J Mater Sci: Mater Med (2007) 18:1037–1042
DOI 10.1007/s10856-006-0081-2

In vitro apatite formation on polyamide containing carboxyl groups modified with silanol groups

Takahiro Kawai · Chikara Ohtsuki ·
Masanobu Kamitakahara · Kayo Hosoya ·
Masao Tanihara · Toshiki Miyazaki ·
Yoshimitsu Sakaguchi · Shigeji Konagaya

Received: 12 April 2005 / Accepted: 3 March 2006 / Published online: 23 January 2007

Abstract Modification of organic polymer with silanol groups in combination with calcium salts enables the polymer to show bioactivity, that is, the polymer forms apatite on its surface after exposure to body environment. However, how modification with silanol groups influences ability of apatite formation on the polymer substrate and adhesive strength between polymer and apatite is not yet known. In the present study, polyamide containing carboxyl groups was modified with different amounts of silanol groups, and its apatite-forming ability in 1.5SBF, which contained ion concentrations 1.5 times those of simulated body fluid (SBF), was examined. The rate of apatite formation increased with increasing content of silanol groups in the polyamide films. This may be attributed to enhancement of dipole interactions. A tendency for the adhesive strength of the apatite layer on the polyamide film to be decreased with increasing content of silanol groups was observed. This may be attributed to swelling in 1.5SBF and having a high degree of shrinkage after drying. These findings clearly show that modification of organic polymers with the functional groups induces apatite deposition, and also determines the adhesive strength of the apatite layer to the organic substrates.

Introduction

Apatite-polymer hybrids have attractive features as candidates for novel bone substitutes because they may show bone-bonding ability and mechanical performances derived from the organic substrate. As a method for fabrication of such a hybrid, Kokubo et al. proposed a biomimetic process that induces apatite formation on the surfaces of organic substrates at ambient conditions in a simulated body fluid (SBF) with ion concentrations similar to those of human extracellular fluid, or related solutions supersaturated with respect to the apatite [1, 2]. In this process, apatite nucleation was induced by a local increase in concentrations of calcium and silicate ions around the surface of the substrates due to dissolution of bioactive glass placed in the vicinity of the substrate under SBF conditions. Locally increased concentration of silicate ions produced heterogeneous nucleation sites for apatite because heterogeneous nucleation of apatite can be triggered by specific functional groups, including –SiOH groups [3, 4]. Release of calcium ions (Ca^{2+}) from the materials enhances apatite nucleation due to an increase in the degree of supersaturation of the fluid with respect to apatite [5]. It was reported that

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T. Kawai · C. Ohtsuki · M. Kamitakahara ·
K. Hosoya · M. Tanihara
Graduate School of Materials Science, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara 630-0192, Japan

Present Address:
M. Kamitakahara (✉)
Graduate School of Environmental Studies, Tohoku University, 6-6-20 Aoba, Aramaki, Aoba-ku,
Sendai 980-8579, Japan
e-mail: kamitaka@mail.kankyo.tohoku.ac.jp

T. Miyazaki
Graduate School of Life Science and Systems Engineering, Kyushu Institute of Technology, 2-4 Hibikino, Wakamatsu, Kitakyushu, Fukuoka 808-0196, Japan

Y. Sakaguchi · S. Konagaya
Toyobo Research Center Co., Ltd., 2-1-1 Katata, Otsu,
Shiga 520-0292, Japan

carboxyl (–COOH) groups are effective in apatite nucleation under biomimetic conditions [6]. Miyazaki et al. reported that polyamide films containing carboxyl (–COOH) groups can deposit apatite on their surfaces after exposure to 1.5SBF with ion concentrations 1.5 times those of SBF, when they contain calcium chloride [7]. On the other hand, it has been revealed that silanol groups are highly effective for induction of apatite formation, according to reported studies of silica gels [3, 4]. Recently, modification of organic polymer with silanol groups in combination with calcium salts was attempted to achieve bioactivity and flexibility. Actually, organically modified silicates and related hybrids can acquire deposits of bone-like apatite after soaking in SBF [8–12]. Therefore, modification of aromatic polyamide with silanol groups is expected to enhance apatite formation in a biomimetic solution. However, how modification with silanol groups influences ability of apatite formation on the polymer substrate and adhesive strength between polymer and apatite is not yet known. In the present study, polyamide containing carboxyl groups was modified with silanol groups and calcium chloride. Its apatite-forming ability was investigated in 1.5SBF and compared with polyamide containing unmodified carboxyl groups.

