation

Figures 1(a) and (b) show thin-film X-ray diffraction patterns and FT-IR reflection spectra of the surfaces of the PESF substrates which were set in contact with Na1Si9 glass in SBF for different periods and then soaked in 1.5SBF for 6 d. Figure 2 shows SEM photographs of the same surfaces. It can be seen from Figs. 1 and 2 that a dense and uniform layer of carbonate-containing hydroxyapatite with a small crystallite and/or defective structure, similar to the apatite in the natural bone, is formed on the PESF substrates by the biomimetic method using even the $\text{Na}_2\text{O-SiO}_2$ glass, similarly to the CaO-SiO_2 -based glass [7]. In this case too, formation of the apatite layer after the second treatment largely depends on the period of the time for the first treatment. When the first treatment was omitted, no apatite was formed even after the second treatment for 6 d. The apatite was formed only sparsely on the substrate subjected to the first treatment for periods shorter than 48 h, and in a continuous layer only on the substrate subjected to the first treatment for longer than 96 h. This indicates that the number of the apatite nuclei formed on the substrate increases with increasing period of time for the first treatment. Here, the time of the first treatment required for forming enough number of the apatite nuclei to make the continuous layer after the second treatment is tentatively defined as the induction period for the apatite nucleation. Thus defined induction period

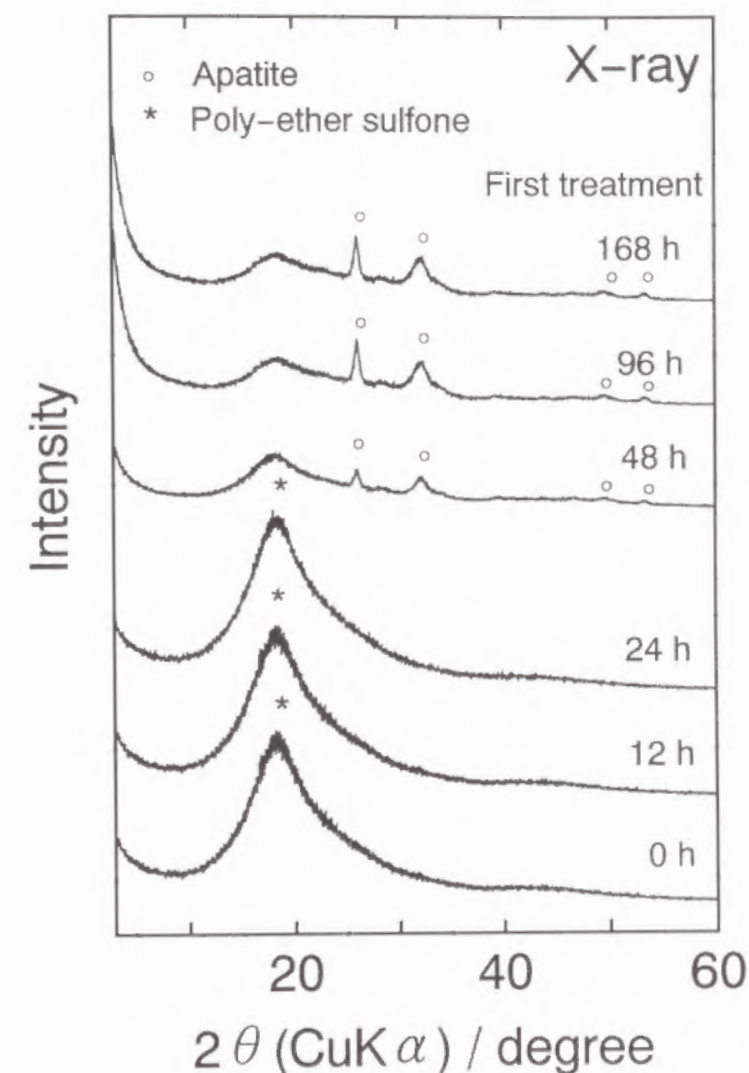


Figure 1(a). Thin-film XRD patterns of the surfaces of the PESF substrates soaked in 1.5SBF for 6 d after soaking in SBF with Na1Si9 glass for different periods.

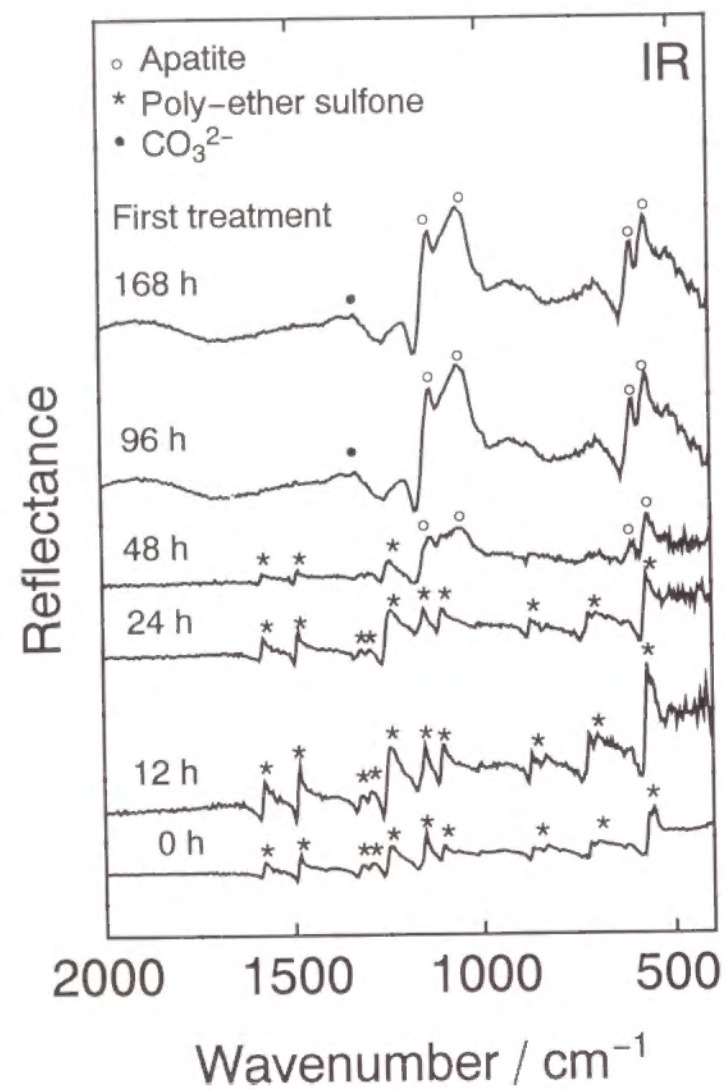


Figure 1(b). FT-IR reflection spectra of the surfaces of the PESF substrates soaked in 1.5SBF for 6 d after soaking in SBF with Na1Si9 glass for different periods.

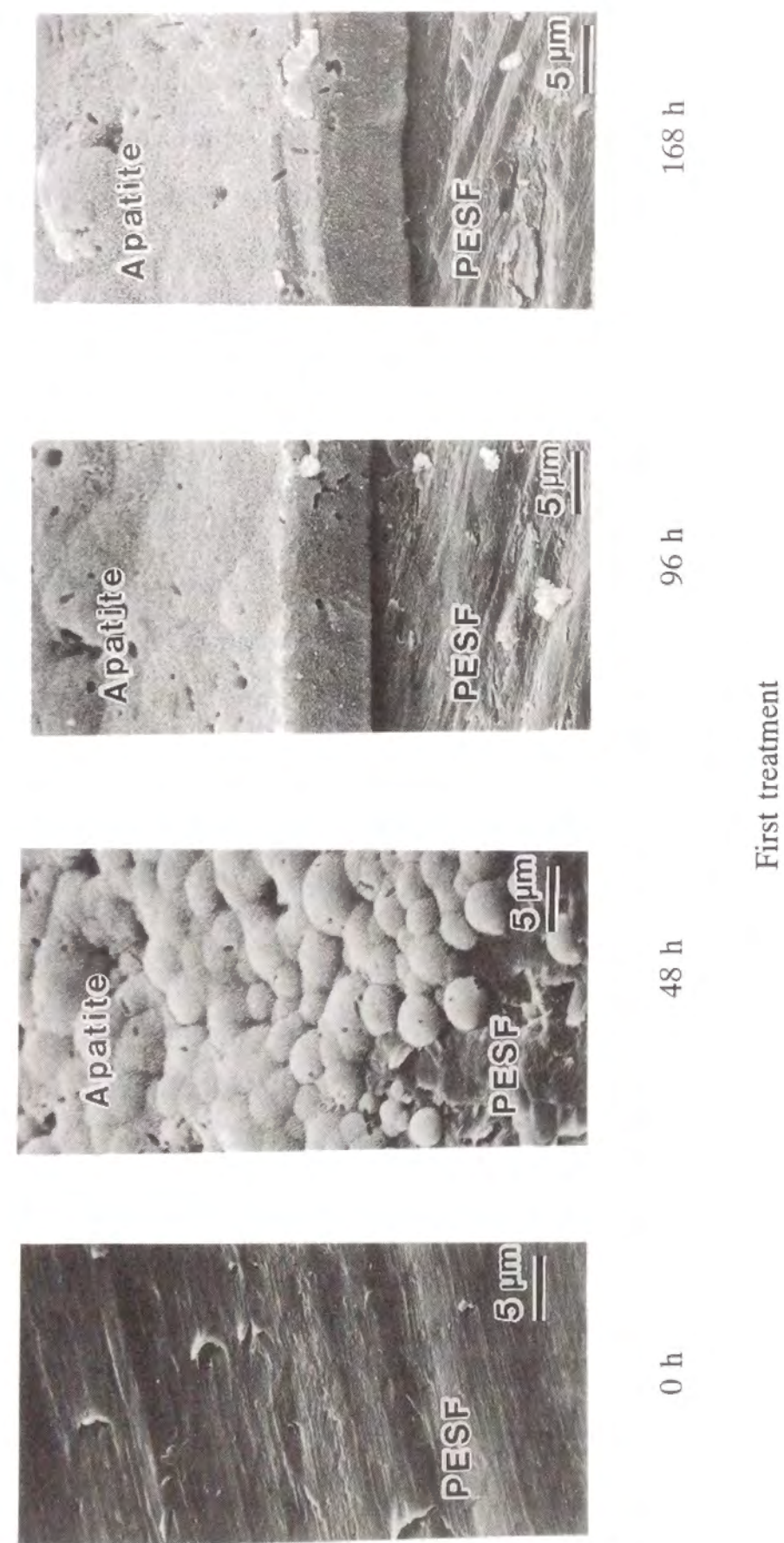


Figure 2. SEM photographs of the surfaces of the PESF substrates soaked in 1.5SBF for 6 d after soaking in SBF with Na1Si9 glass for different periods.

is 96 h in this case.

Figures 3(a) and (b) and Fig. 4 show thin-film X-ray diffraction patterns, FT-IR reflection spectra and SEM photographs, respectively, of the surfaces of the PESF substrates which were set in contact with Na₂Si₈ glass in SBF for different periods and then soaked in 1.5SBF for 6 d. It can be seen from Figs. 3 and 4 that the continuous apatite layer is formed by the first treatment for a shorter period of 48 h. The apatite formed on the substrate became again sparse when the first treatment was prolonged longer than 168 h. Figure 5 shows thin-film X-ray diffraction patterns (a) and FT-IR reflection spectra (b) of the surfaces of the PESF substrates subjected to the same treatments as those described above but using Na₃Si₇ glass. Figure 6 shows SEM photographs of the same surfaces. It can be seen from Figs. 5 and 6 that the induction periods for the apatite nucleation in this case is 1.5 h. When the substrate was subjected to the first treatment for longer periods, other substance was formed on the substrate. This substance was identified to be silica by electron probe X-ray microanalysis. In the case of Na₄Si₆ glass, similar phenomena were observed and the induction period for the apatite nucleation was 0.5 h. Figures 7(a) and (b) and 8 show thin-film X-ray diffraction patterns, FT-IR reflection spectra and SEM photographs of the surfaces of the PESF substrates subjected to the first treatment using the Na₂O-free silica gel for different periods, and then to the second treatment for 6 d. Even in this case, when the substrate was subjected to the first treatment for longer periods than 96 h, the apatite was sparsely formed on the substrate. When the silica glass was used instead of the silica gel, however, no apatite was formed on PESF substrate after the second treatment even when it was subjected to the first treatment for a period as long as 168 h.

