

## The crystallographic influence on the dissolution and aligned crystallites of hydroxyapatite

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The crystallographic influence of hydroxyapatite (HA) on dissolution was investigated by X-ray diffraction (XRD) and X-ray pole figure (XPF). All HA disks were sintered at 1200 °C for 2 h in air with moisture protection and were immersed in 40 ml of pH 7.4 distilled water at 37 °C. In addition to the surface dissolution at grain boundaries, grains themselves were degraded in a specific direction showing the aligned crystallites. XRD analysis showed that the peak intensity of (300) was maintained relatively during immersion compared with the rest of the peaks. XPF analysis revealed that the contour lines of (211) and (112) decreased after immersion, whereas the (300) contour line remained similar before and after immersion. This means that the (300) plane of HA could be relatively stable in a liquid environment. Therefore, it may be concluded that the direction of crystal alignment should be associated with the susceptibility of certain ceramic grains to dissolution.

**Key words:** Hydroxyapatite, Crystal alignment, Dissolution, X-ray pole figure.

### Introduction

Hydroxyapatite (HA) which has a Ca/P ratio of 1.667 with a chemical formula of  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$  has achieved the most significant attention because of its compositional similarities to natural human bone and teeth, and its excellent biocompatibility [1]. In addition, HA is expected to be stable in body fluid because it shows the lowest solubility of calcium phosphate materials in a very narrow pH range of  $>7.3$  [2]. However, numerous studies have confirmed dissolution and degradation of materials during exposure to the *in vitro* and *in vivo* environment [3-7].

Although the factors contributing to the dissolution of HA have not been completely elucidated, a number of factors concerned with physical properties, chemical compositions, and crystal structures have been known to govern the dissolution behavior of HA [8-11]. With respect to crystal structures, the solubility of HA depends on crystallographic structure [12] and degree of crystallinity [13].

de Bruijn et al. [14] reported that the presence of unidirectionally aligned crystallites contributed to the intimate deposition of a newly-formed bone matrix. An *in vitro* study by Yamada et al. [15] also demonstrated the degraded crystals in a single direction by osteoclastic resorption. Both studies referred the incidence of the aligned crystallites to the formation of a highly

acidic micro-environment. However, neither of them discussed the crystallographic dependency on dissolution in more detail.

Therefore, in this study, the crystallographic characteristics of HA to dissolution were investigated. We have made an effort to evaluate which crystal planes of HA are susceptible or stable in a liquid environment.

### Experimental

Commercially-obtained HA powder was uniaxially compacted in a 10 mm cylindrical steel mould under a pressure of 15 MPa and subsequently cold isostatic pressed (CIP) at 220 MPa. The green compacts were sintered at 1200 °C for 2 h in air with moisture protection. After sintering, the disks were polished using a series of SiC sandpapers (#600, #800, #1,000) and subsequently polished to a 1  $\mu\text{m}$  surface finish using diamond paste. For the *in vitro* tests, the polished disks were soaked in 40 ml of pH 7.4 distilled water (buffered using 0.05 M tris-hydroxymethyl aminomethane) at 37 °C for 3, 7 and 14 days. At the end of these time periods all samples were washed with distilled water and with acetone, and then dried at 50 °C overnight for further analysis. For the *in vivo* tests, the disks were also implanted in a subcutaneous site of a male dog for 2-12 weeks. The retrieved samples were freeze-dried after fixing and washing with ethanol. As a function of the immersion period, relative intensities of crystal planes in HA were measured from X-ray diffraction (XRD) patterns and X-ray pole figures (XPF) examined distributions of (211), (300), and (112) crystal planes, which are the three strongest peaks of HA.

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## Results and Discussion

Figure 1 gives XRD patterns of the as-received HA powder and sintered HA. The powder consisted of the HA phase showing broad peaks due to the presence of submicrometer particles. When sintered at 1200 °C, the poorly crystalline HA was crystallized into highly crystalline HA. There was no indication of thermal decomposition and the related presence of any phases other than HA. The reduction of the Ca/P ratio is responsible for the presence of nonstoichiometric HA which can be easily decomposed to secondary phases at elevated temperatures [16, 17]. Hence, thermal stability of HA in this study can be expected by the fact that the Ca/P molar ratio of the powder was  $1.67 \pm 0.02$  which is the stoichiometric value of HA.

SEM micrographs of the as-received HA powders (see Fig. 2) revealed that the presence of soft agglomerates was in turn formed by nano-sized primary particles. The powders appear to have a wide size distribution ranging from approximately 15-30 nm (spherical particles) to 70-150 nm (needle-shaped particles).

