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Concept of Functionally Graded Apatites and Their Application to Biomaterials

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Abstract

Originally, functionally graded materials were developed for use in engineering fields. We successfully synthesized gradational apatite containing fluoride at 80°C and pH 7.4, applying this concept. Furthermore, we designed a gradational synthesis system for applying the graded concept to the synthesis of CO_3Ap containing magnesium to improve the biological properties of materials as bone substitutes. Functionally graded Mg-containing carbonate apatite, FGMgCO_3Ap, was synthesized at 60°C and pH 7.4 using a gradient magnesium supply system. The X-ray diffraction analysis of FGMgCO_3Ap synthesized showed a poorly-crystallized apatitic pattern similar to that of human bone. ESCA analysis clearly showed the negative gradient distribution in Mg1s intensity of magnesium from the crystal surface toward the inner core. In cell adhesion assay, the optical density of FGMgCO_3Ap-collagen composite was higher than that of the CO_3Ap-collagen composite. When both composites were implanted into the periosteum cranii of rats, histological results showed that many more osteoblast-like cells existed at the interface between FGMgCO_3Ap-collagen composite and bone. Therefore, the acceleration effect of magnesium ions on the osteoblast adhesion onto the FGMgCO_3Ap-collagen composite could be demonstrated.

Key words: apatites, fluoride, carbonate, magnesium, functionally graded, osteoblast

1. INTRODUCTION

Biological apatites contain many trace elements [1], and sometimes form heterogeneously. To investigate such complicated phenomena in biological systems, we studied heterogeneous apatite formation. Since it is well known that fluoride contributes to caries prevention [2-5], including biomaterials applications [6, 7] and affects significantly on the enamel maturation, we focused on the fluoridated apatites. When a two-step fluoride system was adopted to simplify the phenomena, two different types of heterogeneous fluoridated apatites were formed [8, 9], hydroxyapatite covered with fluorapatite (H-FAp type apatite), and fluorapatite covered with hydroxyapatite (F-HAp type apatite). These two crystals showed different physicochemical properties; H-FAp type apatite was comprised of slender hexagonal crystals, while those of were typically hexagonal. H-FAp type apatite was less soluble than F-HAp.

Functionally graded (gradient) materials, FGM, have been developed for use in engineering fields [10]. These materials are utilized as adhesive agents, or for their thermal or electronic conductivity. The graded method is very useful because phenomena can be examined more smoothly. In biological systems, bamboo has a typical functionally graded structure: a negative density gradient of fibers in the thickness direction. Bone also has a functionally graded structure from the surface cortical compact toward the inner cancellous material. In human teeth, a negative gradient of fluoride concentration from the surface of tooth enamel toward the dentine-enamel junction [1] can be formed. We speculate that even each crystal composing enamel layer may have a fluoride distribution in the crystal structure. Therefore, we designed a continuous gradient fluoride supply system by developing the previous step-like fluoride supply system.

Recently, one of the main focuses of biomaterials research has been biological adhesion. It has been reported that cationic ions appear to be related to the activity of adhesion molecules such as those of the integrin family [11]. We can speculate that Mg^{2+} ions also play some role in cell adhesion. To investigate the effect of the magnesium ions contained in the apatite crystals on cell adhesion, we considered how to prepare apatite that contained magnesium. It is easy to synthesize CO_3apatite with a certain magnesium content [12]. However, this apatite was poorly crystallized and too soluble. It is also possible to synthesize a type of apatite that has a surface on which magnesium accumulates. This apatite, however, appears to have a significant gap in the crystallographic properties at the interface between the surface layer coated with magnesium and the Mg-free crystal in the inner core.

In the previous study, we synthesized a carbonate apatite, CO_3apatite, which has a crystallinity and chemical composition similar to bone. The CO_3apatite was mixed with collagen, whose antigenicity had been removed by enzymatic treatment. This mixture was formed into composite pellets. Pellets implanted beneath the periosteum cranii of rats showed good biocompatibility [13].

In a continuation of those studies, we now endeavored to synthesize functionally graded CO_3apatite containing Mg, producing a negative gradient of magnesium concentration from the surface toward the core. Then, we investigated the degree of cell adhesion to a composite that was made by mixing the FG$MgCO_3$apatite and collagen to facilitate bonding and processing. Furthermore, we examined the biocompatibility and effect of magnesium on bone formation by implanting the same FG$MgCO_3$Ap-collagen composite into the rat periosteum cranii and rabbit femur.