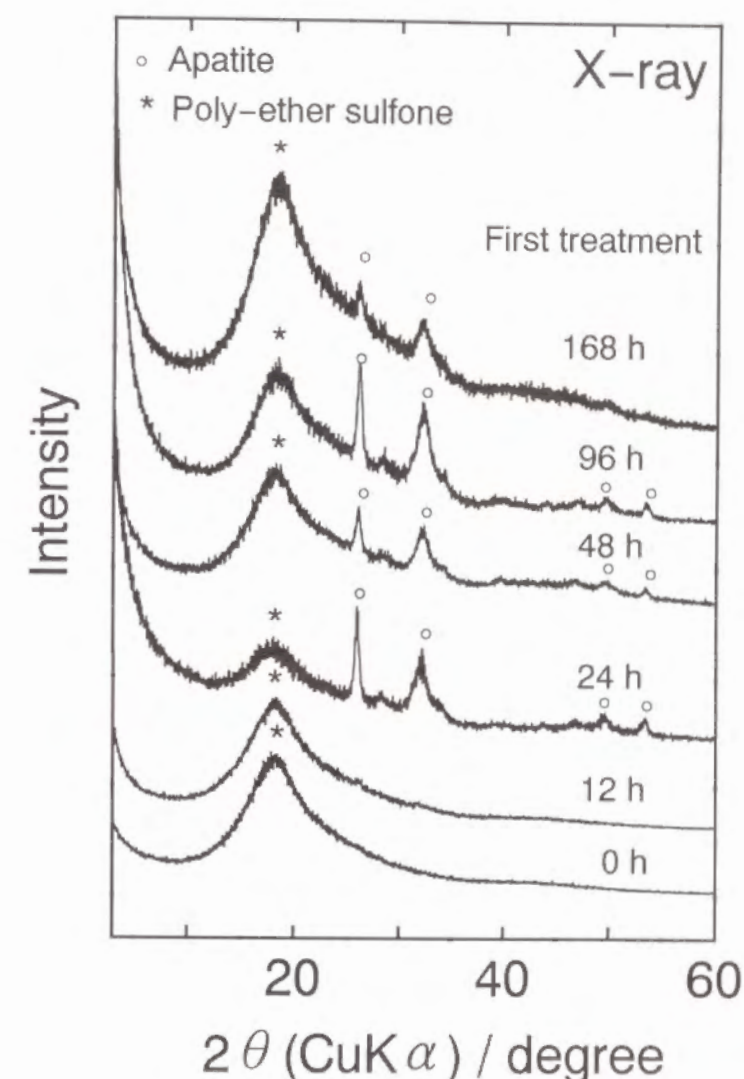


Figure 3(a). Thin-film XRD patterns of the surfaces of the PESF substrates soaked in 1.5SBF for 6 d after soaking in SBF with Na₂Si₈ glass for different periods.

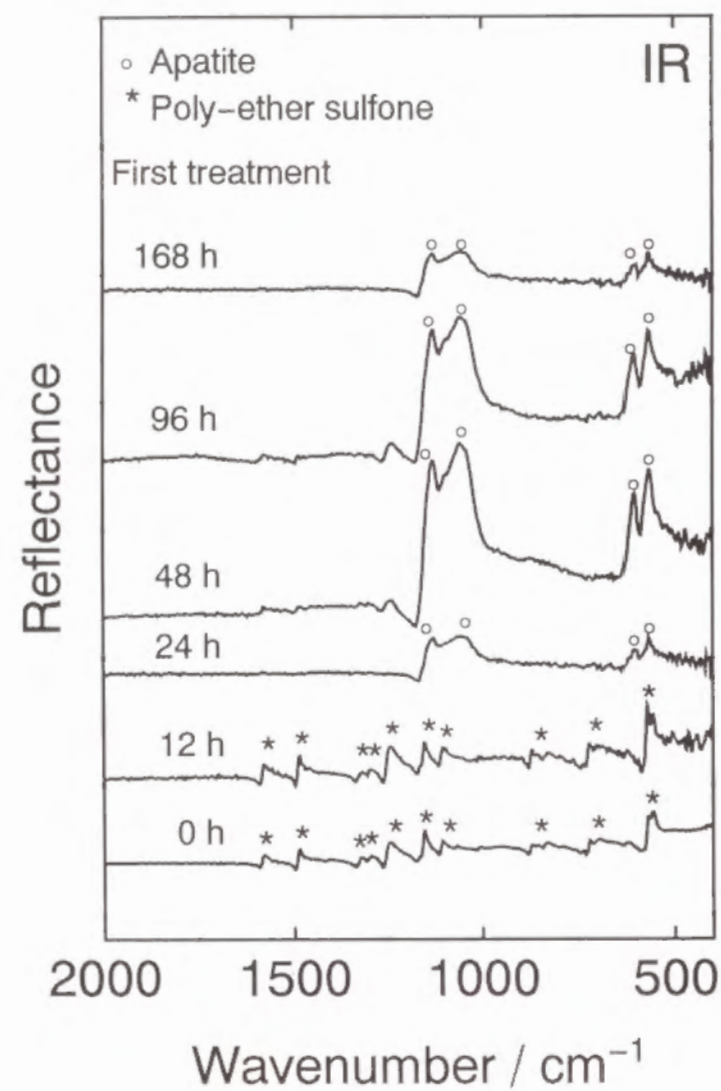


Figure 3(b). FT-IR reflection spectra of the surfaces of the PESF substrates soaked in 1.5SBF for 6 d after soaking in SBF with Na₂Si₈ glass for different periods.

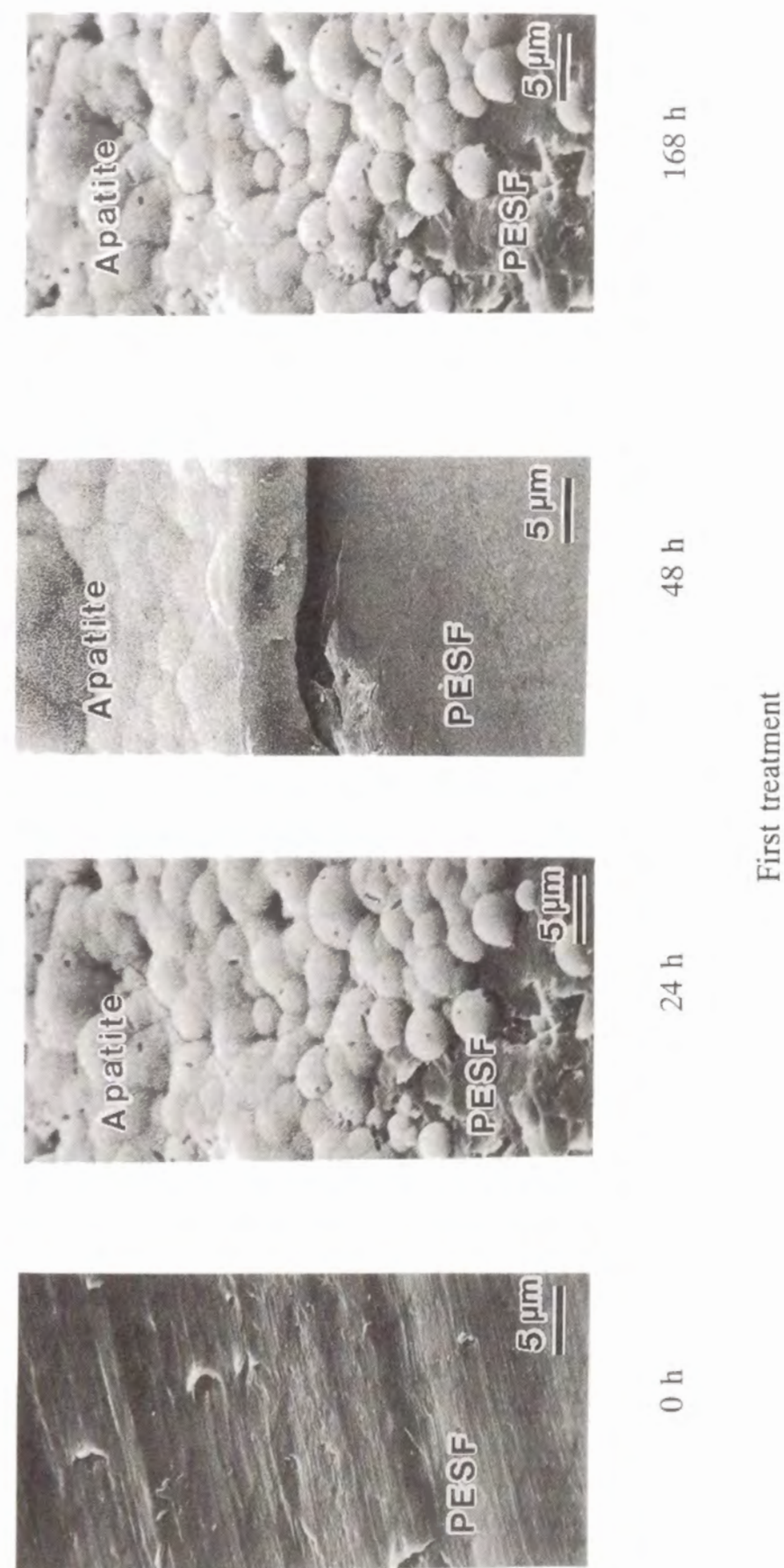


Figure 4. SEM photographs of the surfaces of the PESF substrates soaked in 1.5SBF for 6 d after soaking in SBF with Na₂Si₈ glass for different periods.

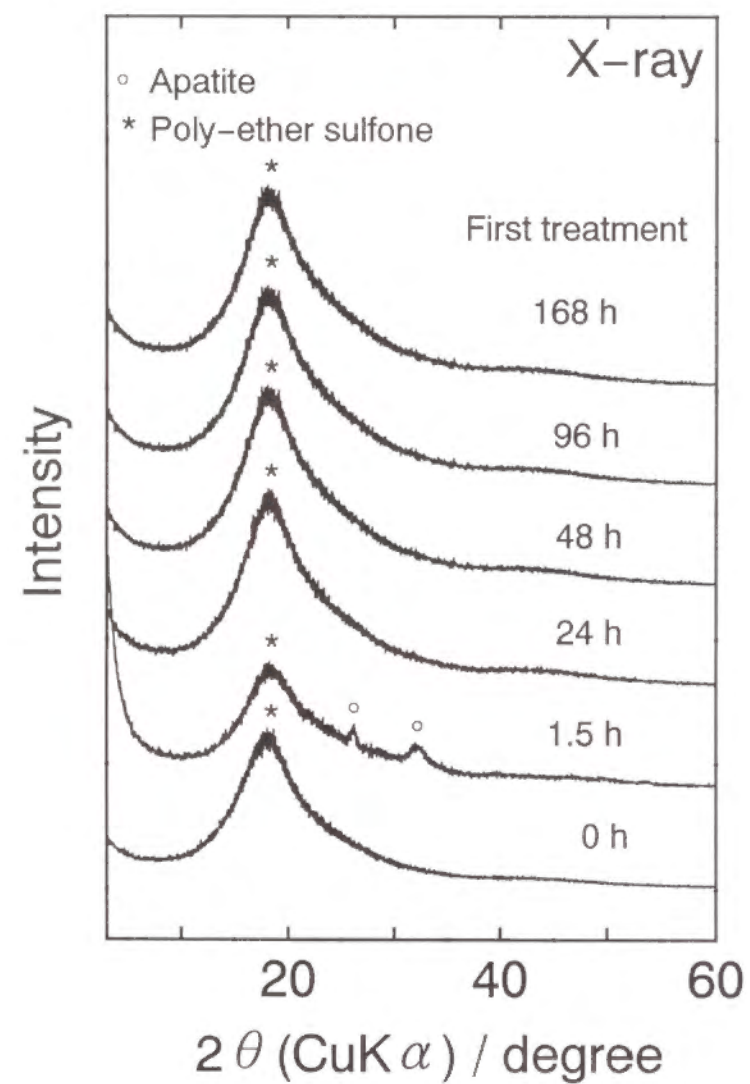


Figure 5(a). Thin-film XRD patterns of the surfaces of the PESF substrates soaked in 1.5SBF for 6 d after soaking in SBF with Na₃Si₇ glass for different periods.

