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Ultrasonic implantation of calcium metasilicate glass particles into PMMA

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Polymer materials for clinical applications should be bioactive and have a bone-bonding ability. In order to provide poly(methyl methacrylate) (PMMA) with bioactivity, granules ($<45\,\mu m$) of a bioactive glass $50CaO{\cdot}50SiO_2$ (mol %) were implanted into PMMA: they were suspended together with a piece of PMMA in a 40 tetrahydrofuran-60 ethanol (vol %) solution and ultrasonically agitated. The granules of $<10\,\mu m$ in size were impregnated at ~ 40–$20\,\mu m$ depth below the substrate surface. Two types were detected on the PMMA surface: (a) a glass-granule layer on PMMA, and (b) an inner granule layer, a PMMA layer, and an outer granule layer on the PMMA. The bioactivity of the implanted PMMA substrates was examined *in vitro* with a simulated body fluid (Kokubo solution). Apatite was precipitated on all glass granules and the whole substrate surfaces within 1 d. After 4 h soaking in the Kokubo solution, aggregates of apatite particles appeared on the substrate surface, independently of those on the glass granules, and they grew and proliferated on the whole subtrate surface in 7 d. Silica gel islands on PMMA due to the silicate anions from the glass were considered to induce nucleation of the apatite particles.

1. Introduction

Most artificial implant materials, such as metals, organic polymers and ceramics, are biocompatible and they are only allowed in human or mammals' bodies when encapsulated by fibrous tissue. It is thus exceptional that limited kinds of lime and silica-based ceramic materials like Bioglass® [1] or Cerabone A-W® [2] bond to living bone through a bone-like apatite layer that spontaneously deposits on their surface in a body environment [3–7]. The bone-bonding ability, called bioactivity, can be confirmed by observing the apatite deposition. It would be much more convenient if organic polymers for biomedical applications, such as polyethylene, polypropylene, or poly(methyl methacrylate: PMMA), were bioactive. Only a few attempts have been reported to provide them with bioactivity or the ability to spontaneously deposit apatite. Bakker *et al.* [8] introduced copolymers of poly(ethylene oxide)–poly(butylene terephthalate) that deposited a bone-like apatite layer when implanted in a rat middle ear. However, Gaillard *et al.* [9] later showed that calcification of the copolymes was a prerequisite for bone-bonding and the precalcification affected the direction of bone apposition with an increased rate. Moreover, it was not clearly stated if the copolymers precalcified or not precalcified, they spontaneously deposited a bone-like apatite layer, if the precalcified ones formed a strong bond with bone, or how strong the bonding was. Tanahashi *et al.* [10] reported an elegant method of chemical coating of biocompatible substrates with a silicate layer that could induce apatite nucleation in a body environment. This process seems complicated and is time-consuming though it is applicable to any material with any shape.

In the present study, we employed a new method of providing organic polymers with bioactivity. The method utilizes an ultrasonic energy to accelerate the kinetic movement of particles suspended in a specific solvent that can swell and soften the surface of a polymer substrate. Because the accelerated particles can be implanted and fixed in the softened layer, the method is termed ultrasonic implantation. Such ultrasonic energy stimulation of the particle motion was first reported by Yugo *et al.* [11] who ultrasonically bombarded silicon single crystals on which diamond films were prepared via plasma chemical vapour deposition, to ensure an anchoring effect. With the present ultrasonic implantation technique, or a physical modification method, one can easily prepare a macroscopic composite surrface layer consisting of bioactive glass particles and the polymer. This technique is expected to be applicable to a wider range of organic polymers, with optimum selection of organic solvents which can soften them. In the present experiment we used PMMA as the substrate polymer material because it is widely used in the biomedical field. $50CaO{\cdot}50SiO_2$ glass (mol %) was selected for implanting, because it

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was one of the simplest compositions among the bioactive ceramics and glasses.

