In vitro apatite formation on organic polymers modified with a silane coupling reagent

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γ-Methacryloxypropyltrimethoxysilane (γ-MPS) was grafted to high-density polyethylene, polyamide and silicone rubber substrates by the emulsion polymerization procedure in order to provide these organic polymers with in vitro apatite-forming ability. The contact angles towards distilled water of the γ-MPS-grafted specimens were lower than those of the original organic polymer specimens, indicating that the grafted substrates were more hydrophilic. The in vitro apatite formation in a simulated body fluid (Kokubo solution) was confirmed for several of the γ-MPS-grafted specimens.

Keywords: graft polymerization; silane coupling agent; emulsion polymerization; in vitro apatite formation

1. INTRODUCTION

When placed in a body environment, several glasses and ceramics (Ogino et al. 1980; Kokubo et al. 1990a) spontaneously deposit an apatite layer. Such ability is denoted as in vivo bioactivity, while it is in vitro bioactivity when such apatite deposition is achieved in a simulated body fluid (SBF) of the Kokubo recipe. SBF contains the same inorganic ions as human plasma in similar concentrations, and reproduces well in vivo surface reactions of materials under in vitro conditions (Ogino et al. 1980; Kokubo et al. 1990a; Tanahashi et al. 1995; Oyane et al. 1999, 2003; Kim et al. 2001). Synthetic organic polymers are the mainstream materials in clinics by virtue of their properties, such as flexibility and ease of shaping, but they are not bioactive. Providing them with bioactivity might be advantageous in fixation to hard and soft tissues, so several surface modification techniques have been proposed. For example, Kim et al. (2001) grafted vinyltrimethoxysilane on polyethylene (PE) substrates by photoreaction of a vinyl group, and subsequently hydrolyzed the methoxy groups with hydrochloric acid to introduce silanol groups at the other end. Although silanol groups are accepted to favour apatite deposition (Ogino et al. 1980; Kokubo et al. 1990a), the vinyltrimethoxysilane-grafted PE substrates were not in vitro bioactive, or they did not deposit apatite on their surfaces in SBF. Oyane et al. confirmed the bioactivity of the ethylene-vinyl alcohol copolymer (EVOH) modified with silanol groups; apatite deposited on the EVOH substrate surface within 1 day in SBF (Oyane et al. 1999, 2003). However, their methods require not only the modification of polymer surfaces with a silane coupling agent but also the deposition of silicate ions. That is, the silicate ions were responsible for the bioactivity, not the grafted molecules (Tanahashi et al. 1995).

The aim of this study is to propose a novel and simpler chemical method of surface modification with which organic polymers would have superior bioactivity or deposit an apatite layer in a body environment within a short period. The emulsion polymerization method is employed here to graft an organo-alkoxysilane (γ-methacryloxypropyltrimethoxysilane, γ-MPS) on to several organic polymer substrates, such as high-density polyethylene (HDPE), polyamide (PA), and silicone rubber. Because the emulsion polymerization proceeds in water, organo-alkoxysilane would be hydrolyzed and be grafted on the polymer surface at the same time. It is one of the most important techniques for the commercial production of polymers (Kroschwitz 1990). In the present study, the optimum conditions of the emulsion polymerization are examined in terms of the grafting of γ-MPS on HDPE, and PA, and are applied to silicone rubber, which is one of the most useful clinical materials. The apatite-forming ability of the γ-MPS-grafted specimens is evaluated by observing the in vitro deposition of apatite in SBF.

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Received 29 October 2004
Accepted 26 April 2005

Published online 16 June 2005
2. EXPERIMENTAL

2.1. Grafting due to emulsion polymerization

Commercially available sheets (Nikko Co., Ltd., Japan) of HDPE and PA were cut into pieces 10×15×1 mm³ in size, and their surfaces were polished with no. 1000 emery paper. Silicone rubber was prepared from a commercially available silicone rubber kit (KE106) supplied by Shinetsu Chemical Co., Japan. It consisted of a room temperature vulcanizing (RTV) type liquid and a solidifying agent, which were mixed well for 3 min at room temperature. Then, the mixture was cast into a mould, and kept for 30 min at 150 °C. Rectangular specimens (10×10×1 mm³) were cut from the sheet of silicone rubber obtained above, which were polished with no. 1000 emery paper and denoted as SR. All samples (HDPE, PA and SR) were washed with distilled water in an ultrasonic cleaner for 3 min and dried at room temperature before use.