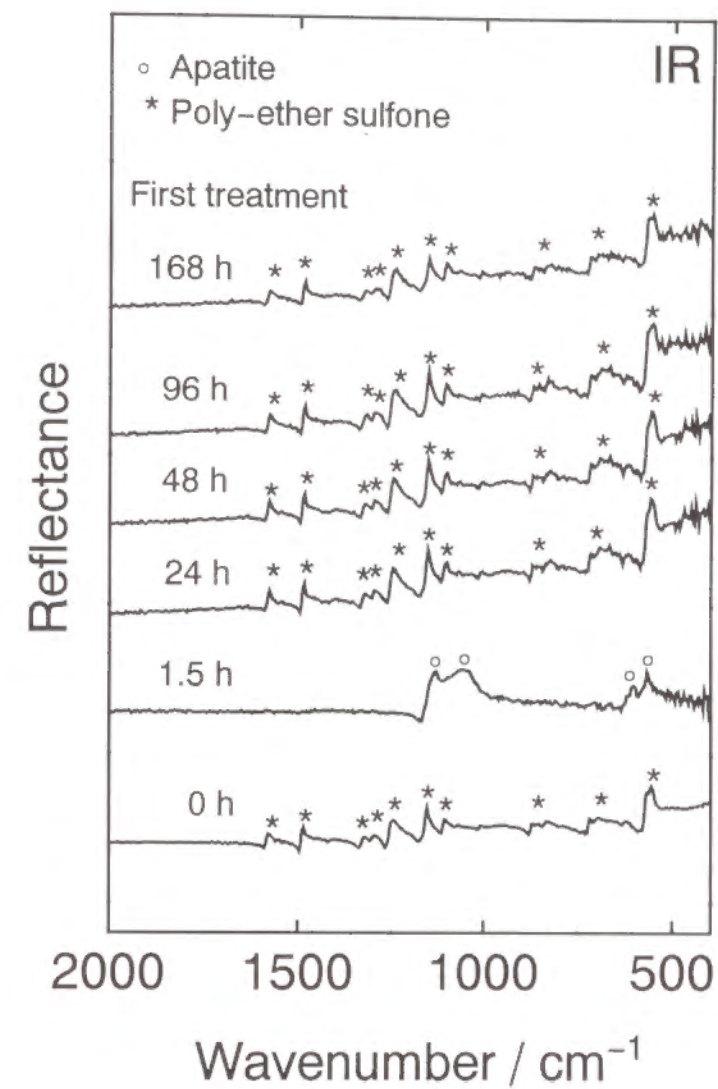
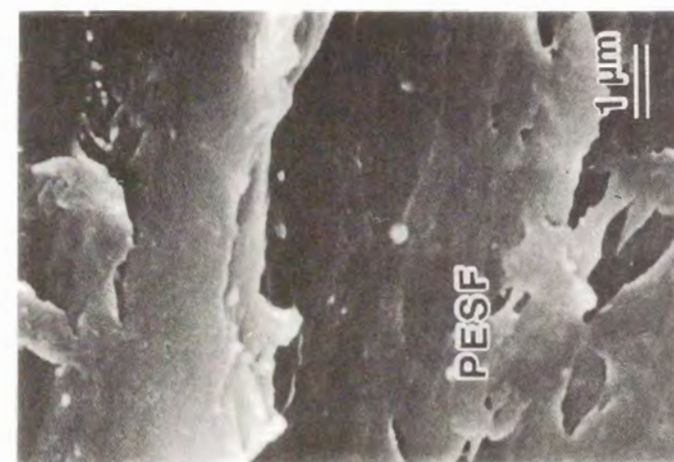
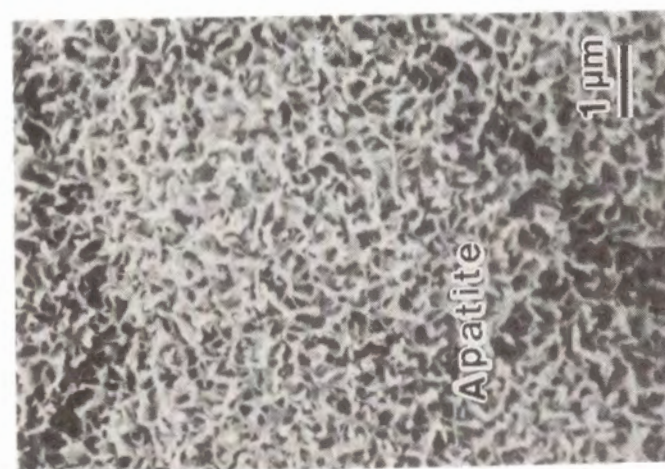


Figure 5(b). FT-IR reflection spectra of the surfaces of the PESF substrates soaked in 1.5SBF for 6 d after soaking in SBF with Na₃Si₇ glass for different periods.

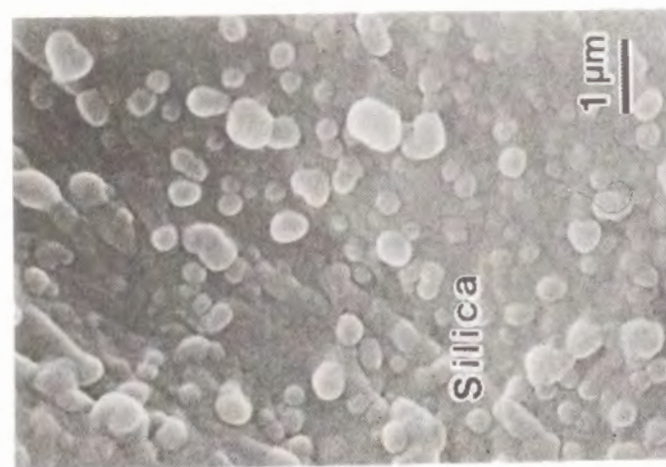


0 h



1.5 h

First treatment



168 h

Figure 6. SEM photographs of the surfaces of the PESF substrates soaked in 1.5SBF for 6 d after soaking in SBF with Na₃Si₇ glass for different periods.

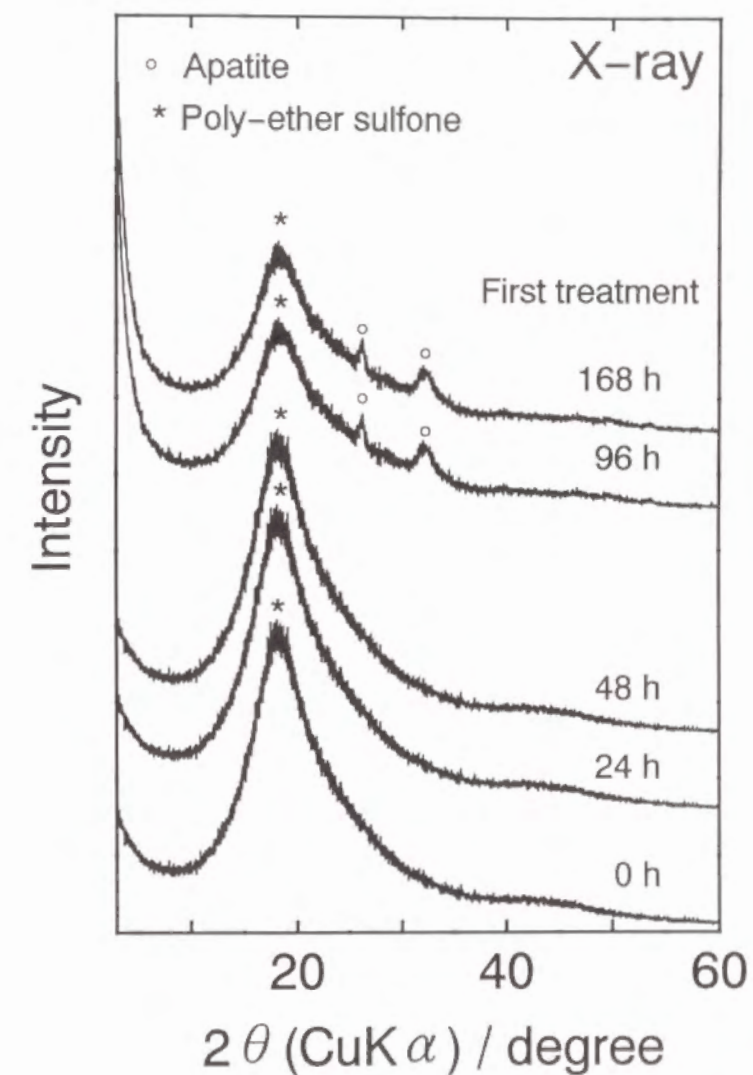


Figure 7(a). Thin-film XRD patterns of the surfaces of the PESF substrates soaked in 1.5SBF for 6 d after soaking in SBF with SiO₂ gel for different periods.

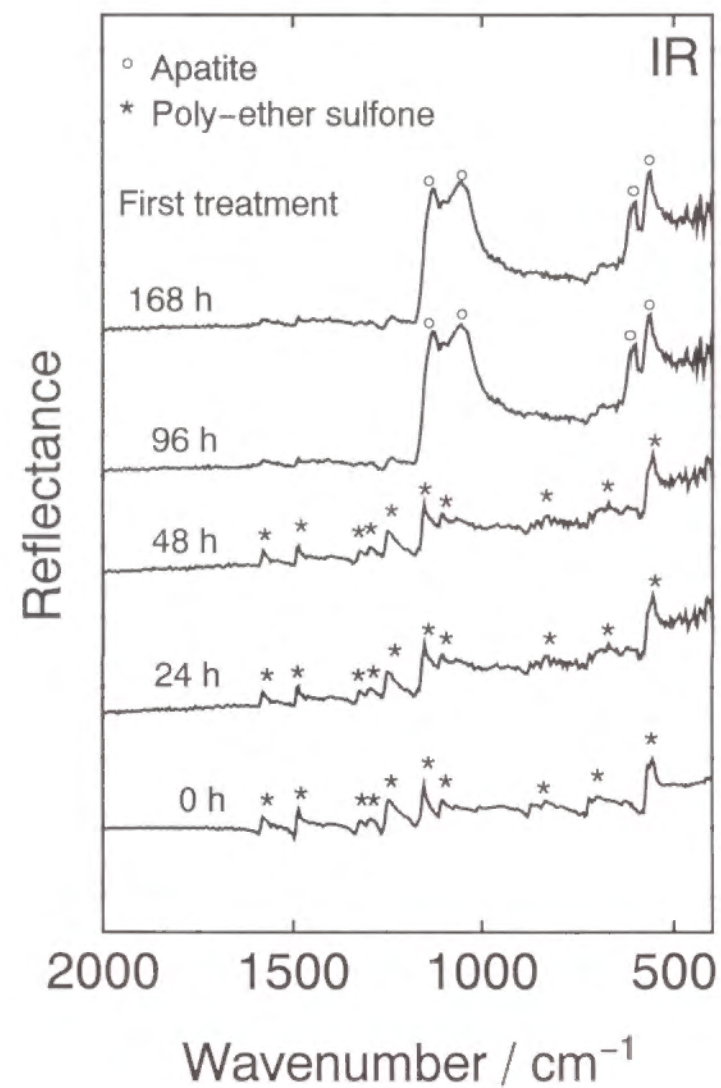


Figure 7(b). FT-IR reflection spectra of the surfaces of the PESF substrates soaked in 1.5SBF for 6 d after soaking in SBF with SiO₂ gel for different periods.



Figure 8. SEM photographs of the surfaces of the PESF substrates soaked in 1.5SBF for 6 d after soaking in SBF with SiO₂ gel for different periods

3.2. Adhesive strength

An apatite layer about 10 μm thick was formed on PESF substrates by using different Na_2O - SiO_2 glasses in the first treatment. The periods for the first treatment were 0.5, 1.5, 48 and 96 h for Na_4Si_6 , Na_3Si_7 , Na_2Si_8 and Na_1Si_9 glasses, respectively. Those for the second treatments were 10 days for Na_4Si_6 and Na_3Si_7 , and 6 days for Na_2Si_8 and Na_1Si_9 glasses. The adhesive strengths of apatite layer formed to the substrate are shown in Fig. 9 as a function of the Na_2O content of the glasses. It can be seen from Fig. 9 that the adhesive strength increases from 1.5 to 6.8 MPa with increasing Na_2O content from 10 to 30 mol% and then decreases down to 2.3 MPa at Na_2O content of 40 mol%.

