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Apatite deposition on polyamide films containing carboxyl group in a biomimetic solution

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The development of organic–inorganic hybrids composed of hydroxyapatite and organic polymers is attractive because of their novelty in being materials that show a bone-bonding ability, i.e. bioactivity, and because they have mechanical properties similar to those of natural bone. The biomimetic process has received much attention for fabricating such a hybrid, where bone-like apatite is deposited under ambient conditions on polymer substrates in a simulated body fluid (SBF) having ion concentrations nearly equal to those of human extracellular fluid or related solutions. It has been shown that the carboxyl group is effective for inducing heterogeneous nucleation of apatite in the body. In the present study, apatite deposition on polyamide films containing various numbers of carboxyl groups was investigated in 1.5 SBF, which had ion concentrations 1.5 times those of a normal SBF. The effect of incorporation of calcium chloride on the formation of apatite was examined. Polyamide films containing ≤ 33 mol % $CaCl_2$ did not form apatite, even after soaking in 1.5 SBF for 7 days, and even when the polymer film contained 50 mol % carboxyl group. On the other hand, those modified with ≥ 40 mass % $CaCl_2$ formed apatite on their surfaces in 1.5 SBF. The ability of the modified film to form an apatite layer increased, and the adhesion of the apatite layer bonded to the film improved, with increasing carboxyl group content. It is concluded that novel apatite–polyamide hybrids can be prepared by a biomimetic process.

1. Introduction

Bioactive ceramics, such as Bioglass®, sintered hydroxyapatite, and glass-ceramic A-W, have been studied extensively for clinical applications, such as bone substitution, because they have the ability to bond directly to bone [1–3]. This specific biological activity has attracted much attention in the development of novel materials for bone repair and regeneration. The problem is, however, that these bioactive ceramics show a higher Young's modulus and a lower fracture toughness than cortical bone. For this reason, they are quite difficult to use as bone substitutes in load-bearing parts of the human skeleton.

Natural bone is an organic–inorganic hybrid made of organic collagen fibers and inorganic apatite crystals with a characteristic structure that leads to specific mechanical properties, such as a high fracture toughness and high flexibility [4]. From this point of view, the fabrication of hybrid materials consisting of apatite and an organic polymer that mimics bone structure opens new avenues for novel bone-repairing materials that have both a bone-bonding ability and mechanical properties similar to those of natural bone.

Kokubo and coworkers have reported on a biomimetic process that utilizes a reaction between bioactive glass and a simulated body fluid (SBF) having ion concentrations nearly equal to those of human extracellular fluid. This is attractive, because a bone-like apatite layer can be coated onto an organic substrate under ambient conditions [5]. In this process, the organic substrate is placed in the vicinity of bioactive glass in the SBF. The dissolved silicate ions and calcium ions (Ca^{2+}) from the glass trigger the selective deposition of bone-like apatite on the polymer substrate. In this process, the dissolved silicate ions provide heterogeneous nucleation sites on the substrate, while the dissolved Ca^{2+} increases the degree of supersaturation of the surrounding solution with respect to apatite. Once the apatite nuclei are formed on the substrate, they can grow spontaneously by consuming the Ca^{2+} and phosphate ions from the surrounding solution. Apatite crystals grown in this way form a dense and uniform layer on the surface of a

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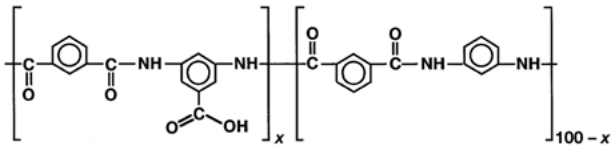

Figure 1 Structural formula of polyamide C(x).

polymer substrate by further immersion in 1.5 SBF, which has ion concentrations 1.5 times those of a normal SBF. This means that apatite deposition on organic polymers can be initiated both by the existence of specific functional groups that are effective for heterogeneous nucleation of apatite, and by an increased concentration of Ca^{2+} in the surrounding solution. It is known that some functional groups have the ability to induce heterogeneous nucleation of apatite in a body environment [6, 7]. In addition, previous studies on apatite deposition on self-assembled monolayers have reported that the carboxyl group (–COOH) also plays an effective role in heterogeneous apatite nucleation [8]. These findings led to the idea of an apatite–polymer hybrid that can be prepared through a process of spontaneous deposition of apatite crystals on an organic polymer in a body environment, where the surface of the polymer is abundant in carboxyl groups and Ca^{2+}. The key to this process is the establishing of a suitable polymer substrate for inducing heterogeneous nucleation of apatite under conditions that mimic those of a body fluid.