Figure 1 illustrates the apparatus set for the present emulsion polymerization. Each specimen was fixed on an aluminium wire grid table that was placed in the centre of a 300 ml three-necked separable flask with 100 ml of distilled water and a magnetic spinner for stirring. The height of the table was adjusted such that the specimen was immersed in the water to half its depth. Two of the three necks were used for N₂. Sodium lauryl sulphate (SLS; Ishizu Seiyaku, Osaka, Japan; 1.5 g) was added as the emulsifier. The solution was stirred at 60 °C for 30 min under flowing N₂. γ-MPS (CHISSO, Tokyo, Japan; 3 ml) was added dropwise into the solution with a syringe at the same neck for the N₂ outlet. Potassium peroxodisulfate (1.5 g) and sodium hydrogen sulfate (0.15 g), both supplied by Wako Pure Chemicals, Osaka, Japan, were dissolved into 50 ml of distilled water. After adding γ-MPS, the solution was added to the flask through another syringe set at the neck for the N₂ inlet. The resultant solution was stirred at 60 °C under N₂ flow for 120 min. All specimens subjected to the grafting were rinsed with ethanol and washed with distilled water in an ultrasonic cleaner for 3 min. The samples were coded as Zi, where Z and i stand for the substrate and the amount of γ-MPS (ml), respectively. Thus, sample PA3 was a PA specimen, subjected to grafting with the solution containing 3 ml of γ-MPS.

2.2. Characterization of the graft layer

The graft layer of γ-MPS was scratched off from the specimens and embedded in a potassium bromide pellet. The structure was examined by Fourier-transform infrared (FT-IR) spectroscopy (model FT-IR300, JASCO, Japan): the signals from 100 scans were accumulated with a resolution of 4 cm⁻¹. FT-IR analysis was also conducted for a γ-MPS homopolymer, derived in ethanol by using benzoylperoxide as the polymerization initiator, after the polymerization procedure described in detail by Yabuta et al. (2000). The hydrophobicity of the samples was evaluated from the static contact angle towards distilled water (1.5 μl) and was measured with an automatic contact angle metre (Kyowa interface science, model CA-V, Japan) at room temperature by the sessile-drop method. X-ray photoelectron spectra (XPS) were measured with an S-probe ESCA (Fisons Instruments, SSX100S, USA; Hayakawa et al. 2000), equipped with a monochromatic X-ray source (Al Kα anode) operated at 10 kV and 210 W. The surface charge owing to the X-ray irradiation was neutralized during the measurement by the combined use of a low-energy flood gun and an electrically grounded Ni mesh screen placed 1 mm above the sample surface (Hayakawa et al. 2000). The binding energy (BE) was normalized to the C1s peak (286.5 eV; 1 eV = 1.602×10⁻¹⁹ J) of adventitious carbon accumulated in the analysis chamber of the spectrometer, where the BE values were presented in eV after convention. The spectral profiles were deconvoluted with a few traces of the Gauss (80%)–Lorentz (20%) mixed function. The full widths at half maximum and the relative area of the component peaks were obtained (Hayakawa et al. 2000).

2.3. In vitro bioactivity

SBF was prepared as described in the literature (Kokubo et al. 1990b; Cho et al. 1995). A similar solution that was 1.5 times as concentrated as SBF was also prepared (Kokubo et al. 1990b; Cho et al. 1995), and denoted as 1.5 SBF. Table 1 shows the composition of SBF and 1.5 SBF. The γ-MPS-grafted specimens were soaked in SBF or 1.5 SBF at 36.5 °C for up to 14 days. Then, they were gently rinsed with distilled water and dried at room temperature. Their surface structure was examined by thin film X-ray diffraction (TF-XRD) and FT-IR reflection spectroscopy. The TF-XRD profiles were taken with an X-ray diffractometer (RAD-II AXE, CuKα, Rigaku, Tokyo, Japan, 40 kV–20 mA) attached with a thin-film diffraction apparatus. FT-IR reflection spectra were measured: the signals...
from 100 scans were accumulated with a resolution of 4 cm$^{-1}$ at 75° reflection angle. The surface morphology was observed under a scanning electron microscope (SEM, JEOL, JMSM-6300) equipped with an energy-dispersion X-ray (EDX) analyser (model DX-4, Phillips).