Experimental

Preparation of polyamide films containing silanol groups

One gram of aromatic polyamide containing –COOH in the polymer molecule, as shown in Fig. 1 [13], was dissolved in 10 ml of *N*, *N*-dimethylacetamide (Wako Pure Chemical Industries, Japan). 3-aminopropyltriethoxysilane (APES: Chisso Company, Japan), 1-ethyl-3-(3-dimethyl-aminopropyl)-carbodiimide (EDC·HCl: Peptide Institute, Japan), 1-hydroxysuccinimide (HOSu: Peptide Institute, Japan) and *N*, *N*-diisopropylethylamine (DIPEA: Applied Biosystems Japan) were added into the solution under the feed conditions listed in Table 1 to obtain polyamide Si(*x*). '*x*' indicates the ratio of silicon atoms included in fed APES to one amide unit (x = 0, 25 and 50). The structural formula is given in Fig. 1. A 40 percent mass of $CaCl_2$ (Nacalai Tesque, Japan) against the (polyamide + $CaCl_2$) was then added to the solution. The solution was stirred for 24 h, and then coated on flat glass plates with a bar coater. The solution coated on the glass plate was kept for drying in a vacuum oven at 60°C under 133 Pa for 8 h. The obtained polymer films were removed from the glass plates and cut into 10 × 10 mm specimens.

Soaking in 1.5SBF

The obtained films were then soaked in 30 ml of 1.5SBF (Na^+ 213.0, K^+ 7.5, Mg^{2+} 2.3, Ca^{2+} 3.8, Cl^- 221.7, HCO_3^- 6.3, HPO_4^{2-} 1.5, and SO_4^{2-} 0.8 mol · m^{-3},

Table 1 Reagents used to synthesize polyamide containing –SiOH groups

Specimen	Reagent			
	APES/ml	HOSu/g	EDC·HCl/g	DIPEA/ml
Si(0)	0	0	0	0
Si(25)	0.24	0.26	0.4	0.4
Si(50)	0.46	0.26	0.4	0.4

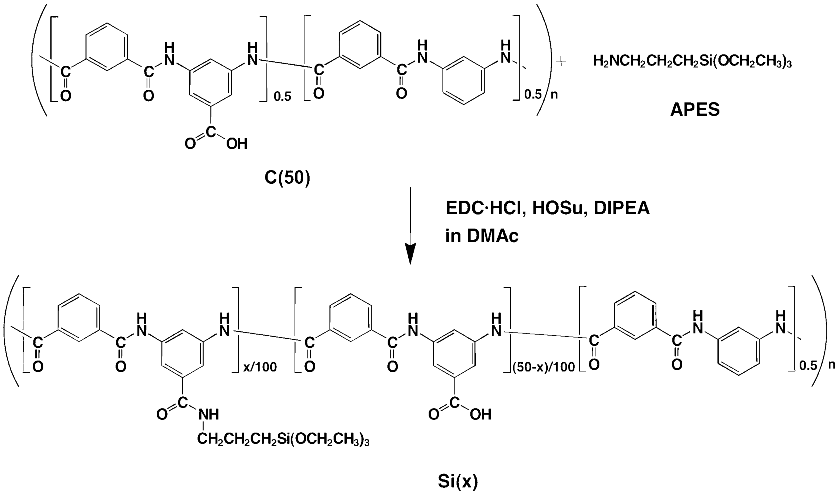

Fig. 1 Modification of C(50) with APES to synthesize Si(*x*) (x = 0, 25 and 50)

pH 7.40) at 36.5°C for various periods. The solution was prepared by dissolving reagent grade chemicals of NaCl, $NaHCO_3$, KCl, $K_2HPO_4 \cdot 3H_2O$, $MgCl_2 \cdot 6H_2O$, $CaCl_2$, and Na_2SO_4 (Nacalai Tesque, Japan) in ultrapure water [2, 14]. The pH of the solution was buffered at 7.40 using 75 mol · m^{-3} of tris (hydroxymethyl) aminomethane (Nacalai Tesque, Japan) along with an appropriate volume of 1 kmol · m^{-3} hydrochloric acid solution. The temperature of the solution was kept at 36.5°C. After soaking for given periods, the films were taken from the solution, washed with ultrapure water, and then dried at room temperature.