2. Experimental procedure

2.1. Preparation of glass and ultrasonic implantation

Reagent grade SiO_2 and $CaCO_3$ were the starting materials. The batches for about 30 g $50CaO{\cdot}50SiO_2$ glass (CS glass) were melted in an $MoSi_2$-electric furnace with a platinum crucible at 1600 °C for 2 h. The melts were quenched on a sheet of stainless steel by being pressed with an iron block. The glasses thus obtained were annealed and pulverized with a zirconia ball mill down to an average size of $<45\,\mu m$. Tetrahydrofuran (THF) was used as the organic solvent that could swell PMMA. Ethanol–THF solutions with various fractons (vol %) were prepared and examined as the suspending media, to find an appropriate mixing ratio: too much THF in the solution would dissolve out the PMMA substrate while too little THF in solutions would not soften the substrate. From a preliminary experiment [12] the optimum ethanol–THF mixing ratio for the suspending medium was 40% THF–60 ethanol. A sheet of PMMA was cut into pieces of 60 mm × 60 mm × 2 mm in size. A piece of the PMMA was immersed in the ethanol–THF solution (0.13 dm^3) held in a 0.3 dm^3 glass container in which 1 g CS glass powder was suspended. The container was positioned at the centre of an ultrasonic cleaning bath filled with water (capacity: 1 dm^3, 40 W) followed by ultrasonic agitation of the suspension for 20 min. It was noticed that the water temperature rose to 40 °C at the end of the ultrasonic treatment. After ultrasonic implantation, the substrate was gently rinsed with ethanol to get rid of the particles that were not fixed in the surface layer. Then the ultrasonically glass-implanted substrate was dried in an oven at 37 °C for 1 h, cut into chips 10 mm × 10 mm × 2 mm in size and gently rinsed again with ethanol.

2.2. *In vitro* bioactivity

A simulated body fluid (the Kokubo solution, SBF) that contained inorganic components similar in concentration to the human blood plasma [13, 14] was prepared after Ohtsuki *et al.* [15] by dissolving the reagents in 0.8 dm^3 distilled water in sequence: NaCl (7.996 g), $NaHCO_3$ (0.350 g), KCl (0.224 g), $K_2HPO_4 \cdot 3H_2O$ (0.228 g), $MgCl_2 \cdot 6H_2O$ (0.305 g), $CaCl_2$ (0.228 g), and Na_2SO_4 (0.278 g). The pH of SBF was adjusted to 7.25 by addition of 50 mmol (6.057 g) *tris*(hydroxymethyl)aminomethane and by subsequent addition of 45 mM (10^{-3} mol dm^{-3}) HCl solutions. The fluid contained no living cells or organic components that should be present in the blood plasma. It is already confirmed [5–7, 15] that in SBF the reactions that would take place *in vivo* near the implant surface are well reproduced *in vitro*, and bone-like apatite is deposited similar to that which forms *in vivo* on bioactive materials.

The glass-implanted PMMA chips were soaked in SBF at 36.5 °C up to 7 d before they were rinsed with distilled water and dried in an air bath at 40 °C. The surface of the chips was then examined by Fourier transform–infrared (FT–IR) reflection spectroscopy, thin-film X-ray diffractometry (CuK_α) and scanning electron microscopy. The IR reflection spectra (75° off-normal) were recorded with a JASCO FT/IR-300 spectrometer with 100 scans at 4 cm^{-1} resolution, using a Spectra Tech attachment Model 501. Thin-film X-ray diffraction spectra were taken with a Rigaku RINT 1400 diffractometer operated under 40 kV–200 mA acceleration and at an angle of incidence of 1°. A scanning electron microscope (SEM), Jeol JSM-6300, equipped with an energy dispersive X-ray analyser (Phillips, EDX-4) was also used for X-ray analysis, as well as for surface and cross-section observation with carbon and gold film coating, respectively.

3. Results

3.1. Changes in surface microstructure of the glass-implanted PMMA in SBF

Fig. 1a and b show, respectively, the X-ray diffraction patterns and FT–IR spectra of the glass-implanted PMMA substrates after soaking in SBF for up to 7 d. The diffraction peaks were found at 25.9° and 31.8° in 2θ for the samples soaked in SBF longer than 12 h: they were assigned to the (0 0 2) and (2 1 1) diffraction peaks of apatite, respectively, according to JCPDS file 9-432 [16]. It has thus been confirmed that the glass-implanted PMMA exhibits bioactivity in a soaking period as short as the deposition time of apatite by the bulk glass of a composition in SBF. The IR peaks in Fig. 1b have been assigned as indicated by Berger and Giehler [17] and Simon and McMahon [18]. It is noted that just 1 h soaking was sufficient for optical Si–O stretching modes (▼) [18, 19] to appear at 1100 and 1250 cm^{-1} showing that a hydrated silica gel layer was produced on the glass granules. The peaks assigned to P–O deformation and stretching (○) at 650 and 1120 cm^{-1} [20], respectively, appeared within 6 h and they grew with the soaking period. Thus the apatite layer was deposited on these glass-granules between 6 and 12 h soaking, and continued to grow over the PMMA surface, as shown by Fig. 2.

