Effect of flowing speed on bone-like apatite formation in porous calcium phosphate in dynamic RSBF

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Simulation *in vitro* is a usual kind of method of evaluating biological performance of implanted materials. Simulation *in vitro* contains ordinarily two modes: static and dynamic simulation. Considering flowing of body fluid, dynamic simulation is a new kind of method, which is a closer simulation to biological environment than static simulation [1, 2]. In osteoinductivity study of materials, porous ceramics were usually implanted in the muscle of animals to eliminate the effect of bone tissue on osteogenesis in materials [3]. The ceramics implanted in animals were wrapped in the muscle or other connective tissue and body fluid flowed through inner interconnected pores of the materials at a certain flowing rate. But in previous dynamic *in vitro* study [2, 4], materials were put in a sample chamber and simulated body fluid (SBF) flowed through the chamber at a physiological flow rate of 2 ml/100 ml min. The SBF flowed basically from the surface of porous ceramics and hardly flowed through inner holes of porous ceramics. The flowing speed of body fluid in inner pores of ceramics implanted in the muscle may be faster than that of ceramics immersed in the sample chamber in dynamic *in vitro* study. Does the difference in flowing speeds between *in vivo* study and dynamic *in vitro* study affect the bone-like apatite formation and osteogenesis in inner pores of ceramics? In this study, the effects of flowing speed of the SBF in inner pores of ceramics on the formation of apatite in the pores were investigated. A modified dynamic system (Fig. 1) was used in this research. In this system, the ceramics, instead of being immersed in the sample chamber, was inserted into the elastic plastic tube in which the SBF flowed through and all of the SBF pumped into the tube must flow through the ceramics. The flow speed of the SBF in inner pores of porous ceramics could be changed easily by changing the flow rate of the RSBF pumped into the plastic tube.

Porous biphasic calcium phosphate ceramics (HA/TCP = 60/40) were synthesized by wet method and sintered at 1200 °C.The samples are cylinders of $\phi 4 \times 8$ mm.The revised SBF (RSBF) [5] used in this research was maintained at a temperature of 37 °C by water bath and at the pH value of 7.4 by filling bubbling of gaseous CO_2 into the RSBF storage tank at a suitable rate. The experiments were performed in the RSBF solution at flowing rates of 2, 5, and 8 ml/min for 2 days. After that, porous ceramics samples were washed with deionized water and dried at 50 °C in the oven. Samples were split into two halves from cylinder diameter line. The microstructures of walls of inner holes in ceramics and surface shallow holes were observed by scanning electron microscopy (SEM) with energy dispersive spectroscopy (EDS), the composition of deposits on the walls was analyzed by Fourier transform infrared (FTIR) spectroscopy.

After immersing 2 days at different flowing rate, the SEM results showed that some crystals were overlaid on the walls of inner pores. The crystal shape changed with the flowing rate of the RSBF. When flowing rates of the RSBF were 2 ml/min, some stamen-like crystals formed on the inner walls of the ceramics (Fig. 2A). In the ceramics in RSBF with the flow rate of 5 ml/min, besides more stamen-like crystals, a thin layer of fine deposits was overlaid on the walls of inner holes and the surface of the stamen-like crystals (Fig. 2B). But when the flow rate was 8 ml/min, only a thick layer of fine deposits on the walls of inner pores could be found (Fig. 2C). By the appearance of the deposit layer, it was a reasonable deduction that the thick layer of deposit was formed by fine particles deposited on the surface of the stamen-like crystals and the pore walls. The deposit layer was so thick that it covered all the inner pore walls and the stamen-like crystal formed in the early stage of deposition. The small sizes (only 100–400 μm in diameter) of pores in the ceramics led to a high ratio of surface area/volume of RSBF in pores. The high surface-to-volume of RSBF resulted in fast dissolution of calcium phosphate. When the RSBF flowed through the pores in porous calcium phosphate ceramics, diffluent tricalcium phosphate (TCP)in the biphasic ceramics dissolved in RSBF and the RSBF was supersaturated with these ions. In the range of flow rates of this study, higher flow rate is propitious to the

