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Apatite coated on organic polymers by biomimetic process: improvement in adhesion to substrate by HCl treatment

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A dense, uniform and highly biologically active 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 $CaO–SiO_2$–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 with ion concentrations 1.5 times those of SBF (1.5 SBF). In the present study, organic polymer substrates were treated with 1 M HCl solution, then subjected to the above mentioned biomimetic process. The induction periods for the apatite nucleation on polyethyleneterephthalate, polymethylmethacrylate, polyamide 6 and polyethersulfone substrates were reduced from 24 to 12 h with the HCl treatment. The adhesive strength of the formed apatite layer to the polyethyleneterephthalate, polymethylmethacrylate and polyamide 6 substrates were 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. It is supposed that highly polar carboxyl group formed by the HCl hydrolysis reaction of ester group in polyethyleneterephthalate and polymethylmethacrylate or amide group in polyamide 6 increased the affinity of the substrates with a silicate ion to decrease the induction period, and also increased the affinity of the substrate with the apatite to increase the adhesive strength. The apatite–organic polymer composites thus obtained are expected to be useful as bone-repairing materials as well as soft-tissue-repairing materials.

1. Introduction

It is 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]. The recent studies of the present authors revealed that the mechanism for the formation of a 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 surface of the glasses and glass-ceramics provides favourable sites for the apatite nucleation. As a result a large number of 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, the present authors have developed the following biomimetic method for forming a bone-like apatite layer of the desired thickness on any kind of organic polymer substrates at normal temperature and pressure. First, a substrate is set in contact with the particles of $CaO–SiO_2$–based glass soaked in a simulated body fluid (SBF) with ion concentrations nearly equal to those of human blood plasma so that calcium ions dissolved from the glass increase the ionic activity product of apatite in SBF, and silicate ions dissolved from the glass adhere to the surface of the substrate resulting in apatite nucleation. Second, the substrate is soaked in another solution with ion concentrations 1.5 times those of SBF

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(1.5 SBF) so that the apatite nuclei formed in the first soaking grow by taking the phosphate and calcium ions from the solution [6]. When rectangular substrates 10 mm × 15 mm × 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 days, a dense and uniform layer of bone-like apatite was formed on the surfaces of the substrates. When the first treatment was omitted, however, the apatite was never formed. Therefore, the period of time necessary in the first treatment for forming apatite nuclei in sufficient numbers to obtain a continuous layer after the second treatment is important and is termed hereafter the induction period for apatite nucleation. The induction periods were previously shown to be 24 h [7] for as-prepared polyethyleneterephthalate (PET), polymethylmethacrylate (PMMA), polyethersulfone (PESF) and polyamide 6 (Nylon 6) substrates, and 12 h for those treated with NaOH solution [8].

The apatite–organic polymer composites obtained by this biomimetic process are expected to be useful not only as bone-repairing materials because of their mechanical properties analogous to those of natural bone as well as high bioactivity, but also as soft tissue-repairing materials because of their ductility as well as high biocompatibility. In biomedical applications of this type of composite, it is also important that the apatite layer adheres strongly to the substrates. The adhesive strengths of the apatite layer were previously shown to vary from almost 0 to 4 MPa [7], depending on the kind of polymers used, and to increase by 3 to 8 times for PET, PMMA and Nylon 6 with NaOH treatment [8]. In the present study, various kinds of polymer substrates were first subjected to 1 M HCl solution, and then to the biomimetic process. The effects of the HCl treatment on the induction periods for apatite nucleation and adhesive strength of the apatite layer to the substrates were investigated.

2. Experimental procedures

2.1. HCl treatment

Rectangular specimens 10 mm × 15 mm × 1 mm of organic polymers listed in Table I were abraded with #400 diamond paste and soaked in 10 ml of 1 M HCl at 20 °C for various periods from 0 to 10 min. After the HCl treatment, the substrates were washed with distilled water, and dried at room temperature.

2.2. Surface characterization

Organic polymer substrates treated with the HCl solution 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 not-treated 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-rays were 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 in the range 5–10 nm. Measured binding energies were corrected by reference to the binding energy of the C1s in CH_2 group (284.6 eV). Overlapping peaks in the XPS spectra were separated by the pattern fitting method using a Gaussian profile for the individual peaks.