The adhesive strengths of the apatite layers similarly formed on various polymer substrates by using Na_3Si_7 glass in the first treatment are shown in Table 3 in comparison with those of the apatite layer formed by using a CaO - SiO_2 -based glass, i. e. MgO 4.6, CaO 44.7, SiO_2 34.0, P_2O_5 16.2 and CaF_2 0.5 wt% glass G, in the first treatment, which was described in Chapter 2. It can be seen from Table 3 that the adhesive strength increases from 3.5 to 5.4, from 1.1 to 1.6, from 4.4 to 6.8 and from 0.6 to 2.9 MPa for PET, PMMA, PESF and Nylon 6, respectively, but does not change for PE and PTFE by replacing glass G with Na_3Si_7 glass.

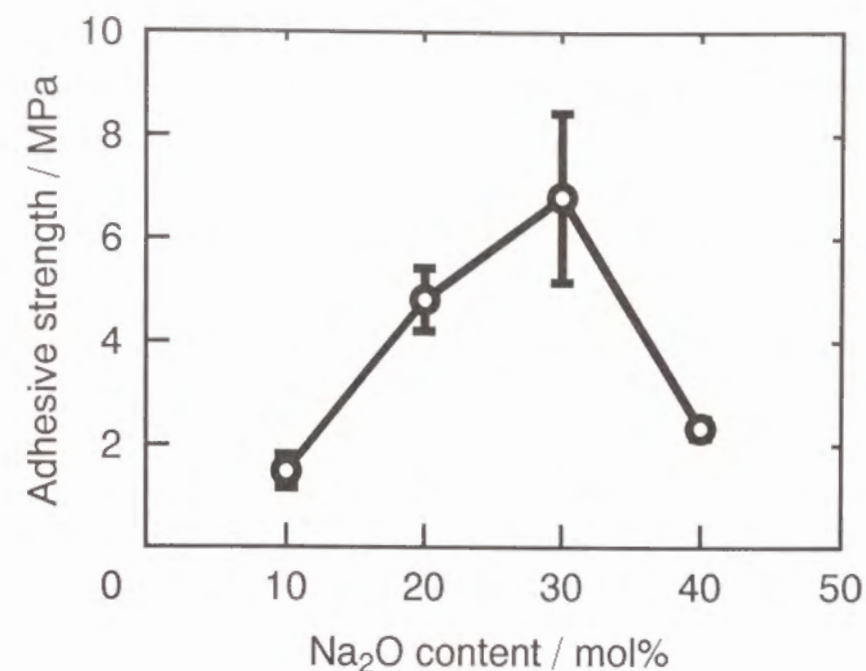


Figure 9. Adhesive strength of the apatite layer to the PESF substrates as a function of Na_2O content of Na_2O - SiO_2 glasses used in the first treatment.

Table 3. Adhesive strengths of apatite layer formed on various organic polymer substrates by using Na_3Si_7 glass in comparison with those formed by using glass G.

Substrate	Adhesive strength / MPa	
	Na_3Si_7 glass	Glass G
PET	5.37 [0.13]	3.48 [0.14]
PMMA	1.63 [0.15]	1.06 [0.17]
PESF	6.80 [3.28]	4.40 [0.28]
Nylon 6	2.91 [0.46]	0.63 [0.02]
PE	1.72 [0.15]	1.93 [0.23]
PTFE	1.1×10^{-2}	1.1×10^{-2}

[] Standard deviation

3.3. Element concentration changes

Figures 10, 11 and 12 show the changes in concentrations of the sodium, silicon and pH value of the SBF, respectively, due to the immersion of Na₄Si₆, Na₃Si₇, Na₂Si₈, Na₁Si₉ glasses, silica gel and silica glass. Those due to the immersion of glass G are also shown in these figures for comparison. It can be seen from Figs. 10, 11 and 12 that Na₄Si₆ and Na₃Si₇ glasses dissolve the sodium as large as 600 mM and silicon in larger amount than 10 mM, to increase pH value to about 12, within 48 h. Na₂Si₈ and Na₁Si₉ glasses also dissolve the sodium and silicon, but their dissolution rates are very small, i. e. the sodium less than 200 mM and the silicon less than 4 mM even after 168 h. Consequently, the increases in pH are also relatively small, i. e. pH less than 11 even after 168 h. The silica gel also dissolves about 2 mM of the silicon, but the silica glass only 0.1 mM of the silicon even after 168 h. It has been previously reported that glass G dissolves appreciable amounts of the calcium and silicon [10]. The results in Figs. 11 and 12 are consistent with the result previously reported.

4. Discussion

It was shown in the experiments described above that the induction period for the apatite nucleation on the PESF substrate is 0.5, 1.5, 48 and 96 h for Na₄Si₆, Na₃Si₇, Na₂Si₈ and Na₁Si₉ glasses which were used in the first treatment. This is explained as follows. These glasses dissolve both the sodium and silicate ions into the SBF as shown in Figs. 10 and 11. The sodium ion increases the ionic activity product of the apatite in the surrounding SBF by increasing pH as shown in Fig. 12, since the apatite

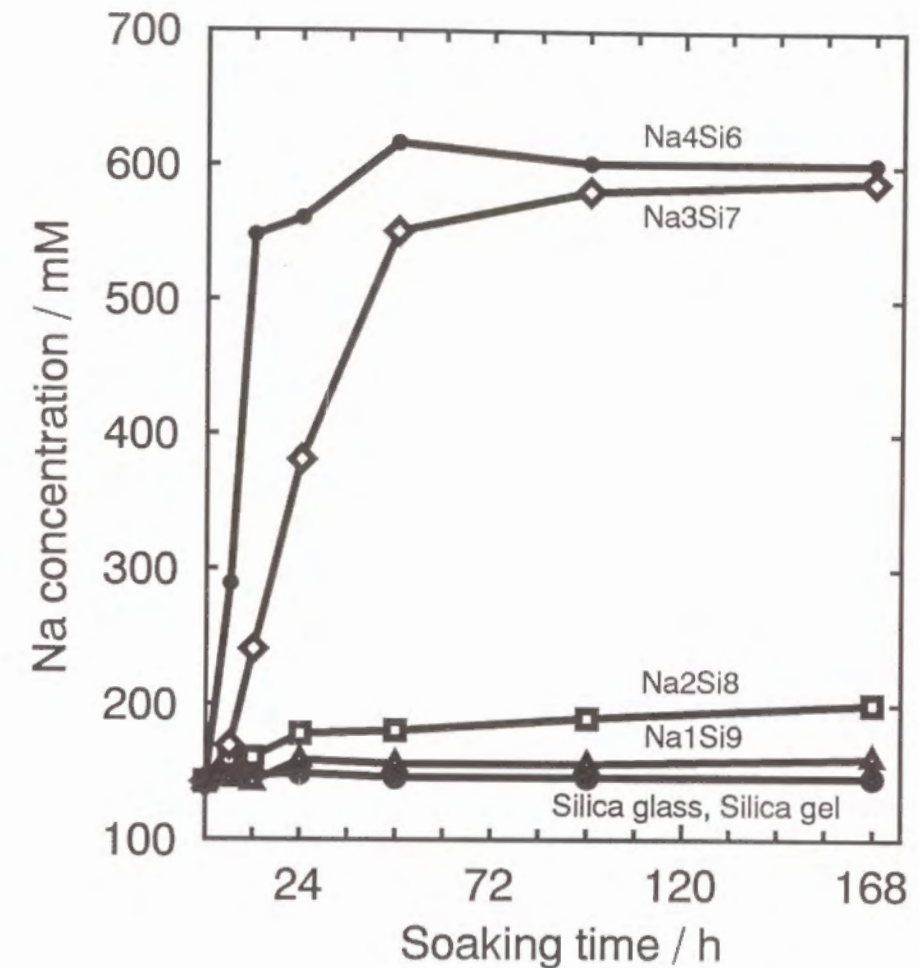


Figure 10. Change in Na concentration of SBF due to immersion of Na₂O–SiO₂ glasses, SiO₂ gel and SiO₂ glass as a function of soaking time.

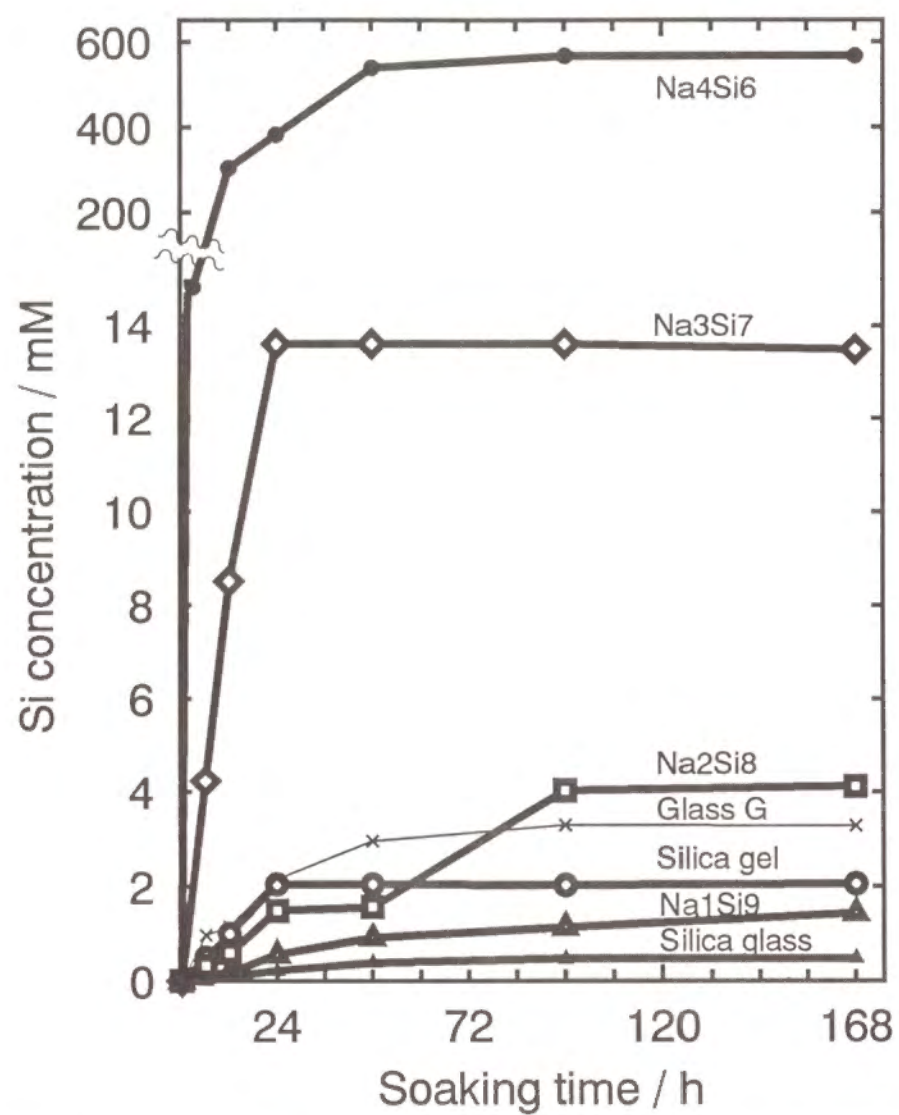


Figure 11. Change in Si concentration of SBF due to immersion of Na_2O - SiO_2 glasses, SiO_2 gel and SiO_2 glass as a function of soaking time.