Fig. 2a shows a glass granule of $\sim 10\,\mu m$ in size ultrasonically implanted in the PMMA surface layer, while Fig. 2b–g represent the surface morphology of the glass-granules after the soaking in SBF for 2 h to 7 d. With comparing Fig 2a and b, the surface roughness, appearing within 2 h soaking, should be noted. This suggests the formation of the silica gel film indicated by the FT–IR spectra in Fig. 1b. With soaking periods longer than 3 h, small flake-like particles, $< 0.4\,\mu m$ in size, appeared on the granule surface exposed to SBF and they covered the whole granule surface after 4 h soaking (Fig. 2c). Fig. 2d indicates that small agglomerated particles also appeared on the substrate surface adjacent to the granules in 7 h. Fig 2c–g show that, with further soaking, the particles increased in number and grew to cover the whole substrate surface in 1 d. On the basis of the previous

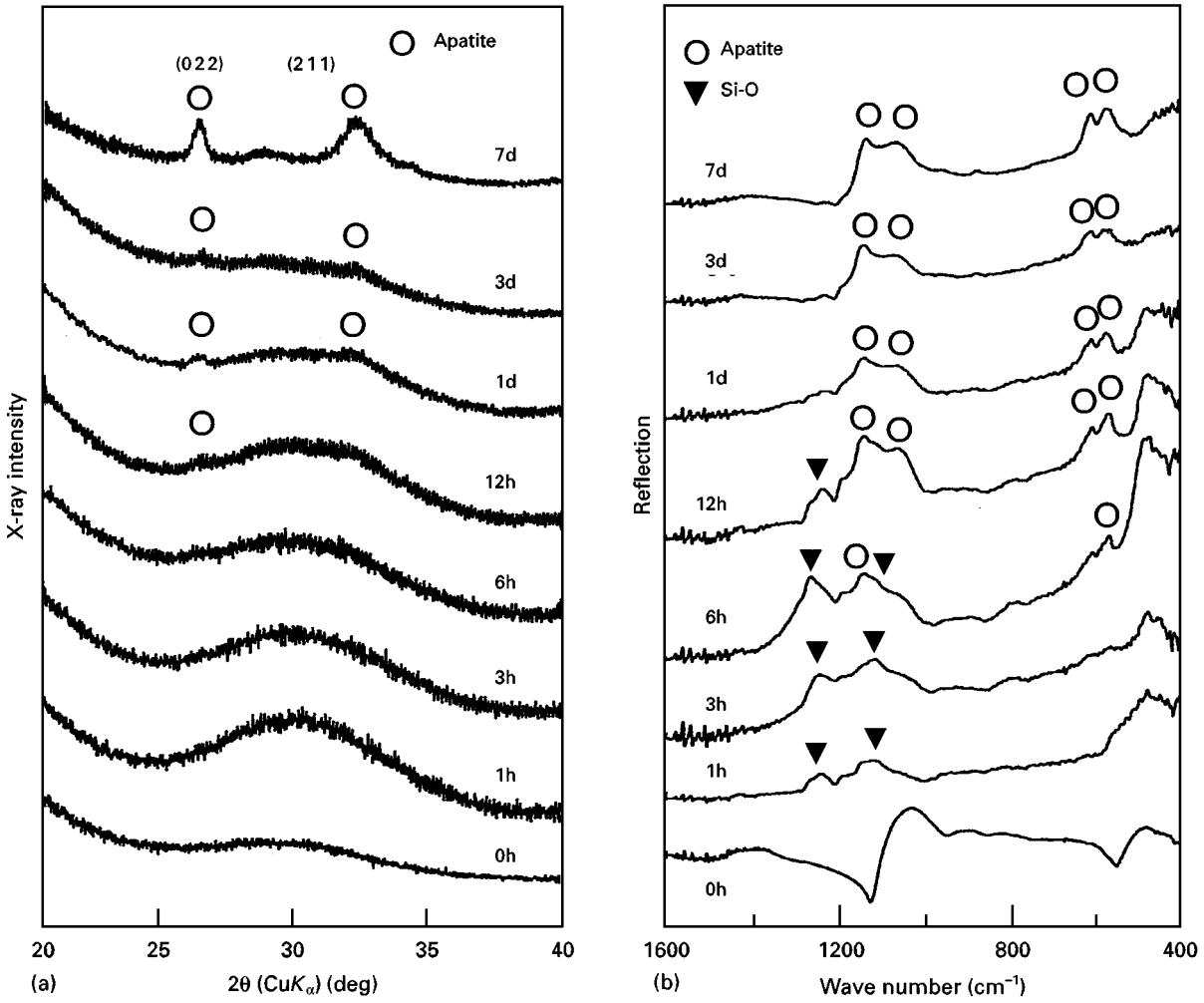


Figure 1 (a) X-ray diffraction patterns and (b) FT–IR spectra of the glass-implanted PMMA substrates after the soaking in SBF for up to 7 d. The X-ray diffraction peaks were of apatite: JCPDS 9–432. IR peaks: (▼) Si–O stretching modes, (○) P–O deformation and stretching.

X-ray diffraction and FT–IR observations in Fig. 1 the flake-like particles were apatite.

3.2. Cross section of the glass-implanted surface layers

Scanning electron micrographs of the cross section of the surface layer indicated two types of microstructure: one involved a single glass-implanted layer on the PMMA substrate, and the other was a triple-layer structure consisting of glass–PMMA–glass layers on the substrate. Fig. 3a–c show a cross-section of the former type of structure: (a) the surface layer of the glass-implanted PMMA before soaking in SBF, (b) after soaking for 1 d. Fig. 3c is a