TABLE I Examined organic polymers

Polyethyleneterephthalate (PET)
Polymethylmethacrylate (PMMA)
Polyethersulfone (PESF)
Polyamide 6(Nylon 6)
Polyethylene (PE)
Polytetrafluoroethylene (PTFE)

2.3. Apatite coating

Bioactive $CaO–SiO_2$–based glass, termed glass G hereafter, of the nominal composition MgO 4.6, CaO 44.7, SiO_2 34.0, P_2O_5 16.2 and CaF_2 0.5 wt % was prepared by the ordinary melting technique. It was crushed by a laboratory planetary type zirconia ball mill and sieved to obtain grains of 150 to 300 μm in size.

Simulated body fluid (SBF) with inorganic ion concentrations nearly equal to those of human blood plasma, and 1.5 SBF with ion concentrations 1.5 times those of SBF, as shown in Table II, were prepared by dissolving the reagents NaCl, $NaHCO_3$, KCl, $K_2HPO_4 \cdot 3H_2O$, $MgCl_2 \cdot 6H_2O$, $CaCl_2$ and Na_2SO_4 in distilled water and buffered at pH 7.25 at 36.5 °C with tris(hydroxymethyl) aminomethane ($(CH_2OH)_3CNH_2$) and 1 M hydrochloric acid (HCl).

For the first treatment, the HCl-treated substrates were set in contact with particles of glass G soaked in 30 ml of SBF at 36.5 °C for various periods less than 24 h. Then, for the second treatment, the substrates were soaked in 30 ml of 1.5 SBF at 36.5 °C for 6 days. A schematic diagram for these treatments is shown in Fig. 1. During the second treatment, 1.5 SBF was renewed every 2 days. After these treatments, the substrates were washed with distilled water and dried at room temperature.

2.4. Analysis of apatite

The surfaces of the substrates were analysed after the second treatment by thin-film X-ray diffraction using RINT-1400 (Rigaku Co., Tokyo, Japan) and Fourier transform infrared (FT-IR) spectroscopy using 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-2500 CX (Hitachi Co. Ltd, Tokyo, Japan).

2.5. Measurement of adhesive strength

The organic polymer substrates on which the apatite layer about 10 μm thick was formed by the first treatment for 24 h and the second treatment for 6 days

TABLE II 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

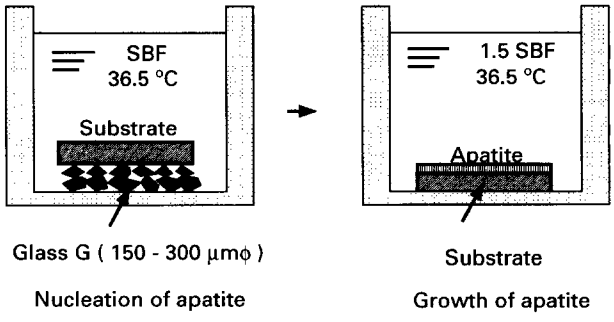
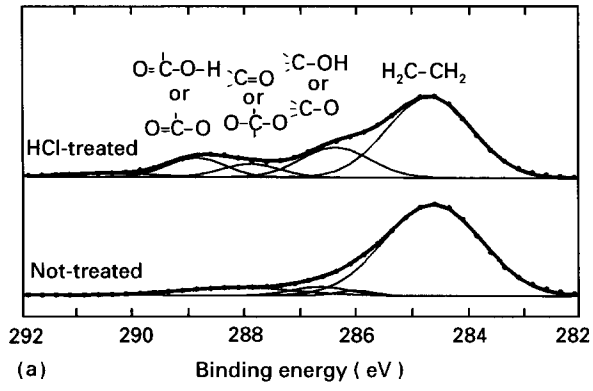


Figure 1 A biomimetic method for forming the bone-like apatite layer on various substrates.