To observe the microstructure, HA was thermally etched at 1150 °C for 0.5 h to delineate the grain boundaries. Figure 3 shows an SEM micrograph of a

polished and etched HA surface. The sample showed a sintered density of 98% above the theoretical density indicating that densification was achieved with very little porosity. A dense microstructure was also evidenced by the presence of the pores, if any, mostly at triple grain junctions. The sample consisted of small grains of 1-2  $\mu\text{m}$ , however there were larger grains of 4-5  $\mu\text{m}$  in diameter. This may be caused by the characteristics of the starting powder which has a wide size distribu-

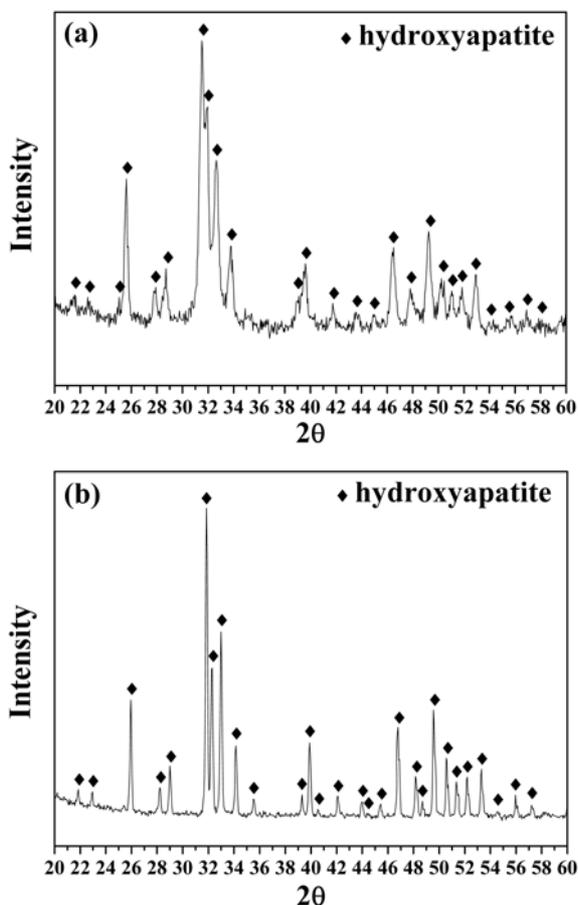


Fig. 1. XRD patterns of (a) as-received powder and (b) sintered HA.

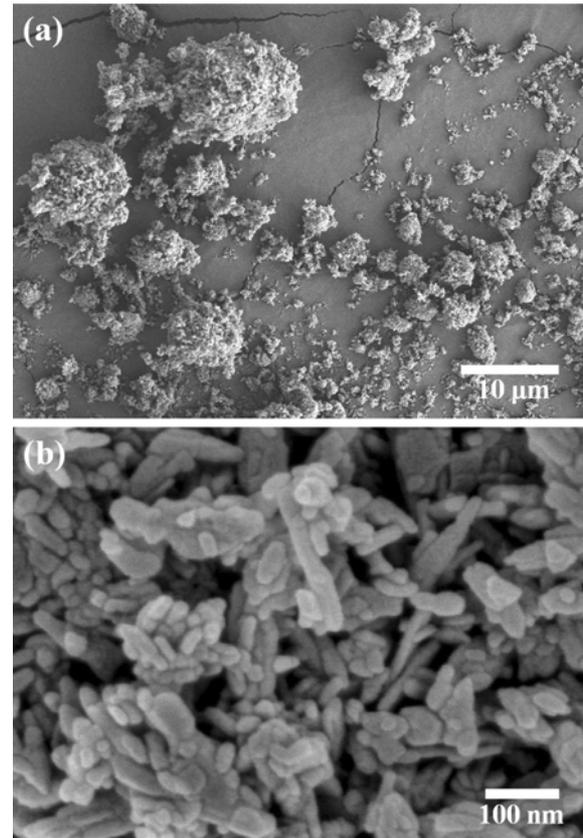


Fig. 2. SEM micrographs of as-received HA powders: (a) The powders exhibit the presence of agglomerates and (b) Highly magnified image reveals that the powders consists of nano-sized primary particles.