schematic representation of (b) after X-ray analysis, indicating the core of a glass granule was covered by a silica gel layer on which apatite was deposited. Here the silica gel and apatite layers were detected on the granule surface at the PMMA–glass interface side as well as on the outer surface directly exposed to SBF. Fig. 4a–c show the cross-section of the latter type structure having the layer sequence glass–PMMA–glass. Fig. 4a is for the implanted substrate before soaking in SBF, showing the PMMA substrate is in contact with an inner glass-granule layer (depth $\sim$40 μm) on which lay an intermediate layer of PMMA ($\sim$20 μm thick) covered by an outer glass-granule layer (10–15 μm thick). Fig. 4b shows the structure after soaking for 1 d while (c) schematically presents the microstructure derived from X-ray analysis. Note that the X-ray analysis detected calcium and phosphorus on the surface of the granules in the inner glass layer in contact with the PMMA substrate, which strongly suggested the presence of apatite on the granules hidden from SBF.

4. Discussion

The present study proved that the ultrasonic implantation of bioactive-glass granules into the PMMA substrate was effective in providing them with bioactivity. The suspended granules passed a 45 μm open sieve, whereas SEM observation indicated granules of, at most, 20 μm in diameter were implanted. The reason for this is that the ultrasonic energy of the present apparatus was so low that it could not agitate the movement of granules larger than 20 μm in size. Moreover, the ultrasonic vibration generated from the vibrator unit caused an energy concentration or produced nodes at specific positions in the cleaning bath, in addition to causing the substrate to float. It is hence considered that the triple-layer structures appear in