2. Functionally Graded Fluoridated Hydroxyapatite, FGFAp

The X-ray diffraction pattern of the precipitate synthesized heterogeneously at 80°C and pH 7.4, FGFAp, was typically apatitic, although its crystallinity was lower than those of homogeneous hydroxyapatite, HAp and fluorapatite, FAp. The expanded (300) reflection peak of FGFAp shifted to the higher direction and approached that of homogeneous FAp. The half-value breadth of the (300) reflection was much lower than those of homogeneous HAp and FAp. The (002) reflection was not markedly different from those of HAp and FAp. Calcium and phosphate contents were not significantly different from homogeneous HAp and FAp. Fluoride content of FGFAp was half of that of homogeneous FAp.

Scanning electron micrographs showed typical needle-like crystals, which were developed to the c-axis,

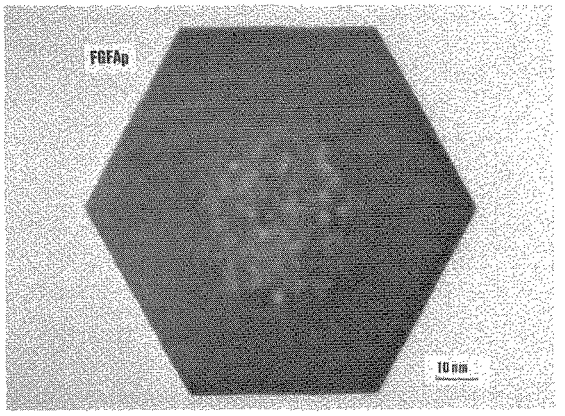

Fig. 1 High-resolution transmission electron micrograph of functionally graded fluoridated hydroxyapatite (FGFAp).

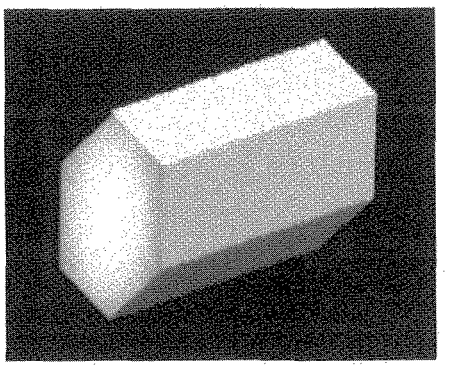

Fig. 2 Scheme of functionally graded fluoridated hydroxyapatite (FGFAp).

and were similar to those of fluorapatite. High-resolution transmission electron microscopy (HR-TEM) of sectional FGFAp crystals (Fig. 1) revealed the typical hexagonal shape similar to that of homogeneous fluorapatite. Structural damage was observed in the inner core, the boundary of which was not clear, although a clear boundary for two-layer H-FAp apatite was observed previously [9]. ESCA analysis clearly showed the negative gradient distribution in F1s intensity (atomic concentration) of fluoride from the crystal surface toward the inner core .

Using our new method of gradient fluoride supply, we synthesized of functionally graded fluoridated hydroxyapatite, FGFAp. The effects of fluoride on tooth enamel have been described in mature enamel [14, 15] and in dental fluorosis [16]. Although fluoride profiles in the enamel layers are known, a fluoride gradient in a single apatite crystal has not been demonstrated. Our findings concerning the gradient of fluoride concentration in the apatite crystal is not entirely satisfactory. Direct comparison of the fluoride gradient in each layer of a single apatite crystal was not possible, although microbeam electron diffraction or the convergent beam electron diffraction technique was adopted. However, with the combined results of HR-TEM and ESCA, we predicted the graded fluoride concentration in the apatite crystal (Fig. 2). The fluoride concentration at the surface of the crystal may be much higher than that of the inner core, and ESCA results supported this speculation. HR-TEM observation showed less imperfect lattice structure toward the outer layer (Fig. 1), probably because of the existence of crystallographically stable fluoridated apatites with higher degree of fluoridation.

3. Functionally Graded Mg-containing CO_3apatite, $FGMgCO_3Ap$

The X-ray diffraction analysis of $FGMgCO_3Ap$ synthesized at 60°C and pH 7.4 showed a poorly crystallized apatitic pattern (Fig. 3) similar to that of human bone and Mg-free CO_3Ap when compared with well-crystallized hydroxyapatite synthesized at 60°C without a carbonate source. SEM observations showed that the $FGMgCO_3Ap$ crystals were smaller and coagulated, while the hydroxyapatite crystals exhibited typically hexagonal plate-like features. ESCA analysis clearly showed a negative gradient distribution of Mg1s

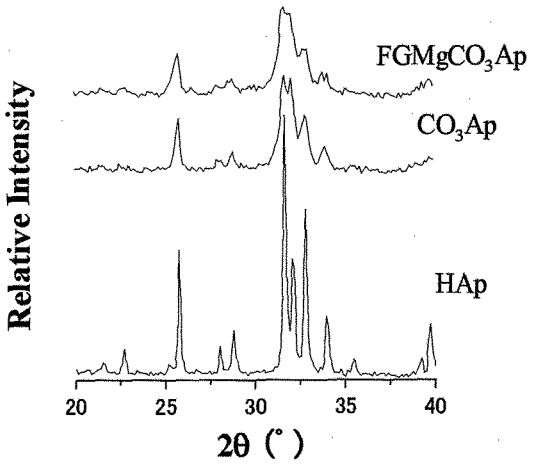

Fig. 3 X-ray diffraction pattern of $FGMgCO_3Ap$, together with HAp and CO_3Ap.