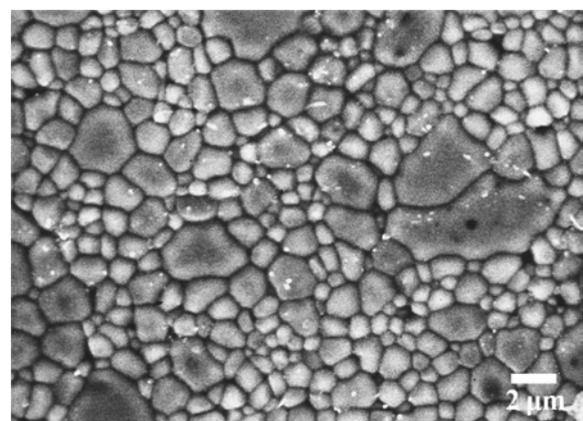
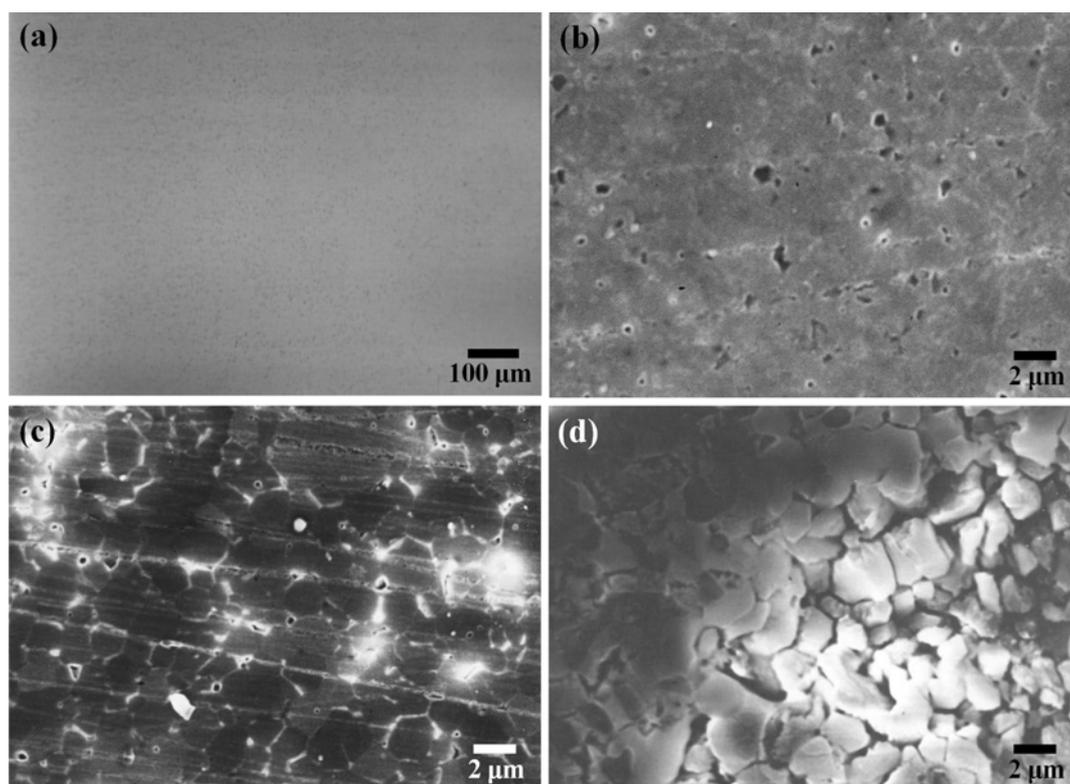


Fig. 3. SEM micrograph of a polished and thermally-etched HA surface sintered at 1200 °C.



