Research Article

Bioactivity and Osteoconductivity of Biphasic Calcium Phosphates

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Abstract Bioactivity and osteoconductivity of calcium phosphates as a function of β-tricalcium phosphate contents, that is, biodegradability, were investigated. Three calcium phosphates, pure hydroxyapatite, biphasic calcium phosphates with different mixing ratios between hydroxyapatite, and β-tricalcium phosphate (60HAp:40β-TCP and 40HAp:60β-TCP in wt.%), were synthesized through the precipitation method using calcium hydroxide and phosphoric acid as starting reactants. After drying, they were sintered at $1100\,°C$ for 3 hours, and then bioactivity and osteoconductivity tests were carried out in simulated body fluid (SBF) and calvarial defect of New Zealand white rabbits, respectively. SBF exposure resulted in the deposition of a layer of carbonate apatite crystals on the surfaces of bicalcium phosphates but not on the hydroxyapatite. New bone forming capacity of the biphasic calcium phosphate with 60HAp:40β-TCP granules was the best, whereas that of the pure HAp was the worst. To this end, it can be concluded that the calcium phosphate with moderate biodegradability is good for producing good osteoconductivity.

Keywords biphasic calcium phosphates; hydroxyapatite; β-tricalcium phosphate; osteoconductivity

1 Introduction

HAp has been widely used as a bone grafting material because it is considered as one of the constituent elements of bone. In fact, however, it is untrue because the apatite found in bone is not HAp but low crystalline hydroxyl carbonate apatite (CO_3Ap) [3]. The apatite exists in bone is constructed at body temperature so its crystallinity is low, grain size is only around tens of nanometers and it contains carbonate ions in its structure, which makes it possible to be involved in the bone remodeling cycle. However, the synthesized apatite is generally made at above $1000\,°C$. Thus, its crystallinity is high, grain size comes to be submicrometers and does not contain carbonate ions in its structure, which makes it difficult to dissolve *in vivo*. As a result, HAp does not show good osteoconductivity compared to CO_3Ap. Despite of many advantages as a bone grafting material, the bulk body of CO_3Ap can hardly be made because carbonate ions escape from the apatite as a carbon dioxide gas during the high temperature sintering process. On the contrary, when decrease the sintering temperature to prevent escaping the carbonate ions from the apatite, it makes decrease in the handling strength of the apatite. Thus, many investigations have been already made to improve the osteoconductivity of the synthetic HAp. One of them is making biphasic calcium phosphates by mixing HAp with resorbable calcium phosphates, such as α- or β-TCPs [1]. The biphasic calcium phosphates have been known to show better osteoconductivity than that of HAp but the reason is still unclear.

In this study, we synthesized the biphasic calcium phosphates composed of HAp and β-TCP in different proportions and evaluated their bioactivity and osteoconductivity to examine the origin of their better osteoconductivity than pure HAp.

2 Materials and methods

HAp and β-TCP powders were synthesized, respectively, by the reaction with calcium hydroxide and phosphoric acid as starting reagents for preparing the biphasic calcium phosphates. Three different mixing ratios between HAp and β-TCP powders were adopted for making different resorbable calcium phosphates. 100HAp:0β-TCP, 60HAp:40β-TCP, and 40HAp:60β-TCP (in wt.%) were prepared by mixing them homogenously using a stirrer in distilled water. Following this, they were sintered at $1100\,°C$ for 3 hours, crushed, and then sieved to make the granules be in the size range between 212 to $1000\,\mu$m.

The phase analysis and measuring the relative content of HAp in the bicalcium phosphates were carried out by X-ray diffractometr (XRD; D8 Advance, Bruker). The functional groups in calcium phosphates were analyzed using Fourier transformed infrared spectroscopy (FT-IR; Spectrum One, Perkin Elmer), and microstructures were observed by filed emission scanning electron microscopy (FE-SEM; S-4700, Hitachi). The bioactivities of the three different specimens were evaluated by their apatite forming abilities in the SBF [2]. SBF was prepared by dissolving reagent grade NaCl, NaHCO$_3$, KCl, K$_2$HPO$_4$ · 3H$_2$O, MgCl$_2$ · 6H$_2$O, CaCl$_2$, and Na$_2$SO$_4$ in ion-exchanged distilled water. The solution was buffered at pH 7.25 with tris(hydroxymethyl) aminomethane ((CH$_2$OH)$_3$CNH$_2$) and 1 M hydrochloric acid (HCl) at 36.5 °C. New Zealand white male rabbits weighing $2 \sim 2.5$ kg (eight animals per test group) were used to assess *in vivo* bone forming capacity. Animals were sacrificed at 8 weeks after implantation. Microscopic examination was conducted using an optical microscope (Eclipse 80i, Nikon) after Multiple Stain (Polysciences).

3 Results and discussion

Figure 1 shows the XRD patterns of the three different calcium phosphates after sintering at 1100 °C for 3 hours. The relative amounts of HAp and β-TCP phases measured based on XRD data were in well accordance with the initially designed composition and they showed high crystallinity. FT-IR spectra of the three specimens after sintering at 1100 °C for 3 hours showed that they did not have carbonate ions at all in their structures, and hydroxyl groups were distinctively observed in all the specimens.

Bioactivity test conducted in the SBF for 1 week showed that flake-like tiny carbonate apatite crystals (confirmed by FE-SEM and FT-IR) were observed to form evenly on the two bicalcium phosphates but not on the pure HAp.