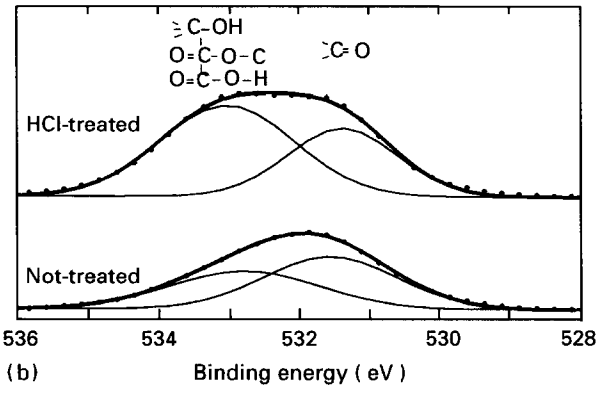


Figure 2 C1s (a) and O1s (b) XPS spectra of polyethyleneterephthalate substrate treated with 1M HCl and non-treated.

were used as the test samples. A brass jig of 8 mm diameter base was stuck to the top surface of the apatite layer and another jig of the same size was stuck to the opposite side of the substrate with cyanoacrylate adhesive. They were left untouched for 1 day for complete solidification of the adhesive. The adhesive strength of the apatite layer to the substrate was measured by applying a tensile stress between the two jigs using an Instron-type testing machine at a cross-head speed of 1 mm/min until fracture occurred. At least five measurements were taken for each data point.

3. Results

3.1. XPS analysis

The C1s and O1s XPS spectra of PET and PMMA treated with HCl for 3 min and those non-treated are shown in Figs 2 and 3, 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 the $-(CH_2)_n-$ group for substrates. The C1s peaks at about 286.8, 287.8 and 288.8 eV are attributed to the carbon in the $\rightarrow$C–O or $\rightarrow$C–OH, $>C=O$ or O–C–O groups and O–C=O or H–O–C=O groups, respectively [9, 10]. These three peaks were 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 the $>C=O$ group, and about 533eV attributed to the oxygen in the H–O–C=O group. The latter were increased by the HCl treatment. These results indicate that the oxidized carbon groups were 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. 4, and it seems that the oxidized carbon groups were also increased by the HCl treatment. Fig. 5 shows the C1s and O1s XPS spectra of HCl-treated and non-treated PESF. In this case, no changes were observed in C1s spectra as a result of the HCl treatment, however, the intensity of O1s peaks at about 531 eV increased. It seems that the carbon skeleton was not changed but the S–O bond changed and the SO_2–OH sulfonic acid group was formed in the surface region. For PE and PTFE no changes in XPS spectra were observed as a result of the HCl treatment. Table III 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 non-treated substrates. It is apparent from Table III that the ratios of $\rightarrow$C–O or $\rightarrow$C–OH, $>C=O$ or O–C–O and O=C–O or O=C–O–H groups in PET, PMMA and Nylon 6 increased as a result of the HCl treatment. This means that the ester group in PET and PMMA and the amide group in Nylon 6 were hydrolysed to the carboxyl group by the HCl treatment.

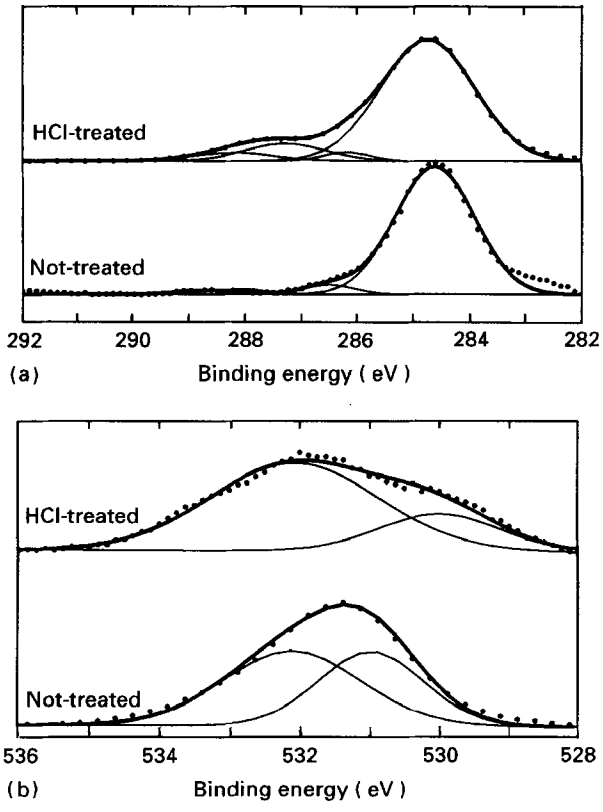


Figure 3 C1s (a) and O1s (b) XPS spectra of polymethylmethacrylate substrate treated with 1M HCl and non-treated.