Characterization

The surfaces of the films were characterized by scanning electron microscopy (SEM: S-3500N, Hitachi, Japan), energy dispersive X-ray (EDX: EMAX ENERGY EX-400, Horiba, Japan) spectroscopic analysis and thin-film X-ray diffraction (TF-XRD: MXP3V, MAC Science Company, Japan), before and after soaking in 1.5SBF. In the SEM observations, a thin Au film was sputtered onto the surface of the specimen. In the TF-XRD measurements, the angle of the incident beam was fixed at 1° against the surface of the specimen. Morphological observation at higher magnification was conducted for typical specimens under field emission scanning electron microscope (FE-SEM: S-4800N Hitachi, Japan). The interface between Si(0) film and the apatite formed on the film after soaking in 1.5SBF was observed using a transmission electron microscope (TEM; JEM2010, JEOL, Japan).

Results

Figure 2 shows TF-XRD patterns of the surfaces of Si(*x*) (*x* = 0, 25 and 50) before and after soaking in 1.5SBF for various periods. Diffraction peaks assigned to apatite were detected in each specimen after soaking for 2 days. The tendency of the apatite formation on the polyamide film after exposure to 1.5SBF is summarized in Table 2, on the basis of the results of TF-XRD. Even when apatite was not detected by TF-XRD, the films containing silanol groups looked opaque after soaking in 1.5SBF even for 1 day. This suggests that apatite had been formed on the films containing silanol groups even after 1 day of soaking in 1.5SBF. Figure 3 shows SEM photographs of the surfaces of Si(*x*) (*x* = 0, 25 and 50) before and after soaking in 1.5SBF for various periods. Spherical particles were found to deposit on the surface for Si(25) and Si(50) within 12 h, whereas this took 2 days for Si(0). The tendency of the adhesive strength of the apatite layer on the polyamide film to decrease with increased content of Si was suggested because the layer was easily peeled off under SEM. Figure 4 shows EDX spectra from the surfaces of Si(*x*) (*x* = 0, 25 and 50) before and after soaking in 1.5SBF for various periods. Peaks assigned to calcium and phosphorus were detected in the spectra of Si(25) and Si(50) within 12 h, while this took 2 days for Si(0). These data indicate that calcium phosphate (implying apatite) was formed on the surfaces of Si(25) and Si(50) within 12 h, and 2 days for Si(0). No peak assigned to apatite was detected in TF-XRD patterns of Si(25) and Si(50) after soaking 12 h despite of the existence of calcium and

Table 2 Tendency of apatite formation evaluated by TF-XRD

Specimen	Soaking period		
	12 h	1 d	2 d
Si(0)	–	–	+
Si(25)	–	–	+
Si(50)	–	–	+

Apatite was formed (+), not formed (–)

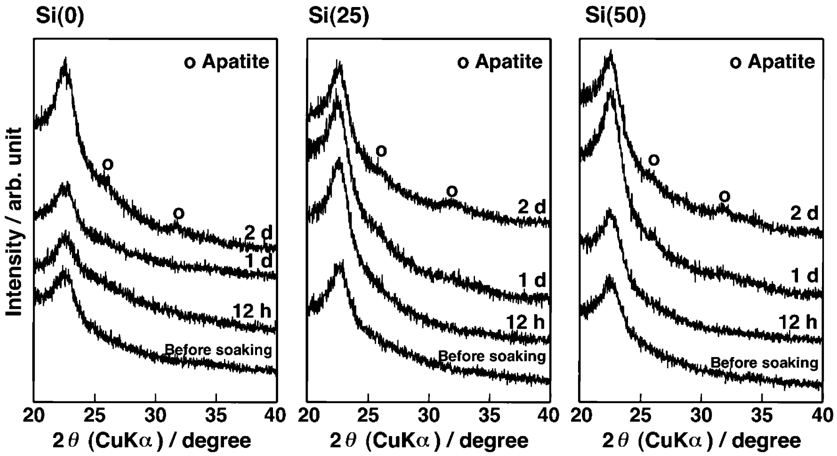

Fig. 2 TF-XRD patterns of surfaces of Si(*x*) (*x* = 0, 25 and 50) films before and after soaking in 1.5SBF for various periods