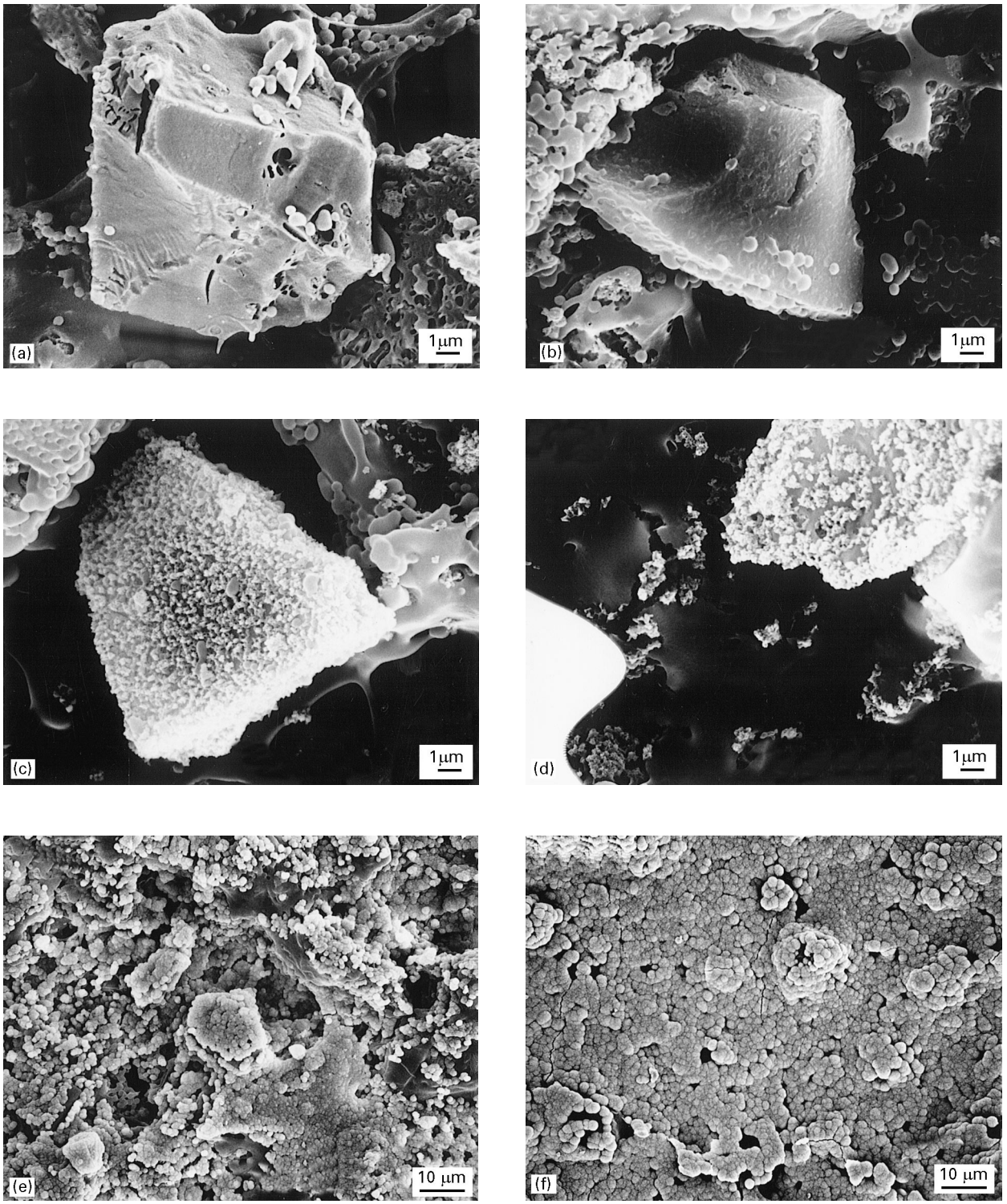
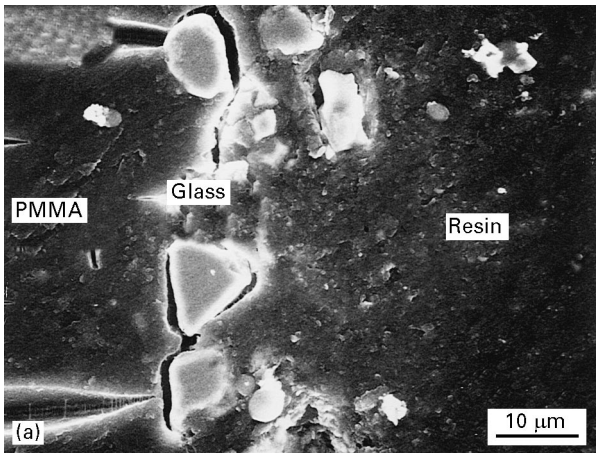
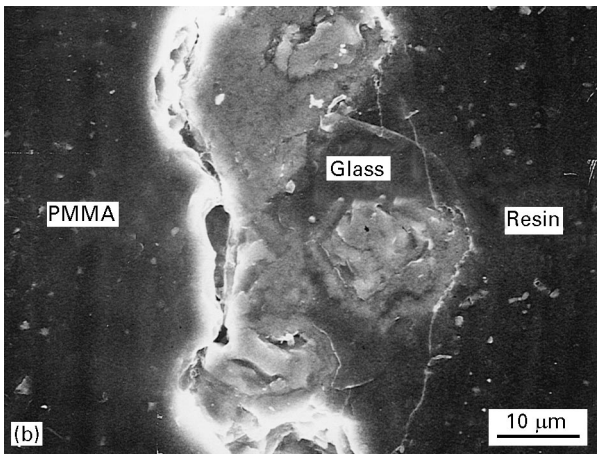


Figure 2 (a) Scanning electron micrographs of a glass granule implanted in PMMA, 0 h, and (b–g) the surface morphology of the glass granules after the soaking in SBF for (b) 2 h, (c) 4 h, (d) 7 h, (e) 12 h, (f) 1 d, (g) 7 d.

the area which receives stronger ultrasonic energy from the nodes and subsequently receives weaker energy due to the movement of the substrate.