In this study, we investigated the potential for apatite deposition on polyamide films containing carboxyl groups after a simple exposure to a solution that had ion concentrations 1.5 times those of a normal SBF solution. Aromatic polyamides [9], shown in Fig. 1, were subjected to these tests, because they have an advantage for fundamental investigations in that their functional groups can be easily modified. Polyamide can also be formed into a film. Thus, polyamide films with various carboxyl group contents were investigated as a polymeric substrate for the deposition of apatite crystals. The effect of incorporation of calcium chloride ($CaCl_2$) onto the polyamide film for apatite formation was also examined.

2. Materials and methods

2.1. Preparation of polyamide films

Aromatic polyamides (Fig. 1) were prepared according to a literature method [9]. Three types of polyamide were examined containing 0, 20, and 50 mol % carboxyl group, and these were denoted as C(0), C(20), and C(50), respectively. Polyamide powder was dissolved in 10 mL of *N*, *N*-dimethyl acetamide, both with and without $CaCl_2$. The $CaCl_2$ was added to the polyamide in various mass ratios of $CaCl_2/(\text{Polyamide} + CaCl_2) = 0$, 0.17, 0.25, 0.33, 0.40, and 0.50. The mixture was then stirred for 12 h to form a homogeneous viscous solution. The obtained solutions both with, and without $CaCl_2$ were then coated onto flat glass plates using a bar coater. The glass plates coated with the solutions were then dried in a vacuum oven at 60 °C at a pressure of 133 Pa for 8 h. The films were then removed from the glass plates, and cut into $10 \times 15\,mm^2$ sections. Polyamide (C(x)) films were prepared by modification with y mass % of $CaCl_2$ to a given total of C(x) and $CaCl_2$, and hereafter, were denoted as C(x)Ca(y).

2.2. Soaking in 1.5 SBF

The obtained films were then soaked in 1.5 SBF (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}) with inorganic ion concentrations 1.5 times those of a normal SBF. This 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 in distilled water [10]. The pH of the solution was buffered at pH = 7.40 using 75 mol m^{-3} of *tris*(hydroxymethyl)aminomethane along with an appropriate volume of hydrochloric acid. The temperature of the solution was kept at 36.5 °C. After soaking for a given period, the films were removed from the solution, washed with distilled water, and then dried at room temperature.

2.3. Characterization

The surfaces of the films were characterized both before and after soaking in 1.5 SBF, using Fourier-transform infrared (FT-IR) spectroscopy (Spectrum GX, Perkin Elmer Ltd., England), thin-film X-ray diffraction (TF-XRD; M18XHF^{22}-SRA, MAC Science Co., Yokohama, Japan) and scanning electron microscopy (SEM; S-3500N, Hitachi Co., Tokyo, Japan). In the TF-XRD measurements, the angle of the incident beam was fixed at 1° against the surface of the specimen. In the SEM observations, an Au thin film was sputtered onto the surface of the specimen.

The adhesion of the apatite layer to the film was evaluated using a peel-off test with Scotch® tape (No. 810-1-12) [11]. In the peel-off test, the tape was attached to the surface of the film and then peeled away from it. The surface of the film after the peel-off test was then observed using SEM.

3. Results

Fig. 2 shows SEM photographs of the surfaces of C(50)Ca(y) films ($y = 0$, 17, 33, 40 and 50) after soaking in 1.5 SBF for 7 d. Assemblies of spherical particles were observed on the films modified with ≥ 40 mass % $CaCl_2$ after soaking. The morphology of the particles on the polyamides was similar to that of the apatite layer that forms on bioactive glasses and glass-ceramics [12]. Fig. 3 shows FT-IR spectra of C(50)Ca(y) films ($y = 0$, 17, 33, 40 and 50) after soaking in 1.5 SBF for 7 days. Broad peaks assigned to P–O stretching were detected at about 1050 cm^{-1} for the films modified with ≥ 40 mass % $CaCl_2$ after soaking. Fig. 4 shows TF-XRD patterns of the surfaces of C(50)Ca(y) films ($y = 0$, 17, 33, 40 and 50) after soaking in 1.5 SBF for 7 days. Peaks ascribed to apatite were observed at $2\theta = 26$ and 32° in the XRD patterns of films modified with ≥ 40 mass % $CaCl_2$ after soaking. The peak at $2\theta = 26°$ is assigned to the 002 diffraction of apatite, while the one at about $2\theta = 32°$ is an envelope of the 211, 112 and 300 diffractions of