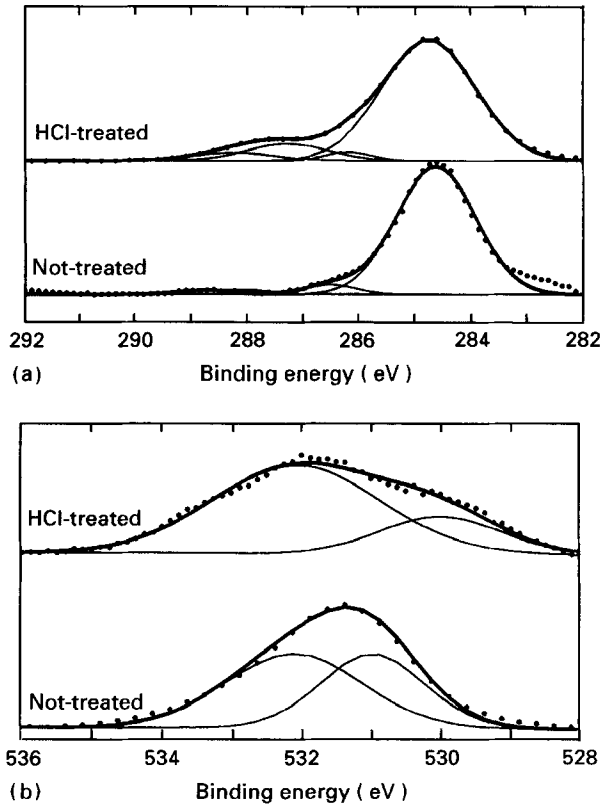


Figure 5 C1s (a) and O1s (b) XPS spectra of polyethersulfone substrate treated with 1M HCl and non-treated.

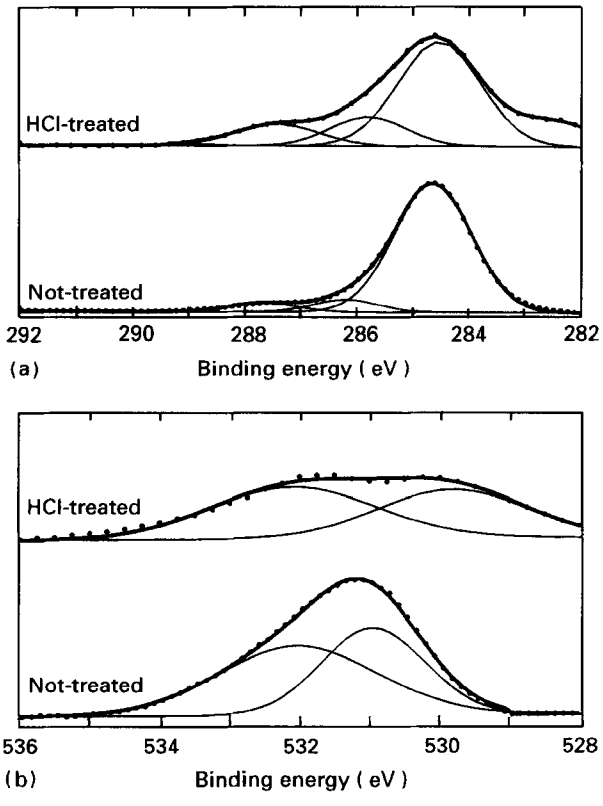


Figure 4 C1s (a) and O1s (b) XPS spectra of polyamide 6 substrate treated with 1M HCl and non-treated.

3.2. Apatite formation

Figs 6 and 7 show thin-film X-ray diffraction patterns and FT-IR reflection spectra, respectively, of the surfaces of the PESF substrates treated with 1M HCl solution for 3 min, then subjected to the first treatment for various periods and to the second treatment for 6 days. It can be seen from Figs 6 and 7 that 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.

Fig. 8 shows scanning electron microscope photographs of the surfaces of PESF substrates subjected to the same treatments described above. It can be seen from Fig. 8 that 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 a first treatment shorter than 6 h. With a first treatment longer than 12 h, a dense and uniform apatite layer was formed on the HCl-treated PESF substrates. Thus the induction period for apatite nucleation, that is the period required for the first treatment to form apatite nuclei in sufficient numbers to give a continuous layer after the second treatment, was determined to be 12 h. The induction period thus measured for various organic polymers in Table I are listed in Table IV. 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.