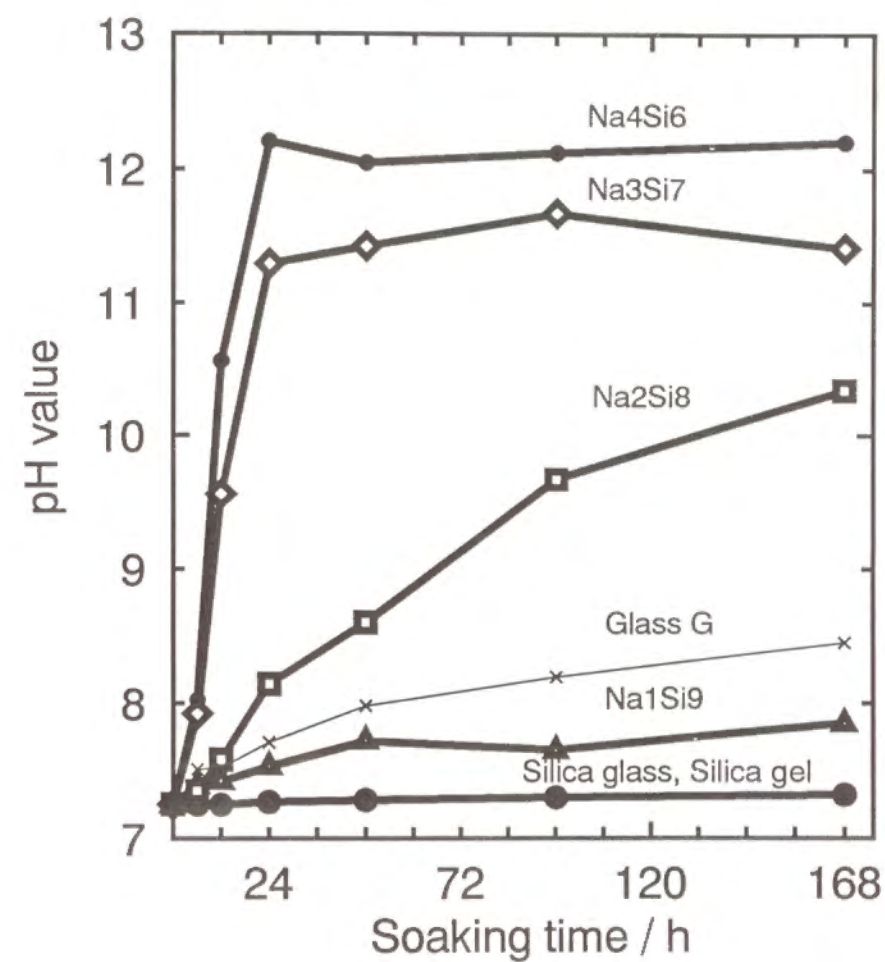


Figure 12. Change in pH of SBF due to immersion of Na_2O - SiO_2 glasses, SiO_2 gel and SiO_2 glass as a function of soaking time.

formation is represented by the following equation.



And the silicate ion attaches on the surface of the substrate to induce the apatite nucleation, similarly in the case of CaO-SiO_2 -based glass [4]. Consequently, the rate of the apatite nucleation on the substrate decreases with decreasing dissolution rates of these ions from the glasses, and hence the induction period for the apatite nucleation increases. According to Figs. 10 and 11, the rate of the dissolution of the sodium and silicate ions decreases in the order of $\text{Na}_4\text{Si}_6 < \text{Na}_3\text{Si}_7 < \text{Na}_2\text{Si}_8 < \text{Na}_1\text{Si}_9$. Therefore, the induction period for the apatite nucleation increases in this order, as described above. In the previous study, the induction period for the apatite nucleation on the PESF substrate was shown to be 24 h when glass G was used in the first treatment [7]. This value is smaller than that for Na_2Si_8 glass, i. e. 48 h, although the rates of dissolution of Si and of increase in pH for the former are lower than those for Na_2Si_8 glass (Figs. 11 and 12). This is attributed to the dissolution of the calcium ion for the former, which can increase the ionic activity product of the apatite not only by increasing pH but also by the presence of itself.

Here, it should be noted that even when the pure silica gel was used in the first treatment, the apatite was formed on the PESF substrate after the second treatment, although it was only sparsely. The silica gel dissolves a certain amount of the silicate ion, as shown in Fig. 11. This clearly shows that the silicate ion can induce the apatite nucleation on the substrates, but is not effective enough for forming the continuous apatite layer after the second treatment. The sodium or calcium ion is required in addition to the silicate ion for forming enough number of the apatite nuclei in the first

treatment to make a continuous layer by their growth in the second treatment. The silica glass dissolves only a slight amount of the silicate ion (Fig. 11), and hence apatite formation was not observed on PESF substrate when the silica glass was used in the first treatment. When the first treatment using Na_4Si_6 or Na_3Si_7 glass was done for a longer period than 0.5 or 1.5 h, respectively, only a silica layer was formed on the substrate after the second treatment. This might be attributed to the deposition of a silica gel layer peeled off from the surfaces of the Na_4Si_6 and Na_3Si_7 glasses. These glasses dissolve a large amount of the sodium ions rapidly. As a result, a thick silica gel layer is formed rapidly on their surfaces and a part of it might be peeled off and deposited on the substrate to cover the apatite nuclei early formed. Therefore, the apatite nuclei can not grow in the second treatment.

The adhesive strength of the apatite layer to the PESF substrate increased with increasing Na_2O content of the $\text{Na}_2\text{O-SiO}_2$ glasses up to 30 mol% which were used in the first treatment. The magnitude of the increase in the adhesive strength is well correlated with the that of the increase in pH value of the SBF due to the immersion of the $\text{Na}_2\text{O-SiO}_2$ glasses (Fig. 12). It was previously shown that the sulfonyl groups on the surfaces of the PESF substrates are hydrolyzed to higher polar group such as sulfonic acid in an alkali environment and form a fairly strong bond with the apatite [11]. In view of this fact, the higher adhesion for the glasses with higher Na_2O contents are attributed to formation of larger amount of the polar group at the surface of the substrate due to larger increase in pH of the surrounding fluid. The $\text{Na}_2\text{O-SiO}_2$ glass with 40 mol% Na_2O , however, gave a low adhesive strength. This might be attributed to the hydrolysis of polymer chains near the surface of the polymer substrate by the excessive hydrolysis in a high pH environment. Consequently, the

surface layer of the polymer substrate was peeled off during the measurement of the adhesive strength.

The adhesive strength of the apatite layer increased against PET, PMMA, PESF and Nylon 6 substrate but did not change against PE and PTFE substrates by replacing glass G with Na₃Si₇ glass in the first treatment. It has been already confirmed that ester group in PET and PMMA, the amide group in Nylon 6 and sulfonyl group in PESF are hydrolyzed to the higher polar groups such as the carboxyl and sulfonic acid groups in an alkali solution [11]. On the other hand, PE and PTFE have no polar groups to be hydrolyzed. Therefore, the higher adhesive strengths for the former four kinds of polymer substrates may be also attributed to the formation of polar groups on their surfaces in the solution increased in pH by the sodium dissolution from the glass.

5. Conclusion

In the previous chapters, it has been stated that a dense and uniform bone-like apatite layer can be formed in arbitrary thickness on any kind and shape of solid substance by the following biomimetic method at normal temperature and pressure: First, a substrate is set in contact with particles of CaO-SiO₂-based glass soaked in a simulated body fluid (SBF) with ion concentrations nearly equal to those of human blood plasma. Second, the substrate is soaked in another solution highly supersaturated with respect to the apatite. In the present chapter, the CaO-SiO₂-based glass as the nucleating agent was replaced by Na₂O-SiO₂ glasses, SiO₂ glass and SiO₂ gel. The induction periods for the apatite nucleation on polyethersulfone substrates were ∞, a little longer than 168, 96, 48, 1.5 and 0.5 h for SiO₂

glass, SiO₂ gel, Na₂O 10·SiO₂ 90, Na₂O 20·SiO₂ 80, Na₂O 30·SiO₂ 70 and Na₂O 40·SiO₂ 60 mol% glasses, respectively, whereas 24 h for MgO 4.6·CaO 44.7·SiO₂ 34.0·P₂O₅ 16.2·CaF₂ 0.5 wt% glass G. The short induction period for the glasses with high Na₂O contents is attributed to high dissolution rates of sodium and silicate ions from them. The adhesive strengths of the formed apatite layer to the PET, PMMA, PESF and Nylon 6 were 5.37, 1.63, 6.80 and 2.91 MPa for Na₂O 30·SiO₂ 70 mol% glass, whereas 3.48, 1.06, 4.40 and 0.63 MPa for glass G. It is considered that highly polar carboxyl or sulfonic acid groups were formed on the polymer surfaces by the hydrolysis of their ester, amide or sulfonyl group in SBF considerably increased in its pH by the Na⁺ dissolution from the glass, and that these polar groups formed a fairly strong bond with the apatite. Thus formed apatite-organic polymer composites are expected to be useful as the bone-repairing materials as well as soft tissue-repairing ones.

References

- [1] T. Kokubo, "Recent progress in glass-based materials for biomedical applications" *J. Ceram. Soc. Japan*, **99**, 965-973 (1991).
- [2] L. L. Hench, "Bioceramics: From concept to clinic" *J. Am. Ceram. Soc.*, **74**, 1487-510 (1991).
- [3] C. Ohtsuki, T. Kokubo, K. Takatsuka and T. Yamamuro, "Compositional dependence of bioactivity of glasses in the system CaO-SiO₂-P₂O₅: Its in vitro evaluation" *J. Ceram. Soc., Japan*, **99**, 1-6 (1991).
- [4] C. Ohtsuki, T. Kokubo, and T. Yamamuro, "Mechanism of apatite formation on CaO-SiO₂-P₂O₅ glasses in a simulated body fluid", *J.*

Non.-Crystl. Solids, **143**, 84-92 (1992)

- [5] P. Li, C. Ohtsuki, T. Kokubo, K. Nakanishi, N. Soga, T. Nakamura and T. Yamamuro, "Apatite formation induced by silica gel in a simulated body fluid", *J. Am. Ceram. Soc.*, **75**, 2094-2097 (1992).
- [6] T. Kokubo, K. Hata, T. Nakamura and T. Yamamuro, "Apatite formation on ceramics, metals and polymers induced by a CaO-SiO₂-based glass in a simulated body fluid", in *Bioceramics* Vol. 4, ed. by W. Bonfield, G. W. Hastings, K. E. Tunner, Butterworth-Heinemann, Guilford, London, pp. 113-120, 1991.
- [7] M. Tanahashi, T. Yao, T. Kokubo, M. Minoda, T. Miyamoto, T. Nakamura and T. Yamamuro, "Apatite coating on organic polymers by a biomimetic process " *J. Am. Ceram. Soc.*, **77**, 2805-2808 (1994).
- [8] F. C. Krasek, "Glass forming region of Na₂O-SiO₂ glass" *J. Am. Chem. Soc.*, **61**, 2869 (1939)
- [9] J. Gamble, in "*Chemical Anatomy Physiology and Pathology of Extracellular Fluid*", 6th Ed. Harvard Univ., Cambridge, pp. 1-8, 1967.
- [10] T. Kokubo, H. Kushitani, C. Ohtsuki, S. Sakka and T. Yamamuro, "Chemical reaction of bioactive glass and glass-ceramics with a simulated body fluid" *J. Mater. Sci: Materials in Medicine*, **3**, 79-83 (1992).
- [11] M. Tanahashi, T. Yao, T. Kokubo, M. Minoda, T. Miyamoto, T. Nakamura and T. Yamamuro "Apatite coated on organic polymers by biomimetic process: Improvement in its adhesion to substrate by NaOH treatment", *J. Appl. Biomater*, **5**, 339-347 (1994).

CHAPTER 7.

ULTRASTRUCTURAL STUDY OF APATITE LAYER FORMED BY A BIOMIMETIC PROCESS AND ITS BONDING TO BONE

1. Introduction

It was shown in the previous chapters that a dense and uniform bone-like apatite layer in desired thickness can be formed on any kind of organic polymer substrate at normal temperature and pressure even *in vitro* by the biomimetic process using CaO-SiO₂, or Na₂O-SiO₂ glasses as nucleating agent [1-2].

The adhesive strength of apatite layer formed to the substrates increases up to 10 MPa at maximum by a pretreatment of the substrates with O₂ gas glow discharge (GD) [3-5]. Thus prepared apatite-coated organic polymer fibers are expected to exhibit mechanical properties analogous to those of the natural bone not only in its mechanical strength, but also in its fracture toughness and elastic moduli, if they could be fabricated into three dimensional structure analogous to those of the natural bone.

In the study of this chapter, the structure of the apatite layer formed on an organic polymer by the biomimetic process and of its interface to living bone after implantation into rat were investigated by means of scanning and transmission electron microscopies.

2. Experimental

2.1. Preparation of substrate

Poly-ether sulfone (PESF) supplied by ICI, PLC (England) was formed into a plate by extrusion method. This plate was cut into a rectangular piece of 10 mm × 15 mm × 1 mm and abraded with #400 diamond pad on both sides. The PESF substrate was subjected to GD treatment in O₂ gas (99.9999%) atmosphere for 300 s to increase the affinity with the apatite [5]. The GD treatment was carried out at a pressure of 30 Pa, under an electric field of 13.56 MHz at 1 W/cm² by using Plasma Polymerization System, Model BP-2 (SAMCO Ltd., Kyoto, Japan).