**Fig. 4.** Surface morphology of HA (a) as-sintered, and immersed for (b) 3 days, (c) 7 days and (d) 14 days.

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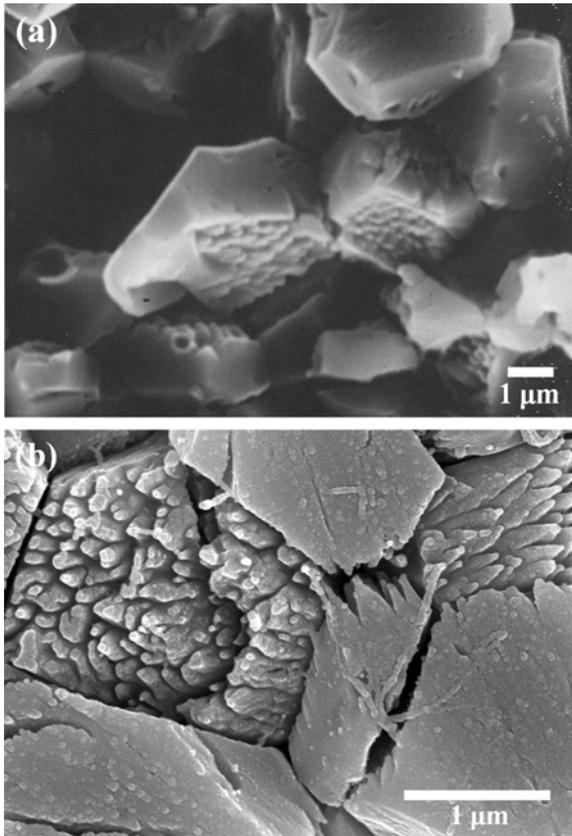
Figure 4 presents the surface morphology of HA as a function of immersion period. The sintered and polished surface (Fig. 4(a)) was initially smooth corresponding to a relatively low density of surface defects, such as residual pores. The surface became rougher and there was a trace of dissolution leaving submicrometer size of pores after 3 days of immersion. After 7 days of immersion (Fig. 4(c)), it can be seen that the grain boundaries appeared due to dissolution in water showing a similar morphology to the etched surface. Following further immersion (Fig. 4(d)), dissolution of HA seemed to have occurred along the grain boundaries and extended into the bulk following these paths resulting in separation of ceramic grains.

HA features structural flexibility to accommodate a wide variety of impurities and thus it seems quite challenging to obtain a “truly” phase pure HA. All variations away from the stoichiometric value of 1.667 will generate impurity phases, such as tricalcium phosphate (TCP) and calcium oxide. TCP and CaO may often be present as a result of decomposition during sintering. Unfortunately, those second phases cannot be detected via X-ray diffraction especially if a trace amount of them are randomly dispersed within the bulk. The presence of TCP and CaO should increase dissolution and related degradation of HA as both are less stable than HA in biological milieu [8, 18, 19].

According to the XRD analysis, the HA used in this experiment was found to be phase pure without ex-

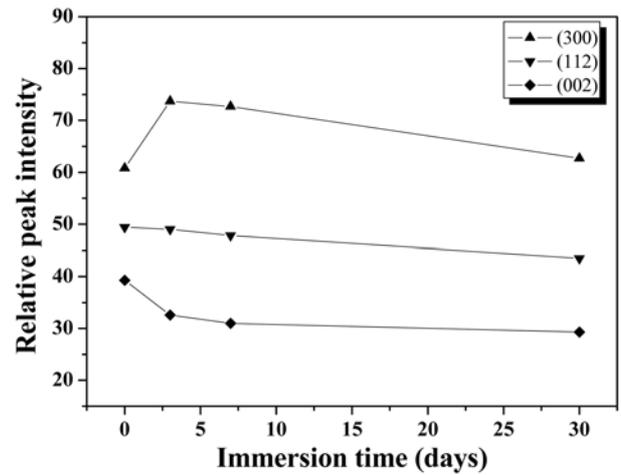
pressing any impurity phases. This means that HA is supposed to be stable in an aqueous solution. However, SEM observation revealed that considerable dissolution occurred on the surface, specifically at grain boundaries. Furthermore, ceramic grains were separated individually as a result of severe grain boundary dissolution. An analytical electron microscopy study by Nonami and Wakai [3] demonstrated that the Ca/P ratio of HA at the grain boundaries was lower than that within the grains, suggesting that there should be the presence of non-stoichiometric composition. Actually, in our previous study [20], high resolution transmission electron microscopy observation revealed that  $\alpha$ -tricalcium phosphate ( $\alpha$ -TCP) existed at grain boundaries of phase pure HA. Therefore, it can be expected that the soluble  $\alpha$ -TCP should induce a loss of mineral at the grain boundaries, accompanied by the overall dissolution of HA.

In addition to the grain boundary dissolution, the surface of ceramic grains was dissolved as shown in Fig. 5. Figure 5(a) shows the pronounced dissolution of a grain itself after immersion for 14 days in water. Ceramic grains were dissolved in a certain direction resulting in aligned crystals within the grains. The *in vivo* experiment also revealed dissolution of specific a grain itself in a single direction perpendicular or parallel to HA surfaces (Fig. 5(b)). The appearance of the dissolved crystals is similar to the previous results by Yamada et al. [15], demonstrating a series of spike-like crystals aligned in a single direction. The results in