3.3. Adhesive strength

Fig. 9 shows the adhesive strength of the apatite layer to the organic polymer substrates as a function of HCl treatment time. For PET, PMMA and Nylon 6, the adhesive strengths were increased from 3.5 to

TABLE III Area ratio of functional groups formed on the surfaces of various organic polymers treated and non-treated with 1M HCl solution for 3 min

Substrate	Non-treated							HCl-treated						
C1s	H_2C-CH_2	$\rightarrow C-O$ or $\rightarrow C-OH$	$>C=O$ or $O-\overset{\vert}{C}-O$	$O=\overset{\vert}{C}-O$ or $O=\overset{\vert}{C}-O-H$	O ‖ O–C–O	F_2C-CF_2 or F_2C-CHF	F_2C-CH_2	H_2C-CH_2	$\rightarrow C-O$ or $\rightarrow C-OH$	$>C=O$ or $O-\overset{\vert}{C}-O$	$O=\overset{\vert}{C}-O$ or $O=\overset{\vert}{C}-O-HO-\overset{\vert}{C}-O$	O ‖	F_2C-CF_2 or F_2C-CHF	F_2C-CH_2
Polyethyleneterephthalate	84	2	5	9	–	–	–	80	6	7	7	–	–	–
Polymethylmethacrylate	72	3	10	15	–	–	–	78	5	11	6	–	–	–
Polyethersulfone	88	12	–	–	–	–	–	89	7	4	–	–	–	–
Polyamide 6	88	7	5	–	–	–	–	88	5	7	–	–	–	–
Polyethylene	100	–	–	–	–	–	–	100	–	–	–	–	–	–
Polytetrafluoroethylene	25	–	–	–	–	69	6	11	–	–	–	–	82	7

O1s	$>C=O$	$\rightarrow C-OH$, $O=\overset{\vert}{C}-O-C$ or $O=\overset{\vert}{C}-O-H$	$-SO-OH$ or $>SO_2-OH$	S–OH	$>C=O$	$\rightarrow C-OH$, $O=\overset{\vert}{C}-O-C$ or $O=\overset{\vert}{C}-O-H$	$\rightarrow SO-OH$ or $-SO_2-OH$	S–OH
Polyethyleneterephthalate	61	39	–	–	47	53	–	–
Polymethylmethacrylate	75	25	–	–	23	77	–	–
Polyethersulfone	–	–	52	48	–	–	67	33
Polyamide 6	60	40	–	–	40	60	–	–
Polyethylene	–	–	–	–	–	–	–	–
Polytetrafluoroethylene	–	–	–	–	–	–	–	–

— Non-detected

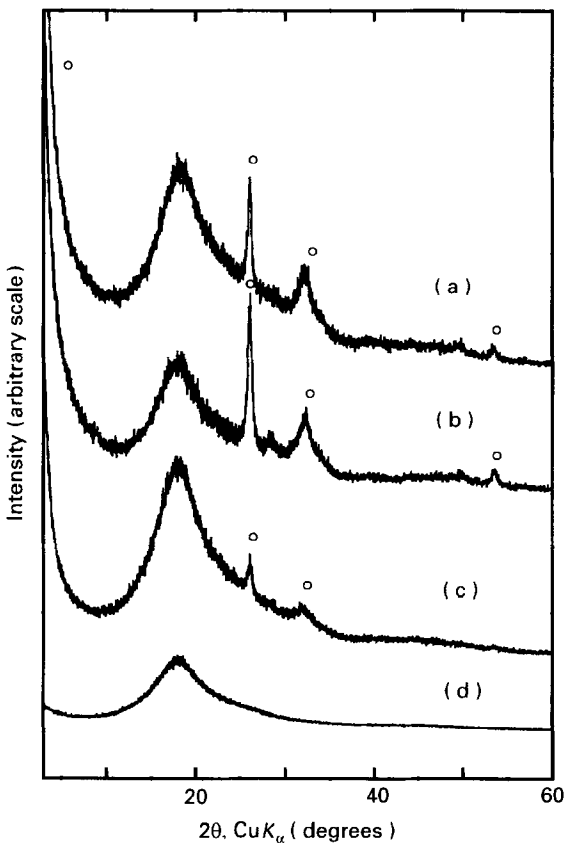


Figure 6 Thin-film XRD patterns of surfaces of HCl-treated polyethersulfone soaked in 1.5SBF for 6 days after soaking in SBF with glass G for various periods. First treatment duration: (a) 24 h; (b) 12 h; (c) 6 h, (d) 0 h. O denotes Apatite.