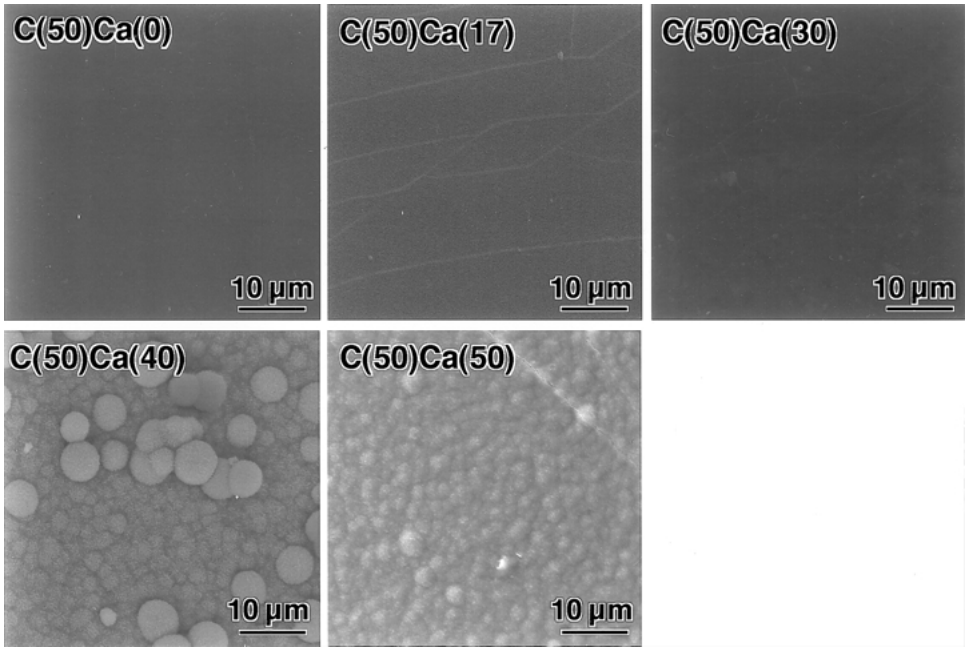

Figure 2 SEM photographs of the surfaces of C(50)Ca(y) films ($y = 0$, 17, 33, 40 and 50) after soaking in 1.5 SBF for 7 days.

apatite. This indicates that the spherical particles observed under SEM were from apatite composed of small crystallites. Furthermore, the phosphate compound detected in the FT-IR spectra is attributable to the formation of hydroxyapatite after soaking in 1.5 SBF. Apatite was therefore formed on the film when $CaCl_2$ was incorporated in a ratio of ≥ 40 mass % in polymer C(50).

Fig. 5 shows SEM photographs of the surfaces of C(x)Ca(40) films ($x = 0$, 20 and 50) after soaking in 1.5 SBF for 7 d. Assemblies of spherical particles were observed to form after soaking. The density of the particles increased with increasing number of carboxyl groups in the films. Fig. 6 shows FT-IR spectra of C(x)Ca(40) films ($x = 0$, 20 and 50) after soaking in 1.5 SBF for 7 days. Broad peaks assigned to P–O stretching were detected for all the specimens at about 1050 cm^{-1} after soaking. The intensity of the peaks increased as the