2.2. Apatite coating

Glass particles named G of the nominal composition MgO 4.6, CaO 44.7, SiO₂ 34.0, P₂O₅ 16.2 and CaF₂ 0.5 wt%, simulated body fluid (SBF) with ion concentrations nearly equal to those of human blood plasma and an aqueous solution with ion concentrations 1.5 times those of SBF (1.5SBF) were prepared by same methods as those described in section 2.1. and 2.2. of Chapter 2.

The GD-treated PESF substrate was first embedded in the particles of glass G soaked in 30 ml of SBF at 36.5 °C for 24 h as shown in Fig. 1. Then, the substrate was soaked in 30 ml of 1.5SBF at 36.5 °C for 12 d. The solution of 1.5SBF was renewed every 2 d. As a result, an apatite layer about 20 μm thick was formed on the substrate. Thus treated substrate was gently washed with distilled water, and dried at room temperature.

2.3. Implantation into living bone

The PESF substrate coated with the bone-like apatite was

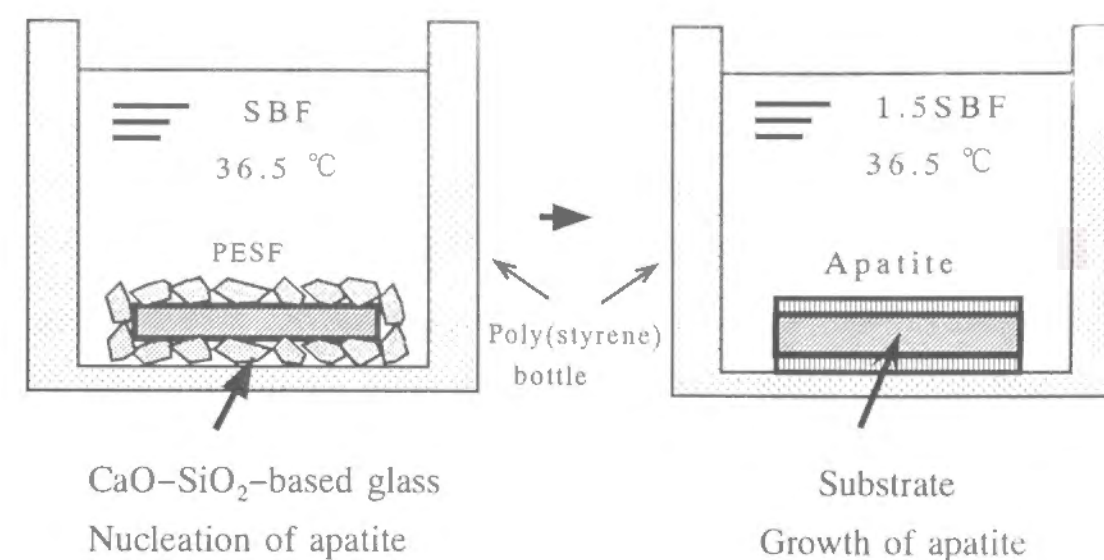


Figure 1. A biomimetic process for forming the bone-like apatite layer on PESF substrate.

conventionally sterilized with ethylene oxide gas, and 1 week later it was implanted into metaphyses of tibiae of a mature male rabbit weighing between 3.0 and 3.5 kg. The rabbit was anesthetized by intravenous injection of Nembutal (50 mg/kg) and local administration of 0.25 % Xylocaine. The operations were performed under standard aseptic conditions.

A 3 cm skin incision was made on the anteromedial aspect of the tibial metaphyses of the rabbit, and the skin, muscle and periosteum were retracted. Using a dental burr and saline coolant, a 15 mm hole was made parallel to the longitudinal axis of the tibial metaphyses. The hole was irrigated with saline before the PESF coated with the apatite was implanted. The muscle and skin were closed in layers.

2.4. Observation by electron microscope

The rabbit was killed 8 weeks after the operation. All the segments

of the tibiae containing PESF coated with the apatite were excised and fixed in 10 % phosphate-buffered formalin solution for histologic processing. Samples were dehydrated in serial ethanol concentrations and embedded in polyester resin. Thin sections were cut by a milling machine with a diamond disc (Maruto Ltd., Tokyo, Japan). Several cross sections at two thickness, 0.5 and 3 mm, were prepared using a band saw.

The surfaces of the 3 mm sections were polished with a diamond paste and coated with a thin layer of carbon. They were then observed under scanning electron microscope (SEM) with Model S-2500CX (Hitachi Co. Ltd., Tokyo, Japan) and analyzed using an energy-dispersive electron probe X-ray microanalyzer (EDX) with Model EMAX-3700 (Horiba Co. Ltd., Tokyo, Japan). Elemental analyses of Ca, P and S were performed across the PESF-apatite-bone interface. The accelerating voltage was 20 kV.

The 0.5 mm sections were ground on a grinding machine with a diamond lap disc to about 30 μm . These 30 μm sections were then embedded in epoxy resin. Ultrathin sections were cut with a diamond knife and stained with uranyl acetate and lead citrate or with uranyl acetate only. When the resin was cut to 30 μm thick, the apatite layer was separated from PESF substrate. The stained sections which contain the interface of apatite-bone were observed under a transmission electron microscope (TEM) with Model JEM-2000FXIII (JEOL LTD, Osaka, Japan). The electron diffraction was performed for both the apatite layer and the bone. The electron diffraction patterns were indexed by comparing their d-spacings with calculated values for same planes of the hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) consisting of a hexagonal unit cell with cell dimensions $a_0=b_0=0.9432$ nm and $c_0=0.6881$ nm [6].

3. Results

Figures 2(a) and (b) show SEM-EDX picture of the interface of PESF-apatite-bone 8 weeks after implantation and SEM photograph of the interface apatite-bone under a higher magnification, respectively. It can be seen from Figs. 2(a) and (b) that the apatite layer synthesized by the biomimetic process is remained on PESF substrate and is continuously bonded to the living bone without intervening layer.

Figure 3 shows TEM photograph of the interface of the apatite layer to the bone. It can be seen from Fig. 3 that the apatite layer consists of very tiny crystals whereas the bone is composed of larger fibrous crystals and that they meet directly. Their interface is not distinct.

Figures. 4 (a) and (b) show electron diffraction patterns at the spots in the apatite layer and in the bone, which are shown as asterisks in Fig. 3. It can be seen from these electron diffraction patterns that both the phases can be identified as the apatite. The interplanar spacings of 2 2 2, 2 1 1 and 0 0 2 reflections for the apatite in the apatite layer were 1.943, 2.814 and 3.440 nm, respectively, whereas those for the apatite in the bone were 1.940, 2.811 and 3.440 nm, respectively. This indicates that the apatite in the synthesized apatite layer is very similar to that in the bone in its structure.

4. Discussion

Surface-active ceramics have been reported to bond to the bone chemically through the bone-like apatite layer formed on their surfaces in the body [7-19]. This bone-like apatite layer is collagen-free and consists

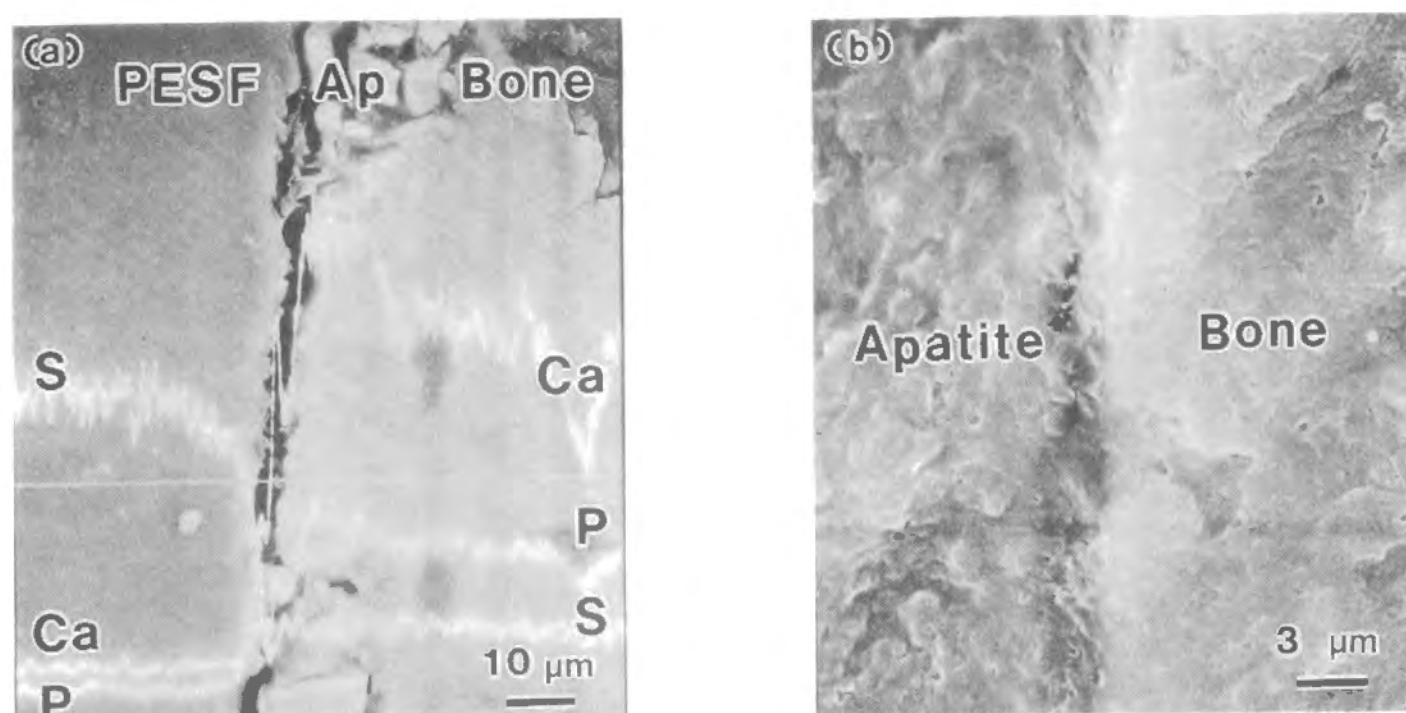


Figure 2. SEM-EDX picture of the interfaces of PESF-apatite layer-bone 8 weeks after implantation (a) and SEM photograph of the interface of apatite layer-bone under a higher magnification (b). Ap: Apatite layer