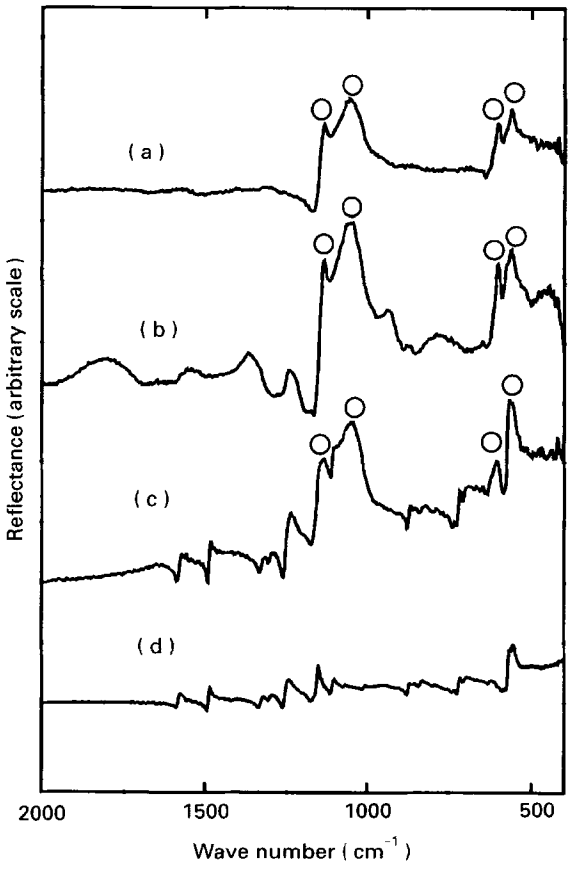
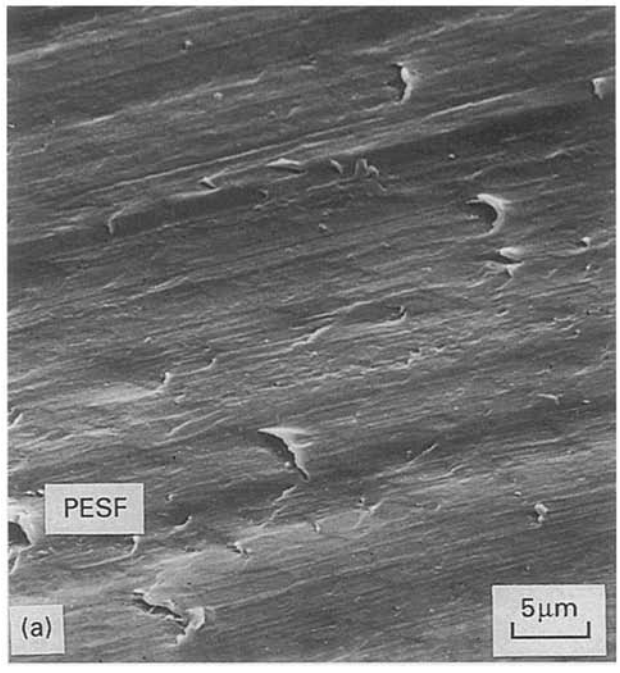


Figure 7 Fourier transform IR reflection spectra of surfaces of HCl-treated polyethersulfone soaked in 1.5 SBF for 6 days after soaking in SBF with glass G for various periods. First treatment duration: (a) 24 h; (b) 12 h; (c) 6 h; (d) 0 h. O denotes Apatite.

7.0 MPa, from 1.1 to 2.8 MPa and from 0.6 to 3.1 MPa, respectively, by HCl treatment for 3 min. They all decreased for HCl treatments 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, at less than 1.1×10^{-2} MPa, was not changed.