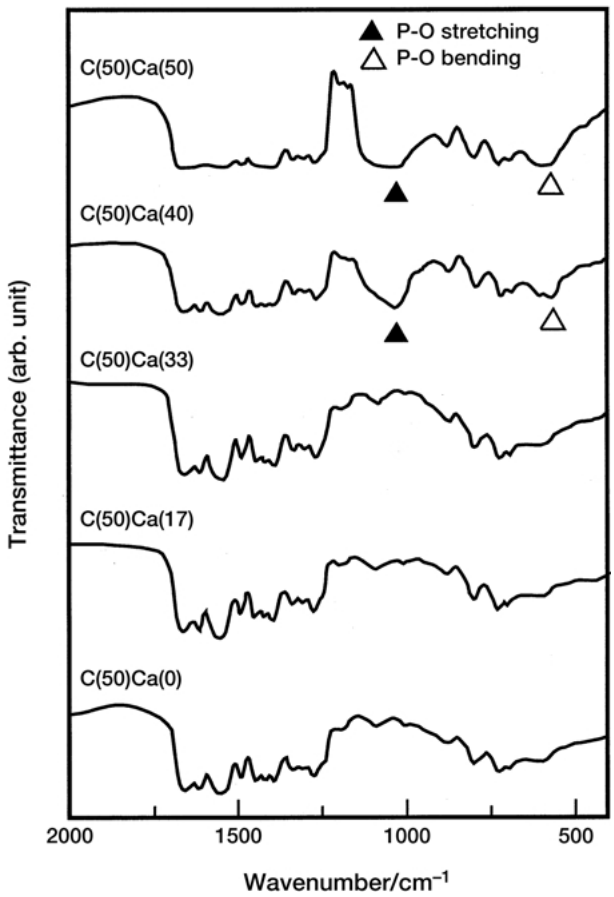

Figure 3 FT-IR spectra of C(50)Ca(y) films ($y = 0$, 17, 33, 40 and 50) after soaking in 1.5 SBF for 7 days.

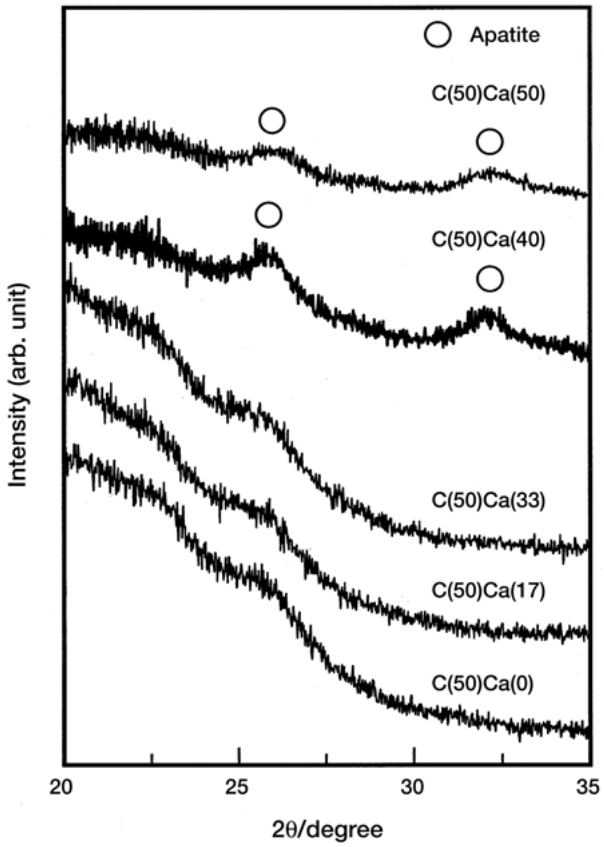

Figure 4 TF-XRD patterns of the surfaces of C(50)Ca(y) films ($y = 0$, 17, 33, 40 and 50) after soaking in 1.5 SBF for 7 days.

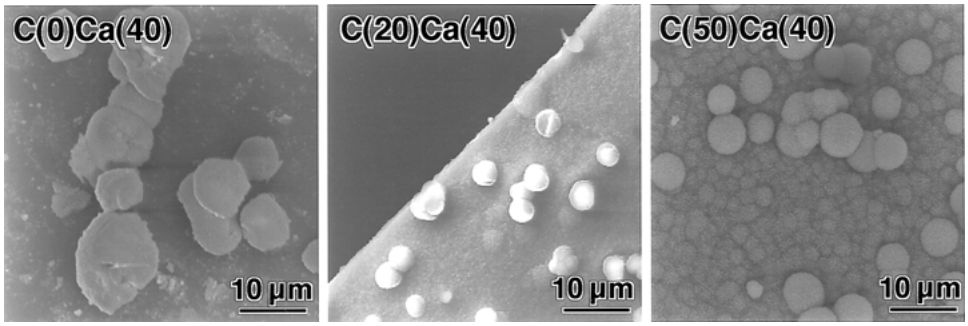

Figure 5 SEM photographs of the surfaces of C(x)Ca(40) films ($x = 0$, 20 and 50) after soaking in 1.5 SBF for 7 days.

