Effects of Solution on Apatite Formation on Substrate in Biomimetic Process

Yoshio ABE, Masakazu KAWASHITA, Tadashi KOKUBO and Takashi NAKAMURA*

1. Introduction

It was previously shown that a substrate which is faced to a CaO, SiO₂-based glass taking a distance shorter than 0.5 mm and soaked in a simulated body fluid forms a dense and uniform layer of the bone-like apatite on its surface. Its modified method which is more convenient for practical uses has been developed and successfully applied to various materials in various complicated forms. In this process, it is speculated that a silicate ion released from the glass is adsorbed on the surface of the substrate to induce the apatite nucleation, and the calcium ions released from the glass accelerate the apatite nucleation by increasing the degree of the supersaturation with respect to the apatite in the surrounding fluid. Once the apatite nuclei are formed, they spontaneously grow in situ by consuming the apatite in the surrounding fluid. The bone-like carbonate apatite was formed even in fluids lacking in calcium or phosphate ions, since those ions and carbonate ions are supplied from the glass and the surrounding fluids, respectively. An apatite was formed in fluids with pH 6.53 to 7.48 but not in those with pH less than 5.00, because the apatite is stable only in neutral to basic environments. Apatite formed in fluids at 30 to 60°C but not below 20°C, since only a small amount of silicate ions is released from the glass at lower temperatures.

2. Experimental

2.1 Preparation of glass

A dense sintered alumina (0.1MgO-99.9mass%Al₂O₃) which was cut into a rectangular piece of 10×15×2 mm³ and their surfaces were polished with 3–4 μm diamond paste.

2.2 Preparation of aqueous solutions

Aqueous solutions with ion concentrations given in Table 1 were prepared by dissolving reagents of NaCl, NaHCO₃, KHCO₃, KCl, Na₃HPO₄, KH₂PO₄, H₂O, MgCl₂, 6H₂O, CaCl₂, Na₂SO₄ into ion-exchanged and distilled water and then buffered to pH 7.25 at 36.5°C with 50 mM tris (hydroxymethyl)aminomethane and about 45 mM hydrochloric acid (HCl). The solution termed SBF is the simulated body fluid with ion concentrations nearly equal to those of the human blood plasma. Solutions which lack in one kind of ions in SBF were also prepared. Solutions with the same ion concentrations as SBF but with different pHs ranging from 4 to 7.5 were also prepared by varying the kind and amount of buffering agents. In the acidic region, 50 mM potassium biphthalate (C₆H₄(COOK)(COOH)) and sodium hydroxide (NaOH) were used and in the basic region 50 mM tris (hydroxymethyl)aminomethane ((CH₃OH)₂CNH₂) and hydrochloric acid were used.

2.3 Soaking in aqueous solutions

A dense sintered alumina (0.1MgO-99.9mass%Al₂O₃) which was cut into a rectangular piece of 10×15×2 mm³ and its surface was polished with a 3–4 μm diamond paste was used as the substrate. The substrate was faced to glass G plate taking a distance of 0.2 mm by an alumina spacer, and bound together with a nylon string as shown in Fig. 1. They were soaked in the aqueous solutions of 30 ml in a polystyrene bottle for 7 d. The temperature was maintained at 36.5°C in most cases, but varied in the range 5 to 70°C in some cases. After the soaking, they were taken out from the solutions and dried at room temperature.

2.4 Analysis of surface of substrate

The surface of the substrate having been faced to glass G was analyzed by thin-film X-ray diffraction (XRD). Fourier transform infrared (FT-IR) reflection spectroscopy and scanning electron microscopic (SEM) observation. The incident beam of X-ray was fixed at 1° against the surface of...
3. Results and discussion

3.1 Effect of ion concentrations

Figures 2 and 3 show thin-film XRD patterns and FT-IR reflection spectra of the surfaces of alumina substrates soaked in various solutions for 7 d being faced to glass G. It can be seen from Figs. 2 and 3 that an apatite is formed on the alumina substrate in all the solutions. It should be noted that the apatite is formed even in the solution lack in the calcium or phosphate ion both of which are components of the apatite. This is because these ions are supplied into the solutions from the glass, as previously shown. All the solutions give broad X-ray diffraction peaks of the apatite. This indicates that the apatite formed is one with defective structure and/or small crystallites, similar to the bone apatite. All the
solutions except for that lack in carbonate ion give FT-IR reflection peak ascribed to the carbonate ion around 1400 cm\(^{-1}\). This indicates that the apatite formed in these solutions is one containing the carbonate ion, similar to the bone apatite. Only the solution lack in the carbonate ion gives the apatite lack in the carbonate ion.

3.2 Effects of pH

Figures 4 and 5 show thin-film XRD patterns and FT-IR reflection spectra of the surfaces of alumina substrates soaked in the solutions with different pHs, being faced to glass G. It can be seen from Figs. 4 and 5 that the bone-like apatite containing the carbonate ion is formed on the alumina substrate in the solutions with pHs 6.53 to 7.48, but not in those with pHs lower than 5.00. This is because the apatite is stable only in from neutral to basic environment.

3.3 Effects of temperature

Figure 6 shows thin-film XRD patterns of the surfaces of alumina substrates soaked in SBF at various temperatures, being faced to glass G. It can be seen from Fig. 6 that the bone-like apatite is formed on the alumina substrate in SBF at 30 to 50°C, but not in below 20°C and above 70°C. Below 20°C, no phase was formed on the substrate. This might be attributed to small amount of the released silicate ion, which plays the essential role in apatite nucleation on the substrate, from the glass at lower temperature. Above 70°C, an unidentified crystalline phase is formed on the substrate. This is because the apatite is not most stable phase above 70°C.

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References


