**Microstructural Analysis on Biological Disintegration of Hydroxyapatite**

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**Statement of Purpose:** Although hydroxyapatite (HA) is used extensively in a variety of clinical settings [1], much of our understanding of their behavior is based on specific cell-based *in vivo* processes [2]. Osteoclastic resorption is probably the most-quoted mechanism for their biological utilization, often to the exclusion of any other potential cellular or extracellular processes. Besides, degradation is often assumed to be uniform over large scales although the microstructure itself may not be. In this study, mechanism on biological disintegration of dense hydroxyapatite both in non-osseous and osseous sites of a dog was investigated.

**Methods:** Phase-pure HA powder was synthesized by a precipitation reaction between mixture of $Ca(NO_3)_2{\cdot}4H_2O$ and $H_3PO_4$, and ammonium hydroxide solutions. HA ceramics were obtained by sintered at 1200 ˚C for 2 h in air with under moisture protection. HA implant with a shape of disk was placed both in subcutaneous and osseous sites of male dogs for 12 weeks. After certain period of implantation, the dogs were anesthetized and killed by intraventricular perfusion with 2% paraformaldehyde aldehyde and 2.5% glutaraldehyde in 0.1 M Na-cacodylate buffer, pH 7.4. Femora were excised, epiphyses removed, and the samples were cleaned with normal saline, immersed in 3% glutaraldehyde-0.1 M phosphorous buffer (pH 7.2) solution for 2-3 h at 4 ˚C, then postfixed in 1% osmium tetraoxide-0.1 M phosphorous buffer solution at 4 ˚C for 2 h, subsequently dehydrated in 50%, 70%, 90%, and 100% ethanol for 1.5 h. And then, they were freeze-dried for field emission scanning electron microscopy (FE-SEM) observation.

**Results:** This study highlight microstructural interactions of HA implant *in vivo*. The implant surface after implantation in femur (osseous site) for 1 week showed that most surfaces did not experienced dissolution, but in some area, grain boundary dissolution was observed. In case of the implant surface after implantation in femur for 4 weeks showed that the region of surface dissolution was extended and aggressive dissolution at grain boundaries occurred. While dissolution occurred extensively, globular accretions formed on the HA implant. The globular materials had fine particle size of 20-30 nm. Ca/P ratio measured by element analysis was 1.65 close to HA. As the biological matrix was further elaborated, the globular accretions were covered by a collagen fiber. The appearance of bone was elaborated. However, it seemed that bone was not firmly bonded to the implant surface due to the severe dissolution on the implant surface. In case of osseous site, osteoclasts were intensively involved in implant dissolution, revealing that osteoclast absorbed on the implant surface showed aggressive implant dissolution by osteoclast resorption. As a result, in the vicinity of osteoclast, a severe and deeper dissolution underwent resulting in formation of smaller and more round particle shape. Less dissolution occurs in a certain distance from osteoclast, but grain boundary dissolution still appears.

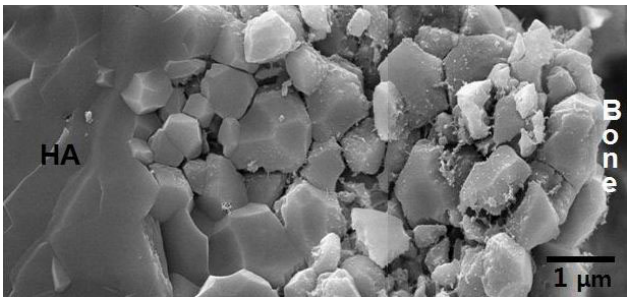

Figure 1. SEM micrographs of fracture surface after implantation in non-osseous site for 12 weeks.

Compared with osseous site, surface dissolution was slow in non-osseous, i.e., subcutaneous site. HA surface after subcutaneous implantation for 4 weeks showed that implant surface was as smooth as the polished one without dissolution. However, dissolution at grain boundaries was observed in some area. After subcutaneous implantation for 12 weeks (Fig. 1), dissolution at grain boundary and grain itself was prominent and it extended into bulk following the grain boundaries. A wide gap between grains near bone was formed, compared with the grains near the implant. Furthermore, grain itself dissolved in a single direction. It should be noted that single crystallites with hexagonal shape appeared and globular accretions were deposited on the crystallites. In case of subcutaneous site, it can be assessed that dissolution of grain boundary and grain itself does not necessarily occur by osteoclastic resorption. Solution-mediated process which is closely related to implant itself could be involved in the dissolution.

**Conclusions:** This study highlights microstructural response and biological disintegration of HA ceramics *in vivo*. The results suggests that an extracellular method of particle generation that does not necessarily require the participation of osteoclasts. It can be concluded that properties of HA itself (chemical composition in this study) affected grain boundary dissolution followed by microstructural disintegration. It was caused by grain boundary dissolution initiated at the implant surface extended into bulk following these paths. This kind of dissolution process apparently evidenced grain boundary dissolving causes particle generation. These results also indicate that long-term bone in-growth and mechanical properties could be dramatically decreased.

**References:**

[1] LeGeros RZ. An Introduction to Bioceramics, World Scientific Publishing, Singapore, 1991;139-180.
[2] Yamada S. J Biomed Mater Res. 1997;37;346-352.