In Fig. 2d, apatite particles were detected on the PMMA substrate surface, which could not ordinarily induce apatite crystallization. They were located near the glass granules but definitely apart from the apatite particles that appeared on the glass-granule surface. Thus their nuclei were formed independently of those on the granules nearby and grew by consuming Ca(II)





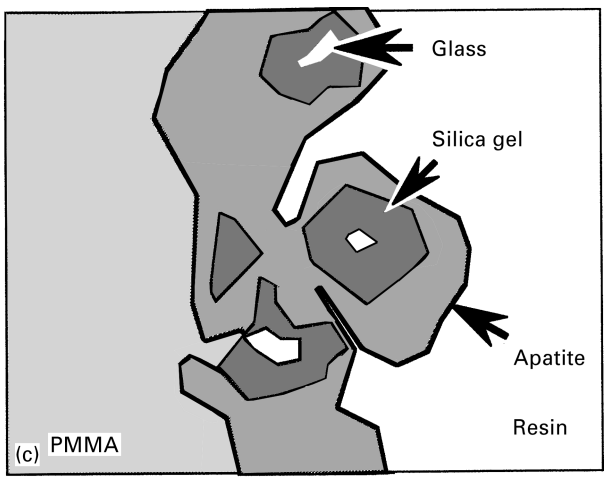
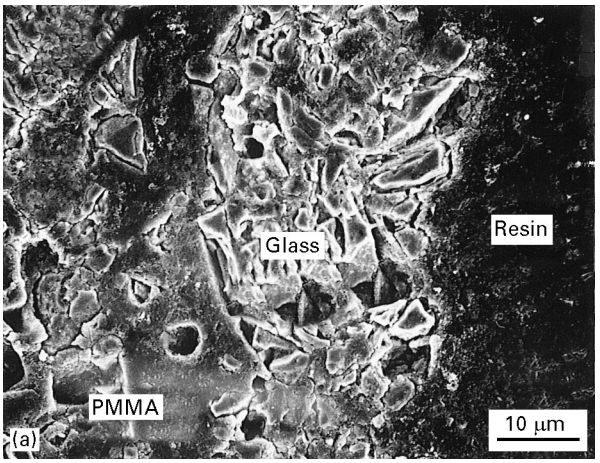
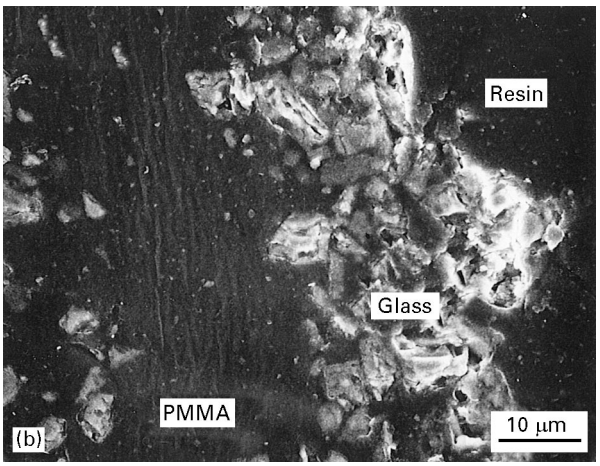


Figure 3 The surface structure (cross-section) comprising a single glass-implanted layer. (a) The surface layer of the glass-implanted PMMA before soaking in SBF, 0 h, (b) after soaking for 1 d, and (c) a schematic representation of (b) after X-ray analysis.





