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A microstructural study of the degradation and calcium release from hydroxyapatite-calcium oxide ceramics made by infiltration

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ABSTRACT

Hydroxyapatite pellets, partially densified in a low-temperature heat treatment, were infiltrated with calcium nitrate solution followed by in-situ precipitation of $Ca(OH)_2$ and $CaCO_3$. The infiltrated bodies were then densified to high relative density and the calcium carbonate transformed to calcium oxide during sintering and resulted in biphasic hydroxyapatite-CaO ceramics. This work investigated the influence of the infiltration on surface morphology, weight change, and microstructural-level degradation caused by exposure to saline at pH = 7.4 and a temperature of 20 °C. The CaO rendered the materials more susceptible to degradation, and released calcium into the saline faster than single phase, calcium deficient hydroxyapatite (HA) that were used as a control. In consequence, these ceramics could be used to release calcium into the culture microenvironments of bone tissue or bone marrow cells next to a scaffold surface.

1. Introduction

In tissue engineering, Hydroxyapatite (HA) and HA-based ceramic composites are widely used biomedical materials, especially in the area of artificial bone and bone substrata. The excellent bioactivity, biocompatibility and osteoconduction [1,2] of HA are desirable biological properties for bone grafts and HA is chemically similar to the inorganic component of human bone [3]. HA also serves as a supply of calcium and phosphate ions for the processes of bone regeneration including osteointegrativity [4] and effects the chemical microenvironment for bone marrow cells, which include hematopoietic stem cells, osteoblasts, stromal cells and endothelial cells. Calcium, in particular, is known to influence osteoblasts [5], hematopoietic stem cells [6] and endothelial cells, which are amongst the main cell types in the bone marrow niche [7,8]. However, compared with tri-calcium phosphate (TCP), and amorphous calcium phosphate (ACP), HA has a much lower degradation rate [9,10] and so fewer Ca^{2+} ions are released in body fluid at pH = 7.4.

To enhance the HA degradation rate, different HA/TCP mixture ratios have been studied and proved to effect the degradation rate as one would expect [11]. However, Suzuki et al. [12] showed that, compared with HA/TCP biphasic ceramics, with ratio: 3–7, 6–4, 8–2, pure HA was more effective at accelerating growth and differentiation of osteoblast-like cells. HA-containing polymer-ceramic composites have also been processed to enhance the degradation rate compared to HA and thereby create a more rapidly resorbed biomaterial [11,13,14]. Finally, Lannutti et al. [15] reported that CaO, another highly soluble Ca-rich phase, could affect HA degradation rate, and calcium release was much faster in calcium rich HA compositions than in TCP. In their study, a relatively small amount of CaO (1.76%) rendered the bulk ceramic far more susceptible to rapid degradation at the microstructural level than the considerably higher volumetric percentages of TCP (26.47%) in HA + TCP biphasic ceramics studied at pH = 6.8– 7.4. However, the effect of phase transformation between CaO and $CaCO_3$ was not mentioned and its contribution to the degradation was not clear for calcium rich HA compositions. Bocaccini et al. also studied the bioactive behavior and the enhancement of HA degradation with CaO [16]. In their study, rapid CaO dissolution assisted in the HCAp layer formation on the surface of HA.

This work aims to create a microstructurally graded biphasic mixture of HA-CaO ceramic which would form $CaCO_3$ at the surface of the ceramic in moist environments and thereby release calcium into a culture microenvironment for bone marrow cells next to the scaffold surface. Ultimately, calcium enriched surface layers on HA scaffolds are desired in order to enhance cell attachment and control cell fate during in-vitro 3D culturing in bioreactors. This could be accomplished without the need to process HA with a highly soluble second phase by mixing. An infiltration process was used to produce a gradient of a CaO minor phase from the surface to the interior of HA ceramics, which in turn affected the degradation behavior of HA-based ceramic and e release of calcium into saline over a longer time period than

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could be achieved with just a surface layer of highly soluble CaO. This material was used to simulate the endosteal bone marrow stem cell niche in-vitro using osteoblasts/hematopoietic stem cells co-cultures The results of the coculture experiments are beyond the scope of this report and will be published separately. Here the emphasis will be on the microstructure mechanism by which the transformation of CaO to $CaCO_3$ creates fragmentation on the ceramic surface, thereby exposing subsurface CaO and maintains enhanced calcium release over a period of 28 days.

2. Materials and methods

2.1. Materials

Hydroxyapatite (HA) powder ($3Ca_3(PO_4)_2 \cdot Ca(OH)_2$, Sigma-Aldrich) was calcined at 900 °C for 1 h to ensure phase purity and no second phases were detected by X-ray diffraction. After calcination, the powder was milled in water for 24 h and was dried and granulated again by pestle and mortar.