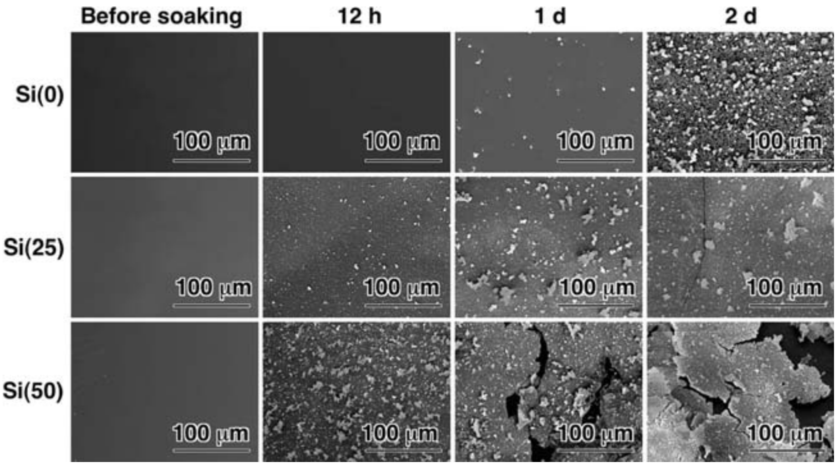

Fig. 3 SEM images of surfaces of Si(x) (x = 0, 25 and 50) films before and after soaking in 1.5SBF for various periods

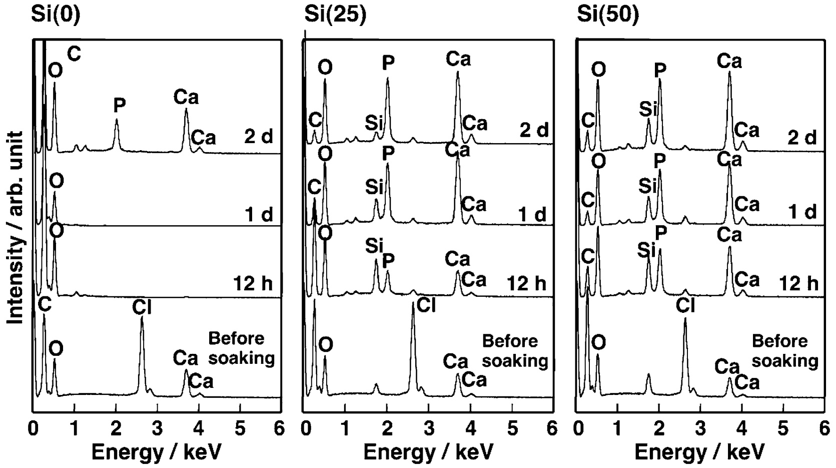

Fig. 4 EDX spectra of Si(x) (x = 0, 25 and 50) films before and after soaking in 1.5SBF for various periods

phosphorus. The particles initially precipitated on the film might have low crystallinity or their amount might be too small to be detected by TF-XRD. It can be seen from the SEM photographs and EDX spectra that the rate of apatite formation increases with increasing silanol group content in the polyamide films. Figure 5 shows morphology of the deposited particles on Si(0), Si(25) and Si(50) films after soaking in 1.5SBF for 2 days, at higher magnification under FE-SEM. This morphology was very similar to that of the deposited apatite on a substrate through biomimetic processing utilizing SBF [2] and their appearance is similar. This means that the bone-like apatite layer formed on the polyamide film containing silanol groups as well as carboxyl group, and the morphology is similar independent of the kind of functional groups and silanol group content. Figure 6 shows the TEM image of the interface between Si(0) film and apatite formed on the film after soaking in 1.5SBF for 7 days. It was observed that nano-sized fibers are intricately intertwined into Si(0) film, as shown in an area surrounded by a dotted frame.

Discussion

It is apparent from the results described above that polyamide containing silanol groups showed a high

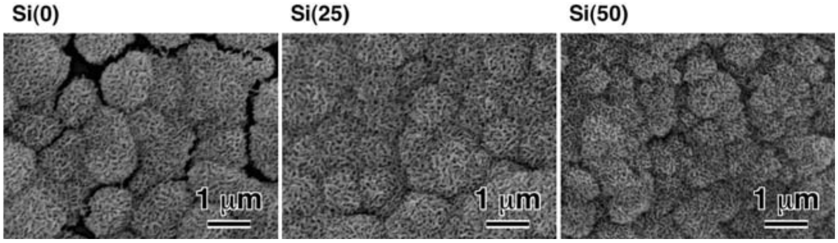

Fig. 5 FE-SEM images of surfaces of Si(x) (x = 0, 25 and 50) films after soaking in 1.5SBF for 2 days