Figure 2 shows the histological sections of New Zealand white rabbit calvarial defects with three calcium phosphates at 8 weeks after surgery. The highest bone forming capacity was observed in 60HAp/40TCP granules, while the lowest one was observed in pure HAp granules. Intervening fibrous tissues can hardly be found on the surfaces of 60HAp/40TCP granules, while they were found a little on the surfaces of 40HAP/60TCP granules. In contrast, HAp granules were mostly covered by fibrous tissues. However, the fastest resorbable rate was observed in the 40HAp/60TCP granules, while the slowest one was observed in pure HAp granules. The original shape could not be found in 40HAp/60TCP granules after 8 weeks of implantation.

From the results, it can be conjectured that better osteoconductivity of a biphasic calcium phosphate than pure HAp came from its carbonate apatite forming capacity.
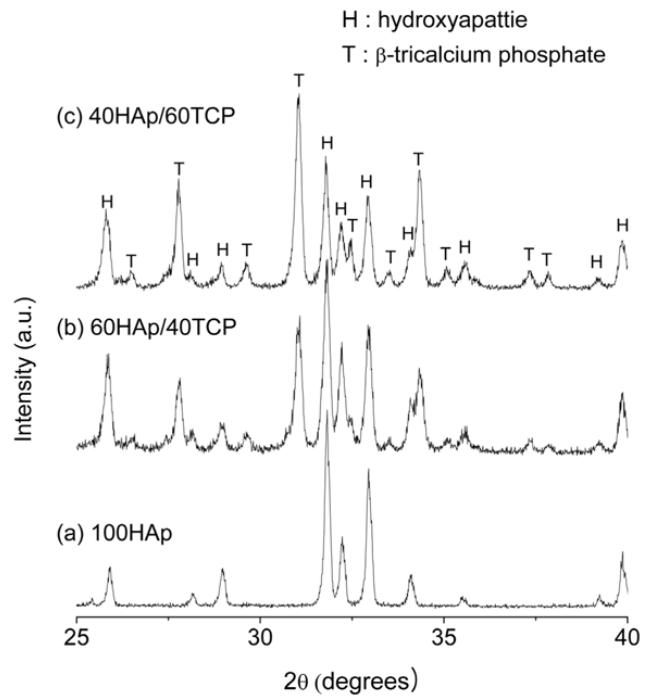

Figure 1: XRD patterns of the three calcium phosphates with different mixing ratios between HAp and β-TCP: (a) 100:0, (b) 60:40, and (c) 40:60 in wt.%.

When a biphasic calcium phosphate is implanted, the TCP phase dissolves faster than HAp phase. Then, it produces calcium and phosphate ions which increase the ionic activity product of apatite in serum, and finally, it induces the formation of low crystalline hydroxyl carbonate apatite on HAp phase, which provokes good osteoconductivity. It means that the currently used method to improve osteoconductivity of calcium phosphates by making biphasic calcium phosphates is quite appropriate because it produces low crystalline hydroxyl carbonate apatite on biphasic calcium phosphate surface.

4 Conclusions

We demonstrated that a biphasic calcium phosphate had a capacity to induce the formation of carbonate apatite in SBF which leads to good osteoconductivity compared to pure HAp itself. The calcium released from β-TCP in biphasic calcium phosphate increased the ionic activity product of apatite in SBF and induced the formation of carbonate apatite on the remained HAp surface. However, when the degradation rate of biphasic calcium phosphate was too fast due to high content of β-TCP, it rather prevented the formation of new bone. To this end, it can be concluded that the improved osteoconductivity of biphasic calcium phosphate compared to pure HAp is likely to originate from its carbonate apatite forming capacity.
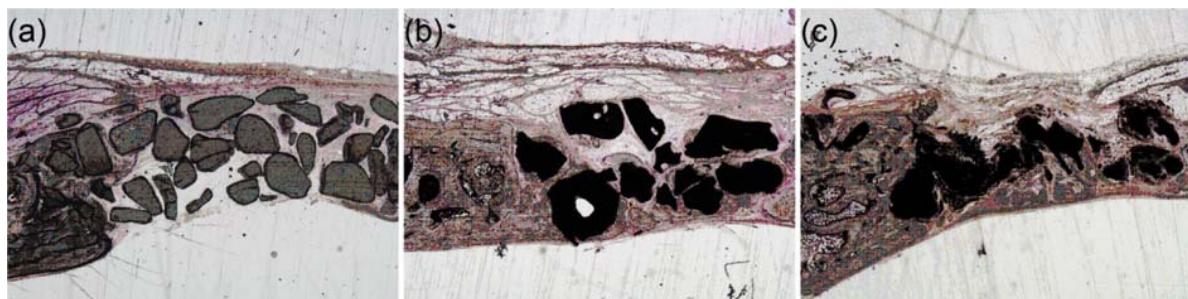
Figure 2: Histological views of the New Zealand white rabbit calvarial defects filled with (a) pure HAp, (b) 60HAp/40TCP, and (c) 40HAp/60TCP granules at 8 weeks after implantation (Multiple staining, original magnification, ×40).

References

[1] G. Daculsi, *Biphasic calcium phosphate concept applied to artificial bone, implant coating and injectable bone substitute*, Biomaterials, 19 (1998), pp. 1473–1478.

[2] T. Kokubo, H. Kushitani, S. Sakka, T. Kitusgi, and T. Yamamuro, *Solutions able to reproduce in vivo surface-structure changes in bioactive glass-ceramic A-W*, J Biomed Mater Res, 24 (1990), pp. 721–734.

[3] B. Wopenka and J. D. Pasteris, *A mineralogical perspective on the apatite in bone*, Mater Sci & Eng, C25 (2005), pp. 131–143.