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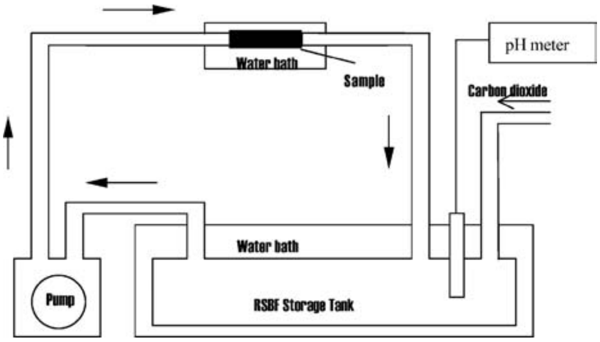

Figure 1 Schematic illustration of dynamic immersion device.

deposition because abundant ions of PO_4^{3-} and Ca^{2+} were brought to the walls of pores. In the experimental condition of low flow rate (2 ml/min), the deposition only began from some sites with a high ion concentration and grew into stamen-like crystals. When the flow rate was high enough (≥5ml/min), the ion concentration of RSBF in pores became more uniform. Nucleation took place on all the surfaces of crystals and the walls of pores and formed a layer of fine particles. Furthermore, the rapid flow of RSBF in pores was unfavorable for the growth of deposited nuclei on the walls. The nuclei could only be developed into fine particles rather than owing into stamen-like crystals.

Because the surfaces of the ceramics were tightly covered by a plastic tube, the flow speed gradually decreased from the central axis of the cylinder sample to the wall of the tube. The flowing speed of RSBF at the surfaces must be very slow. This situation was similar to static immersion mode. The deposits formed on the surface of shallow holes consisted of petal-like crystals (Fig. 3), which are similar to the crystals formed in the static immersion experiment [6, 7].

When the flow rate was 2 and 5 ml/min, petal-like crystals forming in shallow holes of HA/TCP were small (Fig. 3A and B). The petal-like crystals became bigger, denser, and intersected, as the flow speed was increased to 8 ml/min. The network formed by these crystals nearly covered all the shallow holes (Fig. 3C). In the experimental condition of low flow rate (2.5 ml/min), the RSBF flowed very slowly in the shallow pores of the surface. Therefore, the distribution of the ion concentration of the RSBF in pores became uneven. Nearly all the deposits in the shallow pores formed around the margin of HA/TCP crystals, where the ion concentration was higher than other sites. With the increase of flow speed of the RSBF (≥8 ml/min), although the flow speed at the surface of the ceramics sample was still low, the fresh RSBF still brought enough PO_4^{3-} and Ca^{2+} ions to the shallow hole. So, the crystals grew bigger and intersected.

Fig. 4 shows EDS results of the deposits on the inner walls of HA/TCP ceramics after immersion in the dynamic RSBF at the flow rate of 2 ml/min for 2 days. The results showed that the precipitates consist of calcium, phosphor, oxygen, and carbon elements (Fig. 4). The IR spectra suggest that characteristic peaks [7] of CO_3^{2-} (1550, 1500, and 1415 cm^{-1}), PO_4^{3-} (1087, 1046, 962, 631, 602, 574, and 472 cm^{-1}), and OH^- (3572 cm^{-1}) appeared on the surface of HA/TCP ceramics (Fig. 5). PO_4^{3-} and OH^- came from two approaches: one part came from HA/TCP ceramics, the other part came from