2.2. Ceramics processing

The HA powder was pressed into 13 mm diameter pellets at a pressure of 30 MPa with a resulting green density was 49.5% based on the mass and the volume of the pellet determined from its measured dimensions. After pressing, the pellets were sintered in air at 1100 °C for 1 h, to give a relative bulk density of 67% measured by the Archimedes method. Importantly the comparison of the measured apparent density and the bulk density showed that all the porosity was open as would be expected in initial stage and early intermediate stage sintering. The samples were heated at a rate is 5 °C min^{-1} and cooled at a rate of 10 °C min^{-1}.

The infiltrant was prepared from calcium nitrate tetrahydrate ($Ca(NO_3)_2 \cdot 4H_2O$, Alfa Aesar, England) dissolved in DI-water. The concentration studied was 1 mol/L. The partially sintered pellets were then immersed in the calcium nitrate solution and were evacuated to remove air from the pores. After 24 h the pellets were removed from the calcium nitrate solution but they were still saturated when drops of ammonia hydroxide solution ($NH_4(OH)$, J.T. Baker) at pH = 12.5 were applied on to the surface of the pellets followed by immersion of the pellet in the same ammonium hydroxide solution for 30 min. Finally, the infiltrated pellet was allowed to dry in room air for 24 h. The pellets were heat treated at 900 °C to achieve crystallization of CaO [17] and then sintered to high density, 97.8(±1) %, at 1300 °C for 1 h. The remnant porosity was closed. The heat rate was 5 °C min^{-1} while the cooling rate was 10 °C min^{-1}. Also, HA control pellets were prepared with the same processing steps except infiltration.

2.3. Analysis

After final sintering, two groups of pellets were prepared for XRD (using a cobalt target), SEM and degradation analysis. These included: the control HA pellets and the 1 mol/L $Ca(NO_3)_2 \cdot 4H_2O$ solution infiltrated HA pellets. Three technical repeats were prepared for each degradation time point.

All the samples were impregnated with a low viscosity resin and lightly ground to provide a planar surface as close to the original sintered surface as possible. After quickly grinding the surface flat with 45 μm and 30 μm diamonds in water the samples were pre-polished with 15 μm diamonds and 6 μm diamonds with polishing oil and finally with 1 μm diamonds in polishing oil. After polishing the pellets were removed from the mounting resin and each pellet was placed in 25 mL Tris buffered Saline (TBS) (Fisher Scientific) at pH = 7.4. These solutions were sealed and held at 20 °C for 0.5 h, 1 h, 1 day, 3 days, 7 days, 14 days and 28 days. At the end of this period, all samples were taken out, dried and stored in a desiccator.

The sintered surfaces of the pellets were examined by XRD for the phase identification. Scanning electron microscopy (SEM/EDS) (JSM 6610LV, JOEL USA, Peabody, MA) analysis was used to observe the changes in the microstructure on polished surfaces exposed to saline for each time point. In order to quantitatively analyze the degradation behavior Image J (National Institutes of Health, Bethesda, Maryland, USA), was used to threshold the surface damage (pits) on 15 images taken at random positions on the polished surface. Each image contained approximately 8 to 10 pits fully contained within the frame of each image. The area fraction of pits on the surface, their number per unit area and their size distribution was calculated from the processed images.

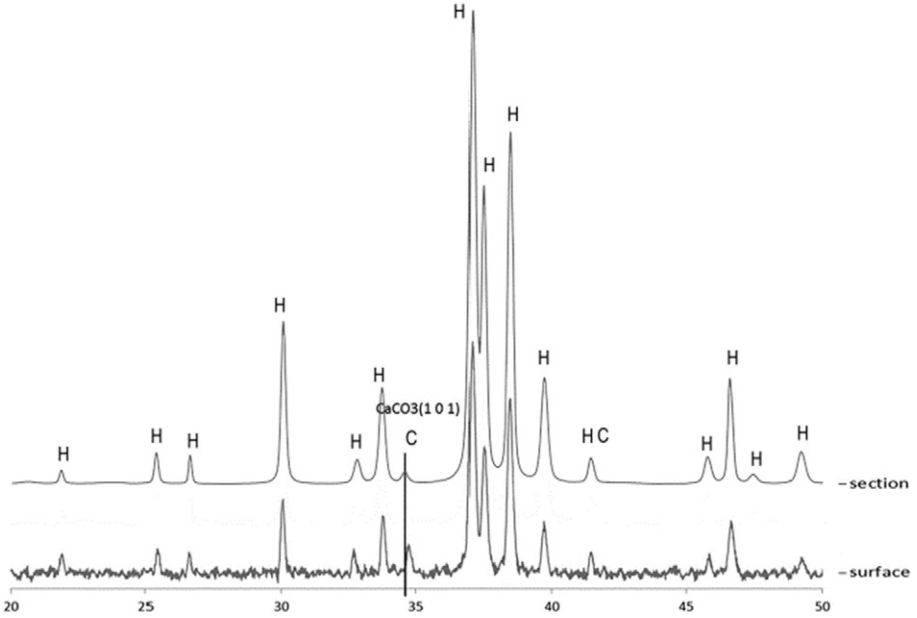

Fig. 1. XRD analysis on the surface and section of 1 M Ca2+ solution infiltrated pellet.