3. RESULTS

3.1. Surface structures

The silane grafted on the samples could not be peeled by the scotch tape. Figure 2 shows the FT-IR transmission spectrum of the $\gamma$-MPS graft layer of PA3. The graft layers on HDPE and SR gave similar profiles. For purposes of comparison, the spectra of the $\gamma$-MPS monomer and $\gamma$-MPS homopolymer are also shown. The $\gamma$-MPS monomer yielded peaks at 1637, 1720 and 2840–3000 cm$^{-1}$ due to $\nu$(C–C), $\nu$(C–O) and $\nu$(C–H), respectively (Hooshmand et al. 2004). The peaks at 1164, 1192, 1297 and 1320 cm$^{-1}$ were assignable to C–O–C of the ester group in $\gamma$-MPS, while the peaks at 816 and 1085 cm$^{-1}$ were assignable to $\nu$sym(Si–O–CH$_3$) and $\nu$asym(Si–O–CH$_3$), respectively. The peak at 937 cm$^{-1}$ was a result of the vinyl group. Homopolymerization introduced several new peaks in the spectra. The peak at 3500 cm$^{-1}$ was attributed to the hydroxyl group in $\gamma$-MPS monomer and $\gamma$-MPS homopolymer. The peaks at 3400–3200 cm$^{-1}$ were attributed to the hydroxyl group in $\gamma$-MPS monomer and $\gamma$-MPS homopolymer. The peaks at 3400–3200 cm$^{-1}$ were attributed to the hydroxyl group in $\gamma$-MPS monomer and $\gamma$-MPS homopolymer.

![Figure 2. FT-IR transmission spectra of $\gamma$-MPS monomer, $\gamma$-MPS homopolymer and product on PA substrate grafted by emulsion polymerization. Open circle, $\nu$(O–H); open square, $\nu$(C–H:CH$_2$); filled square, $\nu$(C–H:CH$_3$); filled diamond, $\nu$(C=O); open triangle, $\nu$(C=C); filled circle, $\nu$(Si–O); open diamond, $\nu$(Si–O); filled down-triangle, $\nu$(Si–O–C); filled up-triangle, $\nu$(C–O–C).](image)

Table 1. Ion concentration and pH of human plasma, SBF and 1.5 SBF.

<table>
<thead>
<tr>
<th>Ion</th>
<th>Concentration (mM)</th>
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<tr>
<td>SO$_4^{2-}$</td>
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</table>

![Figure 3. The contact angles of PA and HDPE substrates before and after emulsion polymerization ($n$=3). Here, the $\gamma$-MPS homopolymer is denoted as $\gamma$-MPS. Open circle, HDPE; open square, PA.](image)
the Si–OH groups derived from hydrolysis of the Si–O–CH$_3$ groups. The peaks at 1100–1040 and 909 cm$^{-1}$ were assigned to the Si–O–Si bridging bonds and the Si–OH group, respectively. The graft product had an IR spectrum profile similar to the g-MPS homopolymer in a low wavenumber region, ca 1100–500 cm$^{-1}$. It is reasonable that the homopolymer had no C–H peak of the SiO–CH$_3$ at 2840 cm$^{-1}$ because the group was lost because of condensation.

Figure 3 summarizes the contact angle data for PA (open squares) and HDPE (open circles) before and after the emulsion polymerization as a function of the g-MPS content in the starting solution. Here, the solid line indicates the contact angle for the g-MPS homopolymer (ca 82$^\circ$). Irrespective of the grafting conditions, the contact angle for the samples decreases of the order:

$$\text{HDPE3}(88.2^\circ) > \text{PA3}(78.4^\circ) \approx \gamma\text{-MPS homopolymer}(82.0^\circ)$$

Figure 5. Thin-film X-ray diffraction patterns (a) and FT-IR reflection spectra (b) PA3 and HDPE3 after being soaked in SBF up to 14 days. (a) Open circle, apatite; (b) filled diamond, v(P–O); open diamond, δ(P–O).

Figure 4. X-ray photoelectron spectra for HDPE3. The profiles were deconvoluted into a few components. (a) The Si peaks in the wide scan suggest that γ-MPS grafted to the HDPE substrate. (b) C1s.