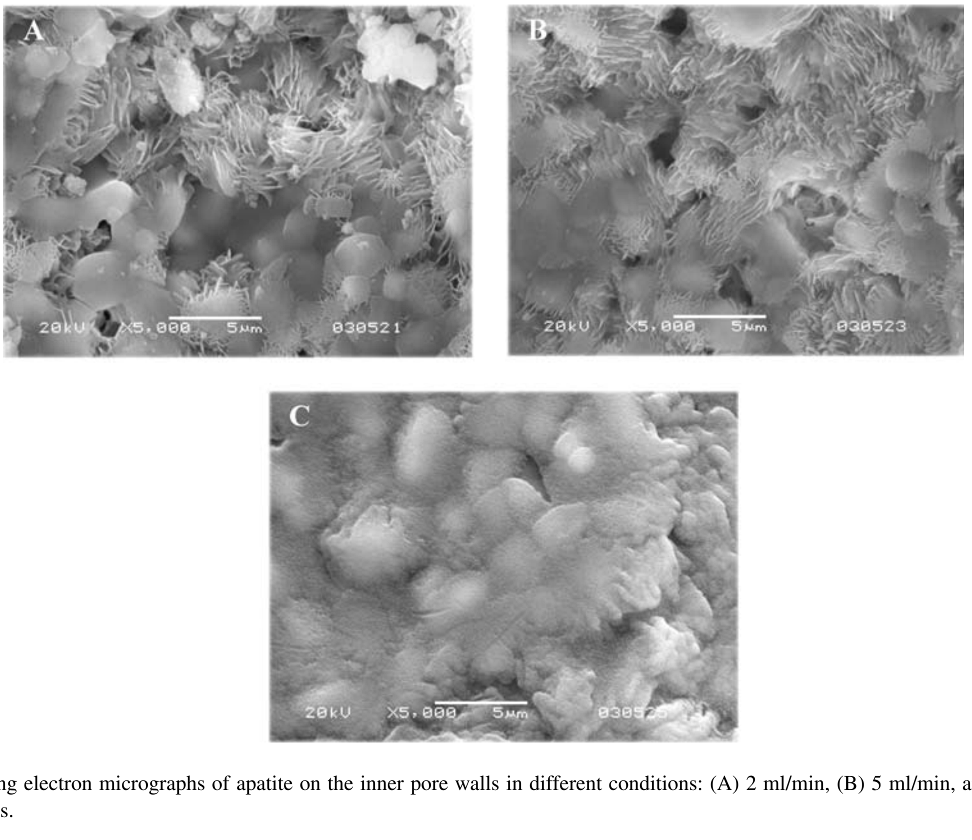

Figure 2 Scanning electron micrographs of apatite on the inner pore walls in different conditions: (A) 2 ml/min, (B) 5 ml/min, and (C) 8 ml/min flowing for 2 days.