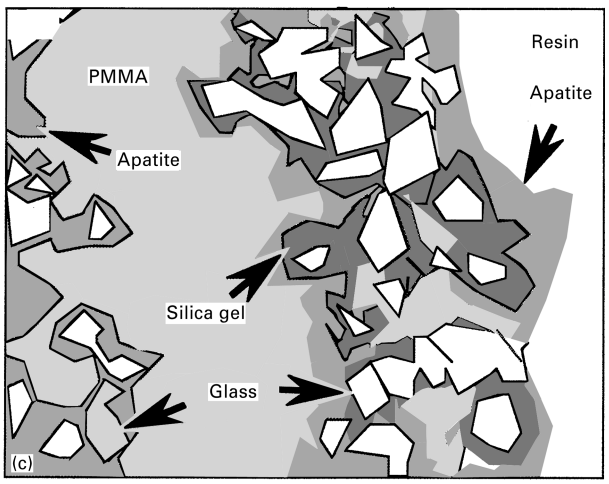


Figure 4 The surface structure (cross-section) comprising the PMMA substrate and the glass layers, in the sequence of the substrate, a glass-granule layer (depth $\sim 40\,\mu m$), an intermediate layer of PMMA ($\sim 20\,\mu m$ thick), and an outer glass granule layer (10–15 μm thick). (a) Before soaking in SBF, 0 h, (b) after soaking for 1 d, and (c) a schematic representation of (b) after X-ray analysis.

and P(V) in SBF. The CS glass reacted with SBF and could release Si(IV) and Ca(II), as illustrated in Fig. 5. If those silicate anions were deposited on the PMMA surface to develop silica gel film islands, they then could induce the nucleation of the apatite as reported by Tanahashi *et al.* [10]. Once the nuclei were formed, they readily grew to apatite particles and to cover the whole surface of the substrate, because SBF was saturated with the component ions of apatite.

When the glass granules were exposed to SBF, surface reactions giving the silica gel layer not only took place at the exposed surfaces but they also proceeded to the area hidden from SBF in contact with PMMA, as indicated from Fig. 3. Thus, the fact that the entire surface of all the granules was covered by a silica gel layer in SBF, as experienced on the surface of

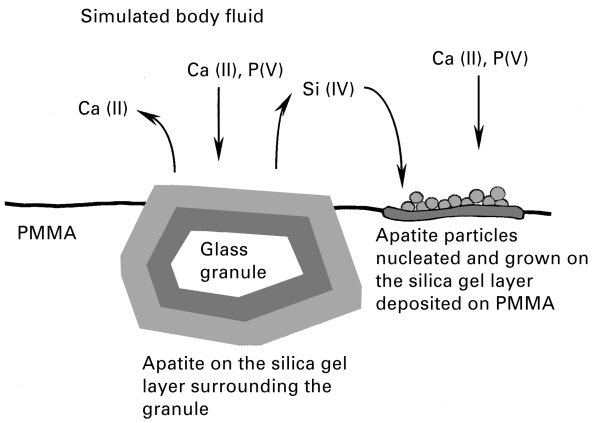


Figure 5 Reactions on the glass-granule surface are schematically presented. Note apatite is deposited on a silica gel island developed on PMMA due to a mechanism similar to that reported by Tanhashi *et al.* [10].

bulk glass, indicates that the silica gel would not disturb the diffusion of the ionic species in SBF, hence apatite was deposited and detected at the glass–PMMA interface.

5. Conclusion

Glass granules of composition $50CaO{\cdot}50SiO_2$ (mol%) of $< 45\,\mu m$ were suspended in 40 THF–60 ethanol (vol%) solution that could soften the poly(methyl methacrylate) (PMMA). Ultrasonic energy was irradiated in the suspension for 20 min so that granules of $\sim 10\,\mu m$ in size were implanted to a depth of ~ 40–$20\,\mu m$ below the substrate surface. Bioactivity was examined for the ultrasonically implanted PMMA substrates by detecting apatite deposition due to thin film X-ray diffraction (CuK_α), scanning electron microscopy, and FT–IR spectroscopy after they were soaked in a simulated body fluid proposed by Kokubo and his co-workers [5–7]. The PMMA substrates showed two types of surface structure: one involved a glass-granule layer, and the other consisted of an inner glass layer, a PMMA layer, and an outer glass layer. Apatite deposited on all the granules, irrespective of the layers involved, and over the whole surface of the granules, indicating that ultrasonic implantation was effective for providing PMMA with bioactivity. Agglomerates of apatite particles were found on the substrate surface independently of the apatite covering the glass granules. The silicate anions released from the glass were considered to develop islands of silica gel and to induce the nucleation of apatite.

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