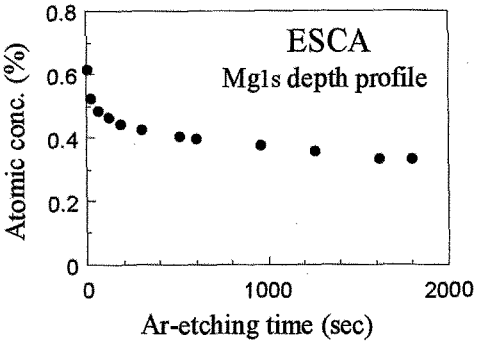

Fig. 4 Mg1s depth profile of electron spectroscopy for chemical analysis (ESCA) of $FGMgCO_3Ap$.

intensity (atomic concentration) of magnesium from the crystal surface toward the inner core (Fig. 4).

In the cell adhesion assay ($p<0.05$), after the nonadhering cells were rinsed off, the optical density of the $FGMgCO_3Ap$-collagen composite was higher than that of the CO_3Ap-collagen composite (Fig. 5). Although the difference in optical density between the composites was not great, there was a significant biological difference. SEM showed that the osteoblast-like cells adhered well to the surface of the $FGMgCO_3Ap$-collagen composite. Fig. 6 shows a cross sectional view with hematoxylin-eosin staining. After 4 wks of incubation, many more osteoblasts adhered to the $FGMgCO_3Ap$-collagen composite than to the CO_3Ap-collagen composite and the layer they formed was thicker.

When the pellet type samples of $FGMgCO_3Ap$-collagen and CO_3Ap-collagen composites were implanted into the periosteum cranii of rats, histological results showed that many more osteoblast-like cells existed at the interface between $FGMgCO_3Ap$-collagen composite and bone (Fig. 7). After 4 wks of implantation, the $FGMgCO_3Ap$-collagen composite had metabolized greatly in comparison with the CO_3Ap-collagen composite. The cranii bone thickness with the $FGMgCO_3Ap$-collagen composite seemed thicker than that with the CO_3Ap-collagen composite.

The results of the hematoxylin-eosin staining, shown in Fig. 6, supported the results of the cell adhesion assay (Fig. 5) and confirmed the existence of the cells on the composites. Thus, the present study demonstrated the effect of the magnesium ions on the acceleration of osteoblast adhesion to the $FGMgCO_3Ap$-collagen composite.

In general, osteoblasts have an important role in the reconstruction of bone defects, through their proliferation, differentiation, and mineralization. This bone formation may originate from osteoblast adhesion to the extracellular matrix. Therefore, bioadhesion is important in the substitution of biomaterials, which attach to neighboring tissues. In addition to biocompatibility, the binding of cells to biomaterials plays an important role in the rapid cure of the defective area. The favorable adhesion of the cells promotes metabolism. In this case, the magnesium ions might act as a catalyst in human metabolism or in general chemical reactions. At first, the Mg^{2+} ions induced the formation of adhesion

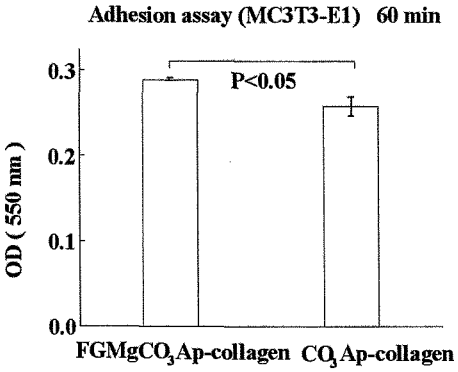

Fig.5 Osteoblast adhesion onto the $FGMgCO_3Ap$-collagen and CO_3Ap-collagen composites

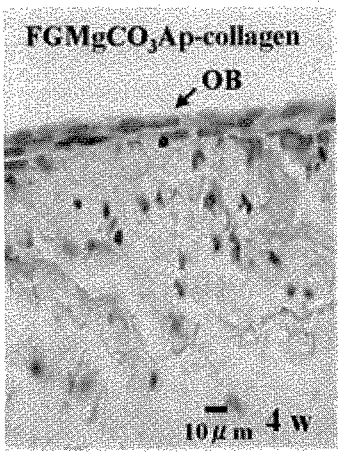

Fig. 6 Hematoxylin-eosin staining, showing osteoblasts adhered to the $FGMgCO_3Ap$- collagen composite after 4 wks incubation

molecules such as osteoblast integrins. Then, the osteoblasts actively formed bone. However, the apatites are easily dissolved and the Mg^{2+} ions finally become a source for the remodeling of the bone.