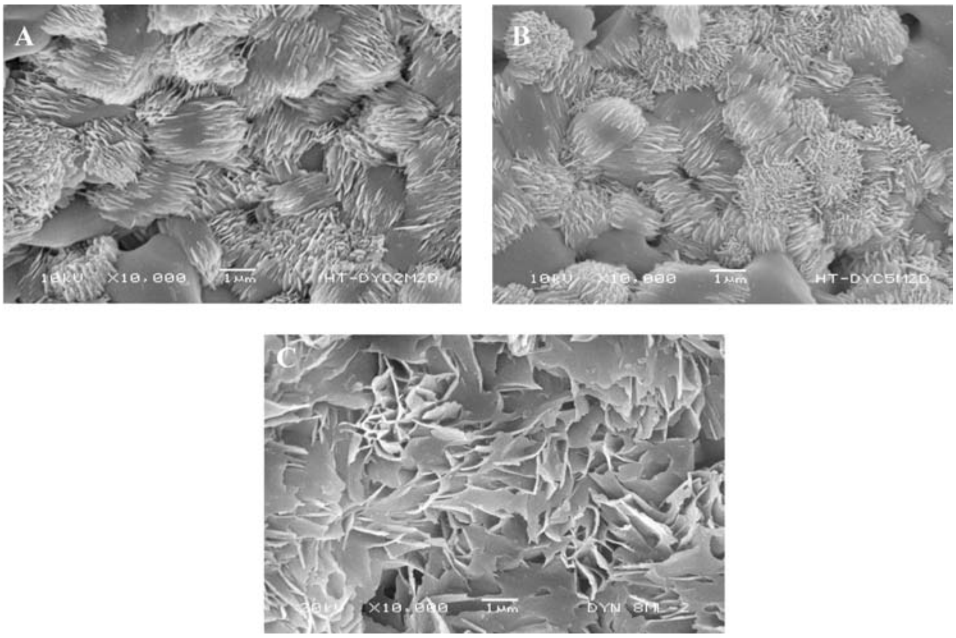

Figure 3 Scanning electron micrographs of the apatite on the wall of surface swallow holes in different conditions: (A): 2 ml/min, (B): 5 ml/min, and (C): 8 ml/min flowing for 2 days.

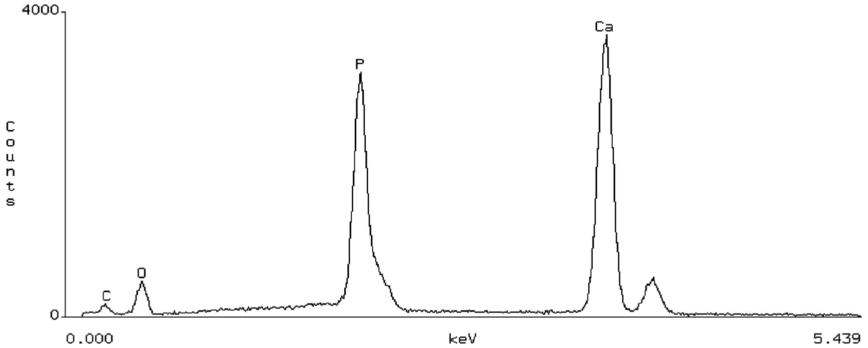

Figure 4 Energy dispersive X-ray chart of deposit on inner hole walls of HA/TCP ceramics at flow rate of 2 ml/min for 2 days.

the formed deposits. The CO_3^{2-}came only from the formed deposits. Therefore, the formed deposits on the hole walls of HA/TCP were carbonated hydroxyapatites (CHA).

Experimental results revealed the precipitates obtained in this study are carbonate hydroxyapatite (CHA); flowing speed of RSBF would affect the formation of apatite.

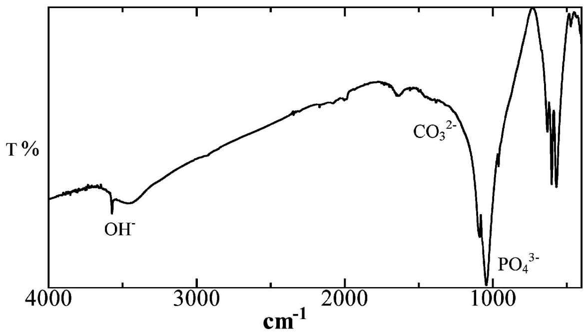

Figure 5 FT-IR spectra of precipitates in HA/TCP ceramics at flow rate of 2 ml/min for 2 days.

In Conclusions: (1) The higher flowing speed of the RSBF in the flow speed ranges of this research facilitated the apatite formation in the pores of calcium phosphate ceramics, because the faster flow brought more abundant CO_3^{2-} and PO_4^{3-} ions for nucleation and growth of the apatite. This phenomenon is different from what happened at the surface of the sample immersed in the SBF of the sample chamber, where the SBF flowing at the higher speed, which brought more CO_3^{2-} and PO_4^{3-} ions dissolved from the surface further away, was disadvantageous to the apatite formation on the surface. (2) The apatite formed on the wall of pores in calcium phosphate ceramics was carbonated hydroxyapatites (CHA).

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