4. Discussion

In the first soaking, glass G dissolves calcium and silicate ions in SBF [11]. It is thought that the calcium ions increase the ionic activity product of the apatite



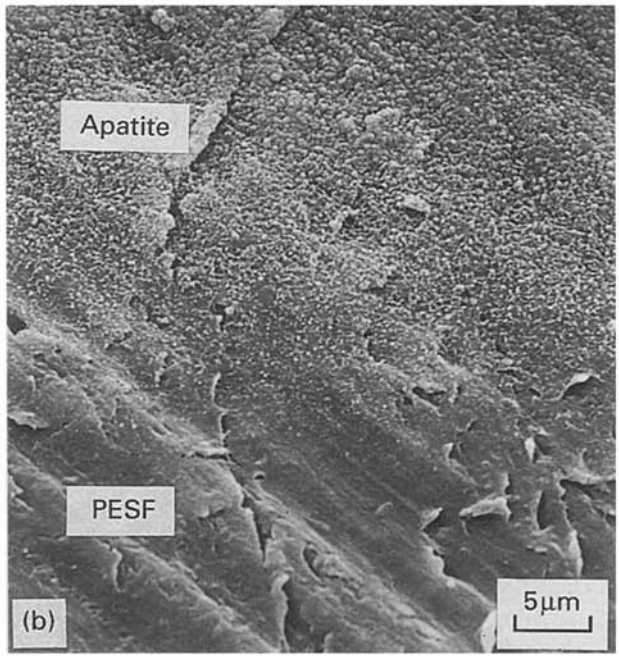


Figure 8 SEM photographs of the surfaces of HCl-treated polyethersulfone soaked in 1.5 SBF for 6 days after soaking in SBF with glass G for various periods: (a) 0 h; (b) 6 h; (c) 12 h.

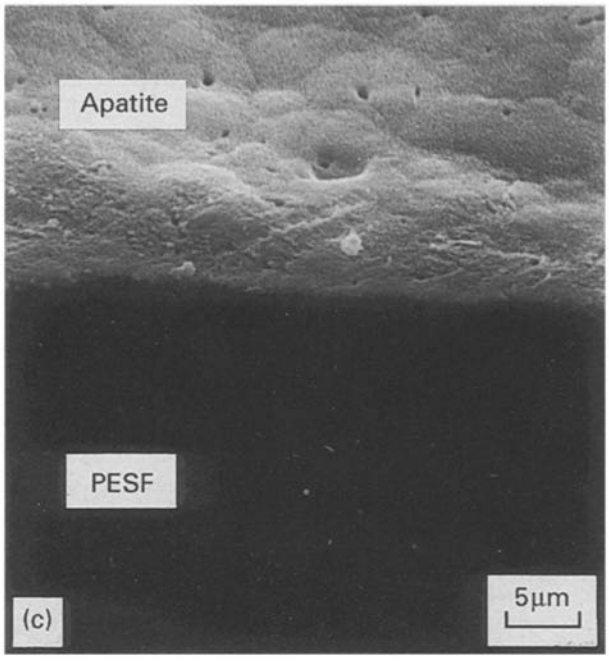


Figure 8 (c)

TABLE IV Induction periods for apatite nucleation on various polymers treated with 1 M HCl solution, in comparison with those not-treated

Substrate	Induction period (h)	
	Non-treated	HCl-treated
Polyethyleneterephthalate (PET)	24	12
Polymethylmethacrylate (PMMA)	24	12
Polyethersulfone (PESF)	24	12
Polyamide 6(Nylon 6)	24	12
Polyethylene (PE)	24	24
Polytetrafluoroethylene (PTFE)	24	24