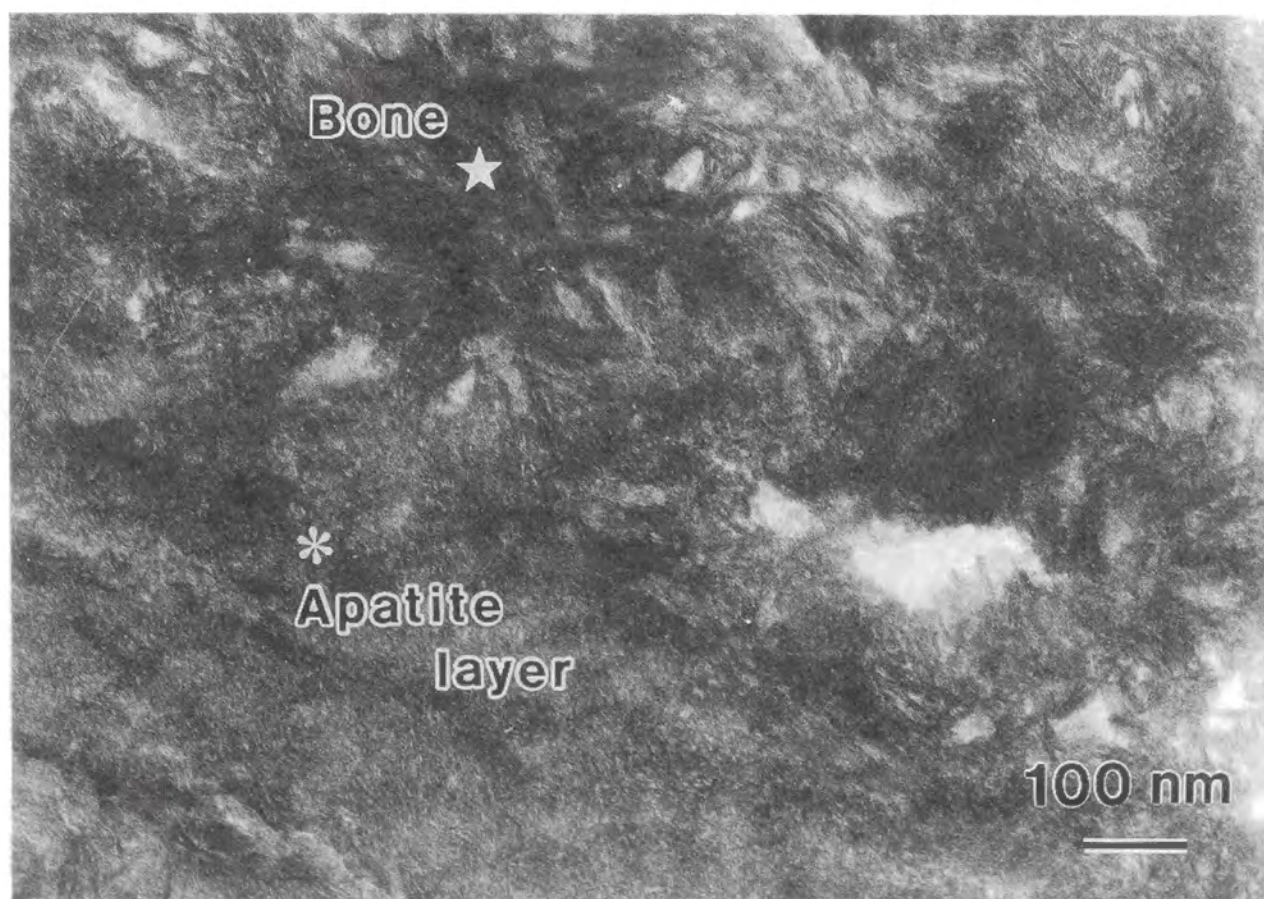


Figure 3. TEM photograph of the interface of the apatite layer to the bone 8 weeks after implantation.

* ☆: Spots for electron diffraction

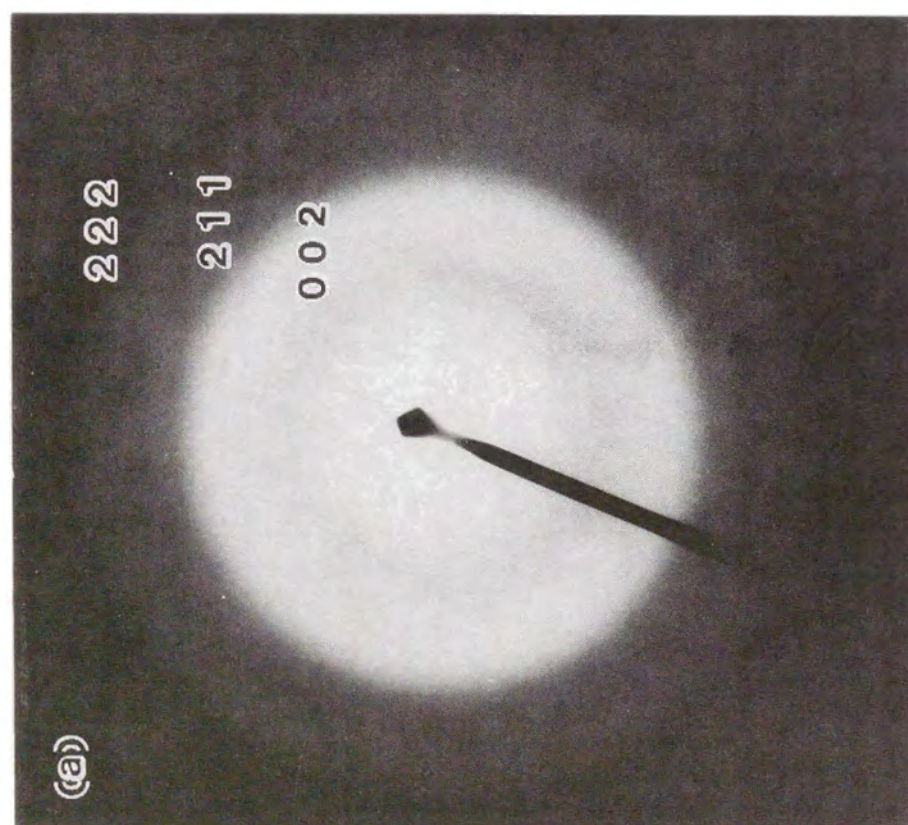
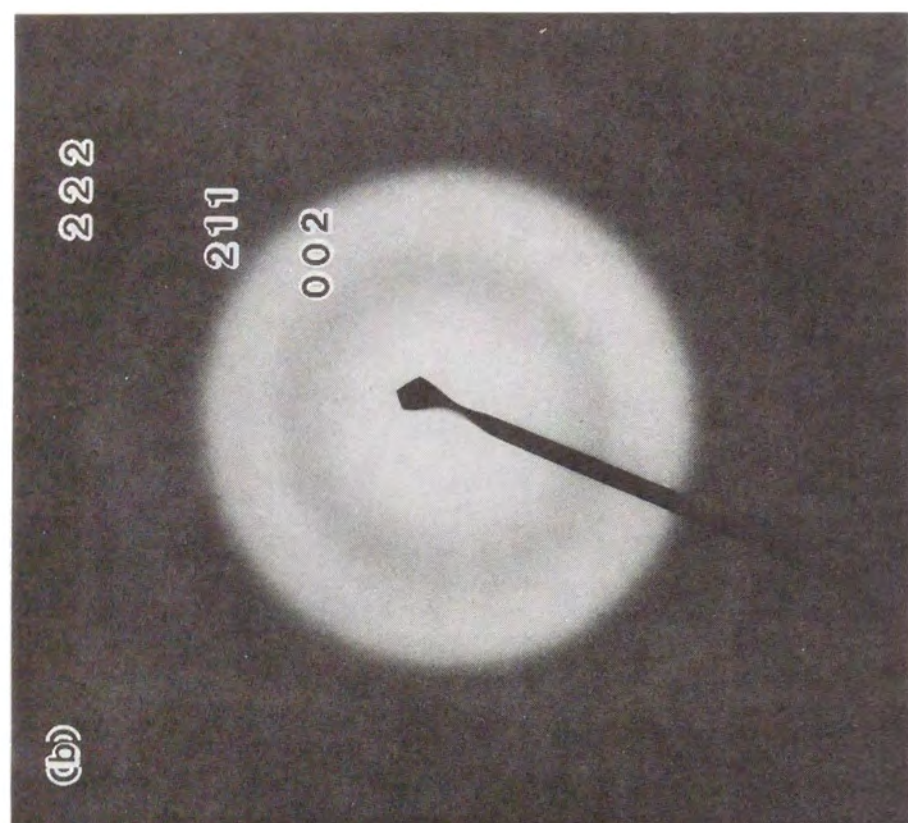


Figure 4. Electron diffraction patterns at the spots in the synthesized apatite layer (a) and in the bone (b).

of fine apatite crystals having similar structure to the apatite in the bone. The apatite layers synthesized by the present biomimetic process have been assumed to be analogous to the bone-like apatite layer formed *in vivo*. This was proved by the present TEM observation. It was also shown in the present studies that the apatite layer synthesized by the biomimetic process remains in the living body and bond to living bone directly. Therefore, the apatite-organic polymer composites obtained by the present biomimetic process are expected to be promising as bone-repairing materials, because they could exhibit mechanical properties analogous to those of natural bone as well as high bioactivity, if they can be fabricated into three dimensional structure analogous to that of the natural bone.

5. Conclusion

A dense and uniform apatite layer of about 20 μm thick was formed on a poly-ether sulfone (PESF) substrate treated with glow discharge in O_2 gas by a biomimetic process. The apatite-polymer composite obtained was implanted into a rabbit tibia and structure of interface of PESF-apatite-bone was observed under a scanning electron microscope and transmission electron microscope 8 weeks after implantation. The apatite layer formed by the biomimetic process was confirmed to consist of small crystals of the apatite with a structure similar to that of the apatite in the bone. The apatite layer remained on the substrate in the body, and bonded to the apatite in the bone directly. Therefore, this type of apatite-organic polymer composites are promising as the bone-repairing materials.

References

- [1] M. Tanahashi, T. Yao, T. Kokubo, M. Minoda, T. Miyamoto, T. Nakamura and T. Yamamuro "Apatite coating on organic polymers by a biomimetic process", *J. Am. Ceram. Soc.*, **77**, 2805–2808 (1994).
- [2] M. Tanahashi, T. Yao, T. Kokubo, M. Minoda, T. Miyamoto, T. Nakamura and T. Yamamuro "Apatite formation on organic polymers by biomimetic process using $\text{Na}_2\text{O-SiO}_2$ glasses as nucleating agent" *J. Ceram. Soc. Japan.*, **102**, 822–829 (1994).
- [3] M. Tanahashi, T. Yao, T. Kokubo, M. Minoda, T. Miyamoto, T. Nakamura and T. Yamamuro "Apatite coated on organic polymers by biomimetic process: Improvement in its adhesion to substrate by HCl treatment", *J. Mat. Sci: Mater. Medicine*. in press.
- [4] M. Tanahashi, T. Yao, T. Kokubo, M. Minoda, T. Miyamoto, T. Nakamura and T. Yamamuro "Apatite coated on organic polymers by biomimetic process: Improvement in its adhesion to substrate by NaOH treatment" *J. Appl. Biomater.*, **5**, 339–347 (1994).
- [5] M. Tanahashi, T. Yao, T. Kokubo, M. Minoda, T. Miyamoto, T. Nakamura and T. Yamamuro "Apatite coated on organic polymers by biomimetic process: Improvement in its adhesion to substrate by glow-discharge treatment" *J. Biomed. Mater. Res.*, in press.
- [6] S. V. Chranjeevijrao, J. Hemmerle, J. C. Voegel and R. M. Frank, *Inorg. Chem. Acta*, **67**, 183 (1982).
- [7] L. L. Hench and A. E. Clark, "Adhesion to Bone", in *Biocompatibility of Orthopedic Implants*, Vol. 2, ed. by D. F. Williams, CRC Press, Boca Raton, pp. 129–170, 1982.
- [8] L. L. Hench, "Stability of ceramics in the physiological environment" in *Fundamental aspects of biocompatibility*, Vol. 1, ed. by D. F. Williams, CRC Press, Boca Raton, pp. 67–85, 1981.
- [9] T. Kitsugi, T. Nakamura, T. Yamamuro, T. Kokubo, T. Shibuya and M. Takagi, "SEM-EPMA observation of three types of apatite-containing glass-ceramics implanted in bone: The variance of a Ca-P-rich layer", *J. Biomed. Mater. Res.*, **21**, 1255–1271 (1987).
- [10] T. Kokubo, C. Ohtsuki, S. Kotani, T. Kitsugi and T. Yamamuro, "Surface structure of bioactive glass-ceramic A-W implanted into sheep and human vertebra" in *Bioceramics*, Vol. 2, ed. by Günther Heimke, Heidelberg, Germany, pp. 113–120, 1989.
- [11] T. Kitsugi, T. Yamamuro, T. Nakamura and T. Kokubo, "Bone bonding behavior of $\text{MgO-CaO-SiO}_2\text{-P}_2\text{O}_5\text{-CaF}_2$ glass (mother glass of A-W-glass-ceramics)", *J. Biomed. Mater. Res.*, **23**, 631–648 (1989).
- [12] K. Ohura, T. Nakamura, T. Yamamuro, T. Kokubo, Y. Ebisawa, Y. Kotoura and M. Oka, "Bone-bonding ability of P_2O_5 -free CaO-SiO_2 glasses", *J. Biomed. Mater. Res.*, **25**, 357–365 (1991).
- [13] C. Ohtsuki, H. Kushitani, T. Kokubo, S. Kotani and T. Yamamuro, "Apatite formation on the surface of Ceravital-type glass-ceramic in the body", *J. Biomed. Mater. Res.*, **25**, 1363–1370 (1991).
- [14] M. Neo, S. Kotani, T. Nakamura, T. Yamamuro, C. Ohtsuki, T. Kokubo, and Y. Bando "A comparative study of ultrastructures of the interfaces between four kinds of surface-active ceramic and bone" *J. Biomed. Mater. Res.*, **26**, 1419–1432 (1992).
- [15] M. Neo, S. Kotani, Y. Fujita, T. Nakamura, T. Yamamuro, Y. Bando, C. Ohtsuki and T. Kokubo, "Difference in ceramic-bone interface between surface-active ceramics and resorbable ceramics: A study by scanning and transmission electron microscopy", *J. Biomed. Mater.*

Res., **26**, 255–267 (1992).