Figure 6. Thin-film X-ray diffraction patterns for SR3 after being soaked in 1.5 SBF up to 14 days. Open circle, apatite.
3.2. In vitro apatite formation

Figure 5 shows TF-XRD patterns (a) and FT-IR spectra (b) of PA3 and HDPE3, after being soaked in SBF. PA3 substrate after 3 days in SBF gave diffraction peaks assignable to apatite and IR reflection peaks assignable to P–O vibrations. The other PA samples (PA1 and PA2) gave similar diffractions and FT-IR spectra (data not shown here), indicating deposition of apatite within 3 days in SBF. HDPE1 and HDPE2 were inferior in bioactivity to PA samples because those two indicated no diffraction peaks assignable to apatite after being soaked in SBF for 14 days (data not shown here). Figure 6 shows the TF-XRD patterns of SR3 after being soaked in 1.5 SBF up to 14 days. The apatite diffraction peaks appeared after 7 days. The longer induction period in 1.5 SBF indicates that SR3 was much less bioactive than the HDPE and PA samples. Figure 7 shows SEM photographs of PA3 (a), HDPE3 (b) and SR3 (c) after being soaked in SBF or 1.5 SBF. Apatite particles were observed on all of the samples. The number of apatite particles on PA3 was greater than that on HDPE3. In figure 7c, apatite particles covered almost all parts of the surface of SR3.

4. DISCUSSION

The wide scan XPS spectrum in figure 4 indicated the presence of the Si component on HDPE3. The Cls component peak at 288.3 eV was assigned to the carbon in O–C=O. This confirmed the grafting of γ-MPS on to the HDPE surface by emulsion polymerization. Owing to the standard mechanism, the initiator is supposed to yield γ-MPS oligomer radicals, which attack the polymer substrates to form grafts. In the present study, the aqueous reaction system had a pH of less than 3, which favoured hydrolysis of –Si(OCH₃)₃ groups to –Si–OH ones and they were further condensed to yield Si–O–Si bonds. Thus, the grafted samples had no IR bands related to the Si–OCH₃ groups that used to be a part of γ-MPS. Those Si–O–Si bridging bonds and residual Si–OH bonds provide the polymers with hydrophilicity, as indicated by the decrease in the contact angle in figure 2. As mentioned above, γ-MPS oligomer radicals attack the polymer substrates to form grafts. Moreover, the substrates with more grafted molecules having –Si–OH groups gave smaller contact angles, accordingly. On the other hand, the surface roughness may reduce by γ-MPS grafted and provide the polymer with hydrophilicity. Thus, the contact angle is a measure of the rate of grafting. The rate of grafting is interpreted in terms of the chemical structure of the polymer substrates. PA3 has smaller contact angle than HDPE3, which is similar to the γ-MPS homopolymer. The decrement of static contact angle can be explained by considering the fact that, since PA has –NH and C=O groups and are easily attacked by γ-MPS oligomer radicals, the amount of γ-MPS grafted on PA increased.

The grafting introduced silanol groups as confirmed by the FT-IR and XPS spectra in figures 2 and 4, which should stimulate the formation of apatite when the grafted samples are soaked in SBF or 1.5 SBF (Ogino et al. 1980; Kokubo et al. 1990a; Tanahashi et al. 1995; Oyane et al. 1999, 2003; Kim et al. 2001). Indeed, PA (PA1, PA2, PA3) and HDPE3 samples deposited apatite in SBF. On the other hand, HDPE1 and HDPE2 were not bioactive, and did not deposit apatite in SBF within 14 days. This suggests that the rate of grafting, the amount of silanol groups on those HDPE samples might be insufficient for inducing apatite formation in SBF. In addition, this method could be applied to silicone rubber in order to provide its surface with in vitro apatite-forming ability.

5. CONCLUSION

An organosilane (γ-MPS) was grafted on to HDPE, PA and silicone rubber substrates by the emulsion polymerization method. The γ-MPS-grafted substrates had in vitro apatite-forming ability. The grafting of γ-MPS by emulsion polymerization is a convenient and uncomplicated method for surface modification of organic polymer substrates in order to provide them with in vitro apatite-forming ability.
REFERENCES


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*J. R. Soc. Interface* (2005)