number of carboxyl groups in the films increased. This indicates that the formation of phosphate compounds was accelerated by increasing the number of carboxyl groups incorporated into the polyamide. Fig. 7 shows TF-XRD patterns of the surfaces of C(x)Ca(40) films ($x = 0$, 20 and 50) after soaking in 1.5 SBF for 7 days. Broad peaks were observed at $2\theta = 26$ and 32° after soaking. These results indicate that a higher number of carboxyl groups in the polyamide films leads to a higher rate of formation of crystalline apatite after soaking in 1.5 SBF.

Fig. 8 shows SEM photographs of a cross-section of the C(50)Ca(40) film after soaking in 1.5 SBF for 7 days. It can be seen that a uniform apatite layer about 2-μm thick was formed on both sides of the film, which itself was about 7-μm thick. All these results support the proposition that aromatic polyamides can form an apatite layer after exposure to 1.5 SBF when it contains 40 mol % of calcium chloride. Fig. 9 shows SEM photographs of the surfaces of C(0)Ca(40) and

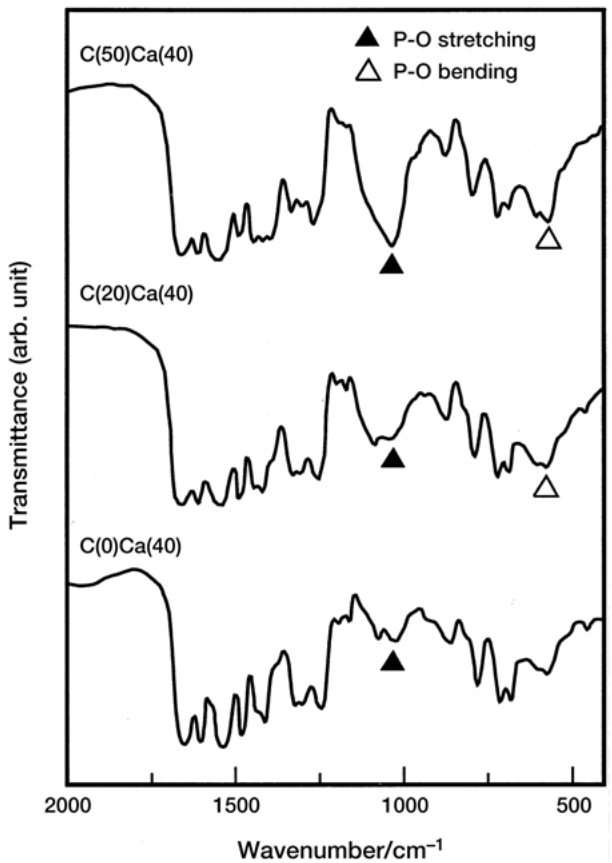

Figure 6 FT-IR spectra of C(x)Ca(40) films ($x = 0$, 20 and 50) after soaking in 1.5 SBF for 7 days.