In the rabbit experiments, a clear difference of bone renewal between the $FGMgCO_3Ap$-collagen composite and control was observed, probably because bone metabolism in the femur is very active. Thus, the rate of biodegradability of the composite is also very much affected by the magnesium.

Finally, as a scaffold material, the $FGMgCO_3Ap$-collagen composite seemed to contribute to bone formation.

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References

[1] Miles AEW. Structural and Chemical Organization of Teeth, Vol.II. Academic Press, New York, 1967.

[2] Volker JF. Effect of fluorine on solubility of enamel and dentine. Proc Soc exp Biol Med 1939; 42: 725-727.

[3] Brudevold F, Steadman LT, Gardner DE, Rowley J, Little MF. Uptake of tin and fluoride by intact enamel. J Am dent Ass 1956; 53: 159-164.

[4] Moreno EC, Kresak M, Zahradnik RT. Physicochemical aspects of fluoride-apatite systems relevant to the study of dental caries. Caries Res 1977; 11(Suppl 1): 142-171.

[5] Okazaki M, Moriwaki Y, Aoba Y, Doi Y, Takahashi J. Dissolution rate behavior of fluoridated apatite pellet. J Dent Res 1981; 60: 1907-1911.

[6] Wiltshire WA, Janse van Rensburg SD. Fluoride release from four visible light-cured orthodontic adhesive resins. Am J Orthod Dentofacial Orthop 1995; 108: 278-283.

[7] van der Reijden WA, Buijis MJ, Damen JJ, Veerman EC, ten Cate JM, Nieuw Amerongen AV. Influence of polymers for use in saliva substitutes on de- and remineralization of enamel in vitro. Caries Res 1997; 31: 216-223.

[8] Okazaki M . Heterogeneous synthesis of fluoridated hydroxyapatites. Biomaterials 1992; 13: 749-754.

[9] Tohda H, Okazaki M, Yanagisawa T, Takahashi J. Transmission electron microscopic observation of heterogeneous fluoridated hydroxyapatites. Biomaterials 1995; 16: 945-50.

[10] Koizumi M. Functionally gradient materials. in Ceramic Transactions Vol.34. Holt JB, Koizumi M, Hirai T, Munir ZA, editors. American Ceramic Society, Westerville, 1993, pp. 3-10.

[11] Albert B, Bray D, Lewis J, Raff M, Roberts K, Watson JD. Molecular Biology of the Cell, 3rd Ed. New York: Garland Publishing, 1994. p. 949-1010.

[12] Okazaki M. Crystallographic behaviour of fluoridated hydroxyapatites containing Mg^{2+} and CO_3^{2-} ions. Biomaterials 1991; 12: 831-835.

[13] Okazaki M, Ohmae H, Takahashi J, Kimura H, Sakuda M. Insolubilized properties of UV-irradiated CO_3apatite-collagen composites. Biomaterials 1990; 11: 568-572.

[14] Daculsi G, Kerebel LM, Kerebel B. Effects of fluoride on human enamel and selachian enameloid in vitro: a high-resolution TEM and electron diffraction study. Calcif Tissue Int 1981; 33: 9-13.

[15] Nelson DG, Jongebloed WL, Arends J. Crystallographic structure of enamel surfaces treated with topical fluoride agents: TEM and XRD considerations. J Dent Res 1984; 63: 6-12.

[16] Huang A, Nakagaki H, Tsuboi S, Ji H, Ohno N, Chen R, Nguyen TT, Kim JB. Fluoride profiles of perikymata in enamel surfaces of human premolars. Arch Oral Biol 1998; 43: 669-677.

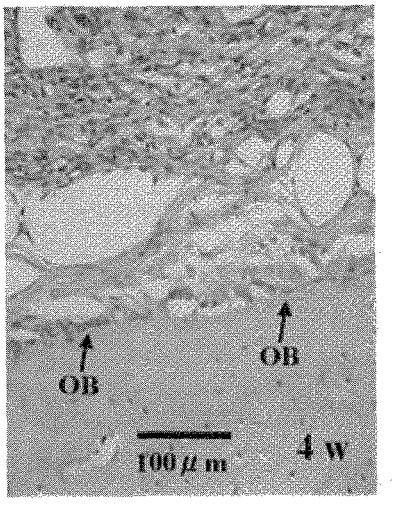

Fig. 7 Hematoxylin-eosin staining of FG$MgCO_3$Ap-collagen composite after 4 wks implantation into the periosteum cranii of rat

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