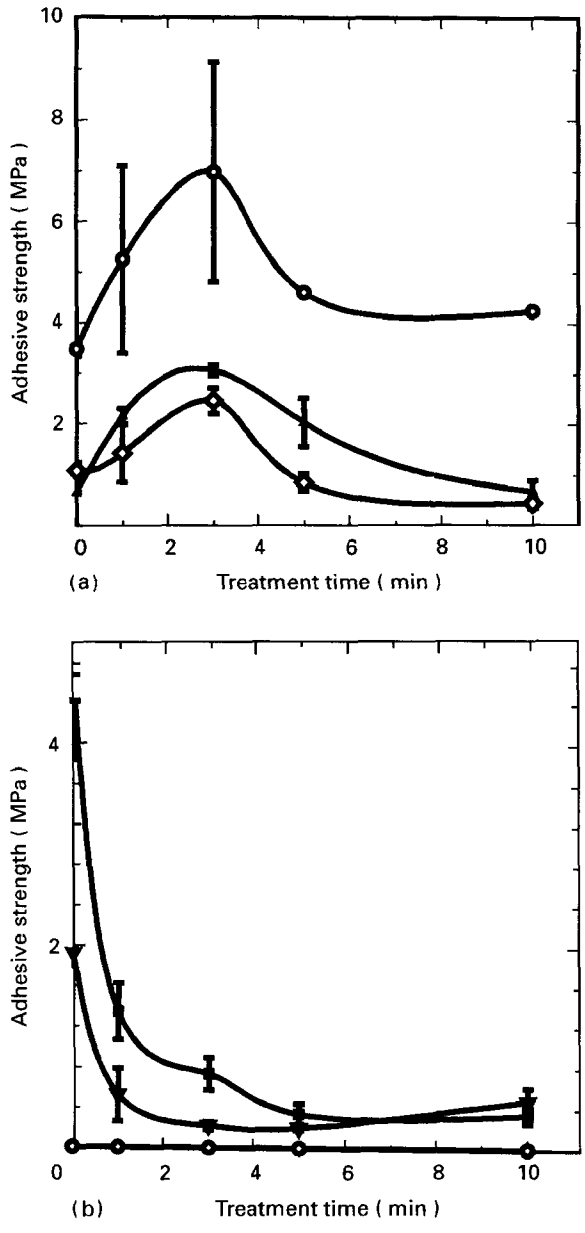


Figure 9 Adhesive strengths of bone-like apatite layer to various substrates as a function of treatment time of 1M HCl. (a) ○ PET; ▲ Nylon 6; ◇ PMMA. (b) ○ PTFE; ■ PESF; ▼ PE.

in the SBF, and the silicate ions diffuse and attach to the surface of the organic polymer substrates, where apatite nuclei are formed by a similar reaction occurring on the surfaces of the bioactive $CaO–SiO_2$–based glass [3–5]. Therefore, it is considered that the induction period for the apatite nucleation is markedly affected by the affinity of the silicate ions to the substrate. The induction periods for apatite nucleation on PET, PMMA, PESF and Nylon 6 were reduced from 24 to 12 h by HCl treatment. The ester group in PET and PMMA and the amide group in Nylon 6 were confirmed to be hydrolysed to the carboxyl group by the HCl treatment, and the sulfonyl group in PESF was hydrolysed to the sulfonic acid group (see Table III). It is considered that these additional polar groups provide favourable sites for the silicate ions to bond to the substrates, and hence accelerate apatite nucleation. In contrast, the induction periods on PE and PTFE were not reduced. Both PE and PTFE have no ester, amide or other groups to be hydrolysed to polar groups by the HCl treatment, and hence it is considered that theHCl treatment did not have the effect on the substrate of increasing the affinity with silicate ions. A similar relation between decrease in the induction period for apatite nucleation and increase in the number of polar groups as also observed for PET, PMMA, PESF and Nylon 6 treated with the NaOH solution. However, the magnitudes of the increase in the number of polar groups using HCl treatment are smaller than those when using NaOH treatment. It should be noted that both NaOH and HCl treatments were effective for PET, PMMA, PESF and Nylon 6 but not for PE and PTFE, in increasing the number of polar groups and hence in decreasing the induction period.

The adhesive strength of the apatite layer to PET, PMMA and Nylon 6 substrates was increased by the HCl treatment. The increase is well 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 polar group formation. It is also consistent with the fact that no increase of adhesive strength was observed for PE and PTFE substrates, for which the induction periods were not reduced by HCl treatment. A similar relation between increase in the adhesive strength and increase in the number of polar groups was also observed for PET, PMMA and Nylon 6 treated with NaOH solution. However, the magnitudes of the increase in adhesive strength using the HCl treatment is smaller than that given by the NaOH treatment. For all these substrates, the adhesive strengths decreased with increasing time of HCl treatment for periods longer than 3 min. This is attributed to the cut-off of the polymer chain by the longer HCl treatment and the release of intertwinements among them during measurement of the adhesive strength. This is very 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 those substrates 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 release the polymer chains. Consequently, decrease in the adhesive strength with increasing time of HCl treatment was observed.

5. Conclusions

By use of HCl treatment, ester groups in PET and PMMA, amide groups in Nylon 6, and sulfonyl groups in PESF were hydrolysed to polar groups, which accelerated 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 were increased by the HCl treatment due to increases in both the number of apatite nuclei and their bonding strength to the substrates. On the other hand, PE and PTFE, which have no functional groups to be hydrolysed to polar groups, showed neither reduction of the induction period for apatite nucleation nor an increase of adhesive strength as a result of 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.

Acknowledgement

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