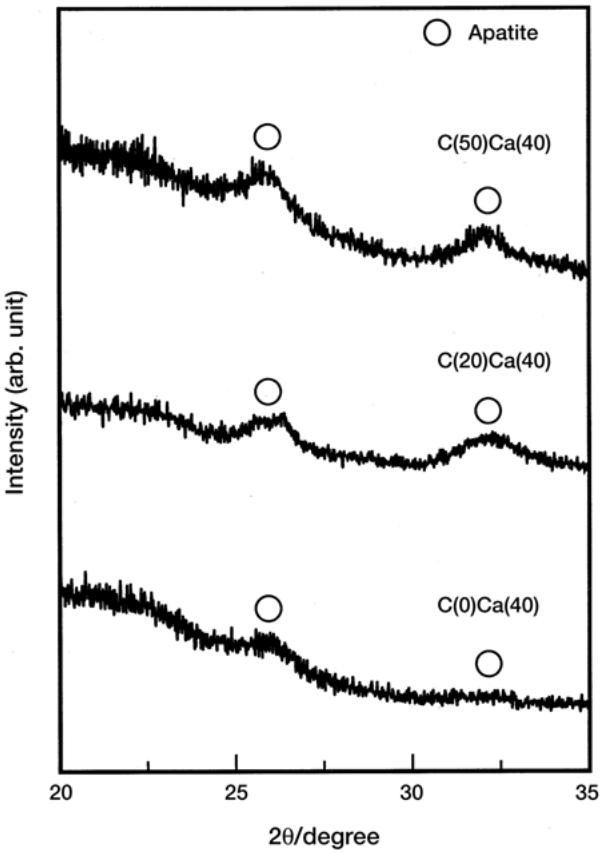

Figure 7 TF-XRD patterns of the surfaces of C(x)Ca(40) films ($x = 0$, 20 and 50) after soaking in 1.5 SBF for 7 days.

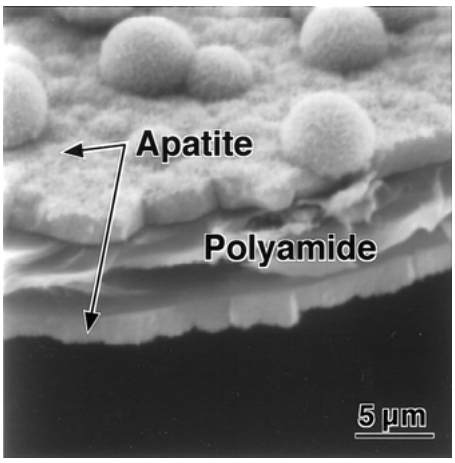

Figure 8 SEM photograph of the cross-section of C(50)Ca(40) film after soaking in 1.5 SBF for 7 days.

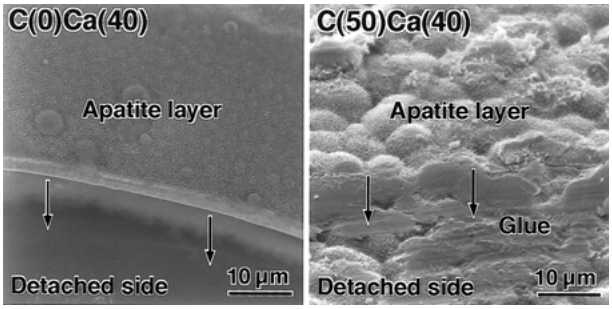

Figure 9 SEM photographs of the surfaces of C(0)Ca(40) and C(50)Ca(40) films after soaking in 1.5 SBF for 7 days, which were then subjected to Scotch® -tape peeling-off test. Lower half side of the surface indicated by arrow was adhered and peeled off with the tape.

C(50)Ca(40) films after soaking in 1.5 SBF for 7 days, which were then subjected to the Scotch® tape peel-off test. The apatite layer that had formed on the C(0)Ca(40) film was almost completely delaminated. In contrast, the apatite formed on the C(50)Ca(40) film was not removed; rather, the glue of the tape was observed to remain on the surface. The increased number of carboxyl groups resulted in a tighter bonding between the polymer substrate and the deposited apatite.

4. Discussion

It can be seen from Figs. 2 to 4 that polyamide films modified with 40 mass % $CaCl_2$ formed apatite on their surfaces after exposure to 1.5 SBF, whereas those containing less than 33 mass % calcium chloride did not. It should also be noted that this apatite formation was enhanced with increasing carboxyl group content. These observations indicate that apatite deposition on the polyamide films is initiated by the incorporation of carboxyl groups onto the film's surface, and by Ca^{2+} ions released from the film into the solution, as the presence of the carboxyl group alone is not enough to induce apatite deposition. The carboxyl group on the film triggers heterogeneous nucleation of apatite by a catalytic effect, while the release of Ca^{2+} from the film increases the degree of supersaturation of the surrounding solution with respect to apatite. It has been revealed from studies on the mechanism of apatite formation on bioactive materials in a body environment that the initial condition for apatite nucleation is the formation of a complex with Ca^{2+} ions and negatively charged functional groups, such as Si-OH [13], Ti-OH [14] and Ta-OH [15]. In the case of the carboxyl group, such a complex with Ca^{2+} may also be formed in the initial stage. Incorporation of $\geq$ 40 mass % $CaCl_2$ is necessary to induce the apatite formation on the films in 1.5 SBF. This shows that the increased degree of supersaturation of the surrounding solution is not high enough to induce apatite nucleation, even after the soaking periods used, when the film contains a low mass % of $CaCl_2$.