- [16] T. Kokubo, H. Kushitani, Y. Ebisawa, T. Kitsugi, S. Kotani, K. Ohura and T. Yamamuro, "Apatite formation on bioactive ceramics in body environment", in *Bioceramics*, Vol. 1, ed. by H. Oonishi, H. Aoki and K. Sawai, Ishiyaku Euro America, Tokyo, pp. 157–162, 1989.
- [17] T. Kokubo, S. Ito, Z. T. Huang, T. Hayashi, S. Sakka, T. Kitsugi and T. Yamamuro, "Ca, P-rich layer formed on high-strength bioactive glass-ceramic A-W", *J. Biomed. Mater. Res.*, **24**, 331–343 (1990).
- [18] C. Ohtsuki, T. Kokubo, K. Takatsuka and T. Yamamuro, "Compositional dependence of bioactivity of glasses in the system CaO–SiO₂–P₂O₅: Its in vitro evaluation" *J. Ceram. Soc., Japan*, **99**, 1–6 (1991).
- [19] C. Ohtsuki, Y. Aoki, T. Kokubo, Y. Bando, M. Neo, T. Yamamuro and T. Nakamura, "Characterization of apatite layer formed on bioactive glass-ceramic A-W", in *Bioceramics*, Vol. 5, ed. by T. Yamamuro, T. Kokubo and T. Nakamura, Kobunshi Kankokai, Inc., Kyoto, pp. 87–94, 1992.

CHAPTER 8.

GENERAL SUMMARY

The present thesis has described the study of the apatite formation on organic polymers by a biomimetic process. The contents of the respective chapters are summarized as follows:

In Chapter 2, it was shown that a dense and uniform bone-like apatite layer could be formed on all of the organic polymers examined such as poly(ethylene terephthalate) (PET), poly(methyl methacrylate) (PMMA), polyamide 6 (Nylon 6), poly-ethersulfone (PESF), polyethylene (PE), poly(tetrafluoroethylene) (PTFE) and poly(vinyl alcohol) hydrogel (PVA-H) in various forms including fabrics of fine fibers by the biomimetic process using a CaO–SiO₂ glass as a nucleating agent. The induction periods for the apatite nucleation were found to be almost 24 h for most of the examined organic polymers. The adhesive strengths of the apatite layer formed to the polymer substrates, however, largely varied with the kind of the polymers, i. e. 3 to 4 MPa for PET, PESF and PVA-H, whereas less than 1 MPa for PMMA, Nylon 6, PE and PTFE.

In Chapter 3, it was found by applying the same biomimetic process to the same polymers but previously treated with 1M-HCl aqueous solution that the HCl treatment reduced the induction periods for the apatite nucleation down to 12 h for PET, PMMA, Nylon 6 and PESF, and increased the adhesive strengths of the apatite layer to the substrates up to 6 MPa for PET, PMMA, Nylon 6. On the basis of X-ray photoelectron spectroscopy(XPS), the reduction in the induction period for the apatite nucleation and the increase in the adhesive strength of the apatite layer by

the HCl treatment were attributed to the formation of polar carboxyl groups on the surface of the polymers by the HCl treatment.

In Chapter 4, it was shown by applying the same biomimetic process to the same polymers but previously treated with 5M-NaOH aqueous solution that the NaOH treatment reduced the induction periods for the apatite nucleation down to 12 h for PET, PMMA, Nylon 6 and PESF, and increased the adhesive strengths of the apatite layer to the substrates up to 9 MPa at maximum for PET, PMMA and Nylon 6. On the basis of XPS, such larger effects of the NaOH treatment were attributed to the formation of the polar carboxyl groups in larger amounts on the surface of the polymers by the NaOH treatment.

In Chapter 5, it was found by applying the same biomimetic process to the polymers pre-treated with the glow-discharge (GD) in O_2 gas atmosphere that the GD treatment not only reduced the induction periods for the apatite nucleation down to 6 h for all the examined polymers, but also increased the adhesive strengths of the apatite layer to the substrates up to 10 MPa at maximum for all the examined polymers. On the basis of XPS, such remarkable effects of the GD treatment were attributed to the effective formation of polar groups such as carbonyl, ester, carboxyl and hydroxyl groups on the polymer surfaces by the GD treatment. These polar groups might combine with the silicate ion dissolved from the $CaO-SiO_2$ glass particles to accelerate the apatite nucleation on the polymer substrates, and also combine with the calcium or hydroxyl ion of the apatite to increase the adhesive strength of the apatite layer to the polymer substrates.

In Chapter 6, it was shown by applying the biomimetic process where the $CaO-SiO_2$ glass was replaced with Na_2O-SiO_2 glasses to PESF that the Na_2O-SiO_2 glasses reduced the induction periods for the apatite nucleation on the PESF substrate down to 30 min at minimum, and increased the

adhesive strengths of the apatite layer to the substrate up to 7 MPa at maximum. Both the reduction in the induction period for the apatite nucleation and the increase in the adhesive strength of the apatite layer were attributed to the formation of the polar sulfonic acid group on the surface of PESF due to the dissolution of Na^+ ion from the glasses.

In Chapter 7, it was shown by observing PESF coated with apatite layer by the present biomimetic process and implanted into rabbit tibia with scanning electron and transmission electron microscopes that the apatite layer consisted of small particles of the apatite with a similar structure to the apatite in the bone, and bonded directly to the apatite in the bone.

From the present studies, it was revealed that a dense and uniform layer of bone-like apatite could be formed on the various kinds of organic polymers even in a form of fabrics by the present biomimetic process, and that the adhesive strength of the apatite layer formed to the polymer substrates increased up to 10 MPa at maximum by a treatment of HCl, NaOH or glow-discharge of the substrates.

The apatite-organic polymer composites obtained by the present biomimetic process are promising as bone-repairing materials, because they exhibit mechanical properties analogous to those of natural bone as well as high bioactivity. In addition, they might be also useful even as soft tissue-repairing materials because they could exhibit ductility as well as high compatibility even with soft tissues.

LIST OF PUBLICATIONS

CHAPTER 2.

"Effect of substrate on apatite formation by a biomimetic process", M. Tanahashi, K. Hata, T. Kokubo, M. Minoda, T. Miyamoto, T. Nakamura and T. Yamamuro, in *Bioceramics* Vol. 5, ed. by T. Yamamuro, T. Kokubo and T. Nakamura, Kobunshi-Kankokai, Kyoto, 1992, pp. 57-64.

"Apatite coating on organic polymers by a biomimetic process", M. Tanahashi, T. Yao, T. Kokubo, M. Minoda, T. Miyamoto, T. Nakamura and T. Yamamuro, *J. Am. Ceram. Soc.*, **77**, 2805-2808 (1994).

CHAPTER 3.

"Apatite coated on organic polymers by biomimetic process: Improvement in its adhesion to substrate by HCl treatment", M. Tanahashi, T. Yao, T. Kokubo, M. Minoda, T. Miyamoto, T. Nakamura and T. Yamamuro, *J. Mater. Sci: Mater. Medicine*, in press.

CHAPTER 4.

"Coating of apatite layer on surfaces of organic polymers by a biomimetic process", M. Tanahashi, T. Yao and T. Kokubo, *J. Jpn Soc. Powder and Powder Metallurgy*, **40**, 1007-1010 (1993) (In Japanese).

"Apatite coated on organic polymers by biomimetic process: Improvement in its adhesion to substrate by NaOH treatment", M. Tanahashi, T. Yao, T. Kokubo, M. Minoda, T. Miyamoto, T. Nakamura and T. Yamamuro, *J. Appl. Biomater.*, **5**, 339-347 (1994).

CHAPTER 5.

"Apatite-polymer composites prepared by biomimetic process: Improvement

of adhesion of apatite to polymer by glow-discharge treatment" M. Tanahashi, T. Yao, T. Kokubo, M. Minoda, T. Miyamoto, T. Nakamura and T. Yamamuro, in *Bioceramics* Vol. 6, ed. by P. Ducheyne and D. Christiansen, Butterworth-Heinemann, Oxford, 1993, pp. 327-332.

"Apatite coated on organic polymers by biomimetic process: Improvement in its adhesion to substrate by glow-discharge treatment" M. Tanahashi, T. Yao, T. Kokubo, M. Minoda, T. Miyamoto, T. Nakamura and T. Yamamuro, *J. Biomed. Mater. Res.*, in press.

CHAPTER 6.

"Apatite formation on organic polymers by biomimetic process using Na₂O-SiO₂ glasses as nucleating agent" M. Tanahashi, T. Yao, T. Kokubo, M. Minoda, T. Miyamoto, T. Nakamura and T. Yamamuro, *J. Ceram. Soc. Japan.*, **102**, 822-829 (1994).

CHAPTER 7

"Ultrastructural study of apatite layer formed by biomimetic process and its bonding to bone" M. Tanahashi, T. Kokubo, T. Nakamura, Y. Katsura and M. Nagano, *Biomaterials*, submitted .

RELATED PUBLICATIONS

"Effects of the addition of F⁻ ions on the properties of fibrous hydroxyapatite grown in the gel system" K. Kamiya, M. Tanahashi, T. Suzuki and K. Tanaka, *Mat. Res. Bull.*, **25**, 63-70 (1990).

"Fibrous hydroxyapatite grown in the gel system: Effects of pH of the solution on the growth rate and morphology" M. Tanahashi, K. Kamiya, T. Suzuki and H. Nasu, *J. Mater. Sci: Mater. Medicine*, **3**, 48-53 (1992).

"Preparation of bioactive materials with different mechanical properties by biomimetic process", T. Kokubo, M. Tanahashi, K. Hata and T. Yao, in *New Functionality Materials: Design, Preparation and Control*, ed. by T. Tsuruta, M. Doyama, M. Seno and Y. Imanishi, Elsevier Sci. Publ. B. V. 1993, pp. 363-368.

"Apatite formation on surfaces of various substrates by a biomimetic process", M. Tanahashi and T. Kokubo, *Chemical Industry*, **44**, 703-709 (1993) (In Japanese).

"Formation of biologically active apatite layer on polymeric materials by a biomimetic method" T. Kokubo, M. Tanahashi and T. Yao, *Proceedings of 2nd Kyoto-Siena Symposium*, ed. by Y. Imanishi, Kyoto Univ., 1993, pp. 37-48.

"Surface modification of metals, ceramics and polymers for inducing bioactivity", T. Kokubo and M. Tanahashi, *Surface Modification Technologies VII*, ed. by T. S. Sudarshan, K. Ishizaki, M. Takata and K. Kamata, The Institute of Materials, London, 1994, pp. 115-130.

ACKNOWLEDGEMENTS

The present thesis has been carried out under the direction of Professor Tadashi Kokubo, Division of Material Chemistry, Kyoto University.

The author wishes to express her sincere gratitude to Professor Tadashi Kokubo for his continuous encouragement and valuable advice all through the duration of the present work. The author also thanks to Dr. Takeshi Yao, Division of Energy and Hydrocarbon Chemistry, Kyoto University for his informative discussion and helpful advice. Hearty thanks are made to all the students of Laboratory of Industrial Solid State Chemistry, Division of Material Chemistry, Kyoto University.

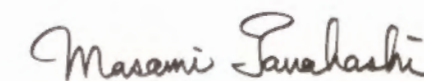
The suggestions from Professor Takeaki Miyamoto and Dr. Masahiko Minoda at Institute for Chemical Research, Kyoto University are also gratefully acknowledged.

The author wishes to thank to Professor Takashi Nakamura, Research Center for Biomedical Engineering, Kyoto University, Professor Takao Yamamuro, Dr. Yoshiaki Katsura and Dr. Masahisa Nagano, Department of Orthopaedic Surgery, Kyoto University for animal experiments.

The author wishes to express the gratitude to Dr. Takehisa Matsuda, Department of Bioengineering, National Cardiovascular Center Research Institute for his useful advice and encouragement.

Finally, the author expresses her sincere gratitude to her parents, Isamu Tanahashi, Mitsuko Tanahashi, and her sisters, Toyoko Fujii, Yoshiko Tanahashi and Yoko Tanahashi.

December 1994



Masami Tanahashi