**Fig. 5.** Microstructures of HA; immersed for (a) 14 days and implanted in a dog for (b) 12 weeks.

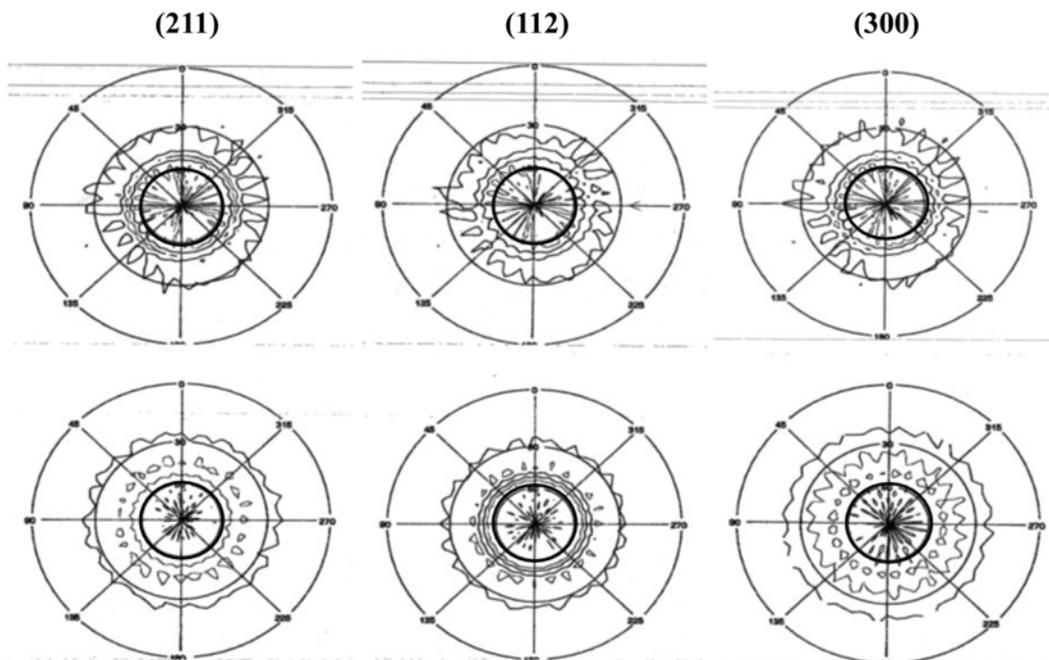
the present study indicate that ceramic grains showed a crystallographic dependence of dissolution, therefore the crystallographic characteristics of HA to dissolution



**Fig. 6.** Variations in relative intensity of crystal planes of HA as a function of immersion period.

should be considered to understand the degradation mechanism of HA in a physiological environment.

To estimate the crystallographic dependency of HA to dissolution, the measured relative intensities of (112), (300), (002) crystal planes from XRD and X-ray pole figures are presented in Fig. 6 and Fig. 7, respectively. The peak intensity of (300) was maintained relatively during immersion compared with the rest of the peaks. In each pole figure, the distribution of the contour lines in the center of the diagram corresponded to the relative intensity of the measured crystal planes. The contour lines of (211) and (112) were found to be decreased after immersion, whereas that of (300) remained similar before and after immersion, meaning that the (300) plane of HA could be stable in water. Accordingly,



**Fig. 7.** X-ray pole figures for (211), (112), and (300) crystal planes of HA. Three diagrams in the upper position represent HA before immersion and the lower diagrams correspond to HA after immersion for 14 days in water.

dissolution of certain HA grains can be explained by a dissolution preference of a certain crystal plane, in our case, (300) plane.

One of the important requirements for HA implant materials is to form a calcium phosphate (CaP)-rich layer on the implant surface in order to bond with surrounding bone. The CaP layer can be formed by either a dissolution/precipitation process or epitaxial growth without pronounced dissolution [21]. These two processes took place in an implant at once. Therefore, it needs to be determined if grain orientation influences the degradation of HA. The results in this study can be useful to understand the underlying mechanism of crystallographic dependency on dissolution and degradation of HA.

### Conclusions

This study has focused on the dissolution behavior of hydroxyapatite affected by the grain boundary structure and crystal orientation. It suggests that there would be non-stoichiometric phase at the grain boundaries of hydroxyapatite even though the material was phase pure and this non-stoichiometric phase initiated grain boundary dissolution. The surface dissolution at grain boundaries, followed by separation of the ceramic grains and particle loosening can lead to a distinct decrease in the long term mechanical stability of bioceramics, therefore a preparation of truly phase pure HA is of primary importance. Notably, a certain crystal plane in HA had resistance to dissolution, therefore the crystallographic influence should be considered to understand the mechanism of HA degradation.

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