The carboxyl group incorporated into the polyamide films has another important role. It can be seen from Fig. 9 that the apatite formed on the C(50)Ca(40) film has a higher resistance to peeling than that formed on the C(0)Ca(40) film. This indicates that the adhesion of the apatite layer to the film is enhanced by the incorporation of a carboxyl group. As described above, apatite nucleation on bioactive materials is initiated by the selective binding of the Ca^{2+} in the surrounding solution to a functional group on the surface. It has been reported that the carboxyl group binds to Ca^{2+} in a body environment much more tightly than an amide group does [8]. The C(50)Ca(40) film contains both a carboxyl and an amide group in its structure, whereas the C(0)Ca(40) film contains only an amide group. The strong interaction between the carboxyl group on the C(50)Ca(40) film and Ca^{2+} leads to a tight bonding between the apatite layer and the film. These results indicate that the carboxyl group not only provides a site for heterogeneous nucleation of apatite, but also contributes to a tight adhesion of the apatite layer to the film.

Modification of the polyamide films with appropriate numbers of carboxyl groups and Ca^{2+} was found to be effective for inducing the deposition of apatite on the film surfaces in 1.5 SBF. The findings in the present study point to a novel technique for obtaining apatite–polyamide hybrids for bone-substitute materials. This type of material design can be applied to fabricate hybrids composed of apatite and natural organic polymers, since some of these are in the form of polyamides. In the future, apatite deposition on polyamide films modified with other functional groups should be examined to reveal the general principles for designing apatite–polyamide hybrids.

5. Conclusions

Apatite formation was induced on polyamide films in 1.5 SBF by modification of the carboxyl group and the Ca^{2+} content of polymer films. Our results provide fundamental information on an effective surface structure for designing novel apatite–polyamide hybrids.

Acknowledgments

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References

1. L. L. HENCH, *J. Am. Ceram. Soc.* **81** (1998) 1705.
2. T. KOKUBO, *J. Ceram. Soc. Japan* **99** (1991) 965.
3. H.-M. KIM, *ibid.* **109** (2001) S49.
4. J. B. PARK and R. S. LAKES, in ''Biomaterials; An Introduction'', 2nd edn (Plenum Press, New York, 1992) p. 194.
5. M. TANAHASHI, T. YAO, T. KOKUBO, M. MINODA, T. MIYAMOTO, T. NAKAMURA and T. YAMAMURO, *J. Am. Ceram. Soc.* **77** (1994) 2805.
6. P. LI, C. OHTSUKI, T. KOKUBO, K. NAKANISHI, N. SOGA and K. DE GROOT, *J. Biomed. Mater. Res.* **28** (1994) 7.
7. T. KOKUBO, H.-M. KIM, M. KAWASHITA, H. TAKADAMA, T. MIYAZAKI, M. UCHIDA and T. NAKAMURA, *Glastech. Ber. Glass Sci. Tech.* **73C1** (2000) 247.
8. M. TANAHASHI and T. MATSUDA, *J. Biomed. Mater. Res.* **34** (1997) 305.
9. S. KONAGAYA and M. TOKAI, *J. Appl. Polym. Sci.* **76** (2000) 913.
10. C. OHTSUKI, T. KOKUBO, M. NEO, S. KOTANI, T. YAMAMURO, T. NAKAMURA and Y. BANDO, *Phosphorus Res. Bull.* **1** (1991) 191.

11. C. WEAVER, *J. Vac. Sci. Tech.* **12** (1975) 18.
12. C. OHTSUKI, H. KUSHITANI, T. KOKUBO, S. KOTANI and T. YAMAMURO, *J. Biomed. Mater. Res.* **25** (1991) 1363.
13. H. TAKADAMA, H.-M. KIM, T. KOKUBO and T. NAKAMURA, *Chem. Mater.* **13** (2001) 1108.
14. H. TAKADAMA, H.-M. KIM, T. KOKUBO and T. NAKAMURA, *J. Biomed. Mater. Res.* **55** (2001) 185.
15. T. MIYAZAKI, H.-M, KIM, T. KOKUBO, C. OHTSUKI, H. KATO and T. NAKAMURA, *Biomaterials* **23** (2002) 827.

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