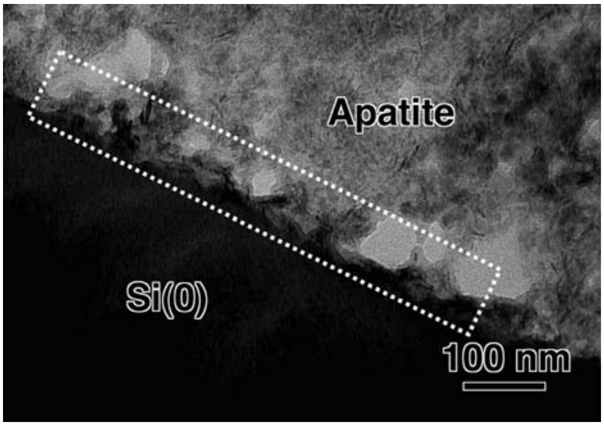

Fig. 6 TEM image of interface between Si(0) film and apatite formed on the film after soaking in 1.5SBF for 7 days

capacity for apatite formation on its surface when it contained calcium chloride, and its apatite-forming ability appears greater than that of carboxyl groups. These results indicate that silanol groups could accelerate the apatite formation. Substitution of carboxyl groups with silanol groups enhanced the heterogeneous nucleation on the surface of the polyamide. This means that modification with silanol groups caused increasing numbers of sites for heterogeneous nucleation of apatite on the surface of polyamide films. However, the deposited apatite was more easily peeled off from Si(50) film than Si(25), especially the films soaked for one and 2 days, as seen in Fig. 2. In contrast, apatite deposited on Si(0) had hardly peeled off from the film. The adhesion strength between the deposited apatite and polyamide film also depends on the types of the functional groups in the polyamide films. It is assumed that silanol groups show higher potential in induction of apatite nucleation than carboxyl groups in 1.5SBF. Almost 99% of carboxyl groups dissociate to be $-COO^-$, while 0.4% of silanol groups dissociate to be $-Si(OH)_2O^-$ at pH 7.4. Almost no dissociation to $-Si(OH)(O^-)_2$, and $-Si(O^-)_3$ occurs in such a solution. From this assumption, modification of carboxyl groups with silanol groups makes access of the groups by calcium ions difficult. On the other hand, calcium ions can be easily adsorbed to silanol groups to form Si–O–Ca bonds [15]. The electronegativity of silicon is lower than that of carbon. This may enable calcium ions to access near lone electron-pairs of oxygen atoms in silanol groups in a dipole interaction. Moreover, one APES molecule consumes one carboxyl group and then provides three silanol groups. The modification of polyamide with APES increases the total content of functional groups. One unit of the silanol groups possesses six lone electron-pairs, while one carboxyl group has five. This may also have contributed to easier access of calcium ions to the integrated silanol groups than that to carboxyl groups. Thus –SiOH group react with calcium ions around pH 7 as shown the formulae below,

$$2(-SiOH) + Ca^{2+} \rightarrow (-SiO)_2Ca + 2H^+$$

$$(-SiOH) + Ca^{2+} \rightarrow (-SiO)Ca^+ + H^+$$

or

$$x\left(-\ SiO^{\delta-}H\right) + Ca^{2+} \rightarrow (-\ SiOH)_xCa^{2+}$$

to form silicate complexes and then these complexes induce nuclei of apatite.

There appeared lower adhesive strength of the apatite layer with polymer substrate containing silanol groups than that with polymer substrate containing carboxyl groups. The weak strength may be attributed to less interaction between the functional groups and Ca^{2+} ions. It is therefore assumed that the formation of $(-SiOH)_xCa^{2+}$ complex at the nucleation stage. Another possible explanation is that increasing content of –SiOH causes the polyamide to swell readily in 1.5SBF and to show a high degree of shrinkage after drying, resulting in low adhesion of the film surface to apatite. Therefore, the adhesive strength seems to be governed not only by a chemical interaction of functional groups with Ca^{2+}, but also by the mechanical property of the polyamide film itself. Actually, from the observation of the cross-section between a polyamide film containing carboxyl groups and apatite formed on the film surface by TEM observation, the interface was found to be constructed by an intertwining structure (see Fig. 6). These factors are assumed to act together to form an apatite layer with high adhesion strength against the surface of the polyamide.

These findings show well that modification of organic polymers with the functional groups induces apatite deposition, and also determines the adhesive strength of apatite layer to the organic substrates.

Conclusions

Apatite formation on aromatic polyamides was accelerated by modification with silanol groups. Modification of polyamide film with silanol groups accelerates the rate of apatite nucleation but decreases the adhesive strength of the formed apatite layer with the substrates. Appropriate amounts of silanol groups and calcium chloride can lead to fabrication of a new apatite-polymer hybrid under biomimetic process using physiological solutions.

Acknowledgments This work was conducted under the auspices of the research project, "Technology Development for Medical Materials Merging Genome Information and Materials Science", in the Kansai Science City Innovative Cluster Creation Project, supported by the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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