Development of Standardized Test Method for Evaluation of Nano-Crystalline Hydroxyapatite Forming Ability of Orthopedic, Dental and Craniofacial Device Materials

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Statement of Purpose: In bone repair surgery, grafts are often used to replace missing or diseased bone, particularly in instances where the defect is of a size where the bone would not be expected to heal on its own. One of the desired properties of bone grafts is their ability to promote integration with native soft and hard tissues by depositing a layer of nanocrystalline hydroxyapatite on its surface. This property is known as "bioactivity" and is frequently used when describing bioactive glasses. The most common method for testing for the presence of this property is to soak a device material in simulated body fluid (SBF) and evaluate the surface for the formation of the apatite layer. Recent studies have shown that variations in SBF preparation (ion concentration, pH, soaking time) can induce apatite deposition with potential for false positive results[1,2]. The goal of this study was to evaluate the role of testing conditions on bioactivity.

Methods: In brief, SBF was prepared according to ISO 23317[3]. Variations of SBFs were produced such as 1.5X and 2.0X concentration and modified SBF with increased Ca^{2+} and PO_4^{2-} (2X, and 4X) ions in solution. Several different materials were soaked in the solution at time points up to 4 weeks. Bio-inert surfaces such as titanium alloy (Ti64 ASTM F136 grade), anodized alumina (Al_2O_3), and ultra-high molecular weight polyethylene (UHMWPE) were used as negative controls and were not expected to form an apatite layer. Bioactive glasses 45S5 and S53P3 were used as positive controls for apatite deposition. Bio-inert materials were also modified with varying degrees of nano-scale roughness to observe the effect of surface modification on non-specific apatite deposition.

The formation of apatite on the surface of the materials was characterized by the amount and phases deposited. These analyses consisted of field-emission scanning electron microscopy (FE-SEM), energy dispersive x-ray spectroscopy (EDX), atomic force microscopy (AFM), Raman spectroscopy, x-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR and laser scanning confocal microscopy.

Results: Modified SBF (mSBF) with 2X and 4X Ca^{2+} and PO_4^{2-} concentrations resulted in decreased pH of the final SBF solutions. Significant apatite deposition was seen on the 20 nm diameter nanoporous anodized Al_2O_3 after 21 days of incubation with all SBF formulations. 1X SBF deposited dense flaky apatite formations (Figure 1A), while the structure of 2X (Figure 1B) and 4X (Figure1C) mSBF apatite was visibly smoother. No apatite deposition was observed on 200 nm nanoporous Al_2O_3 (Figure 1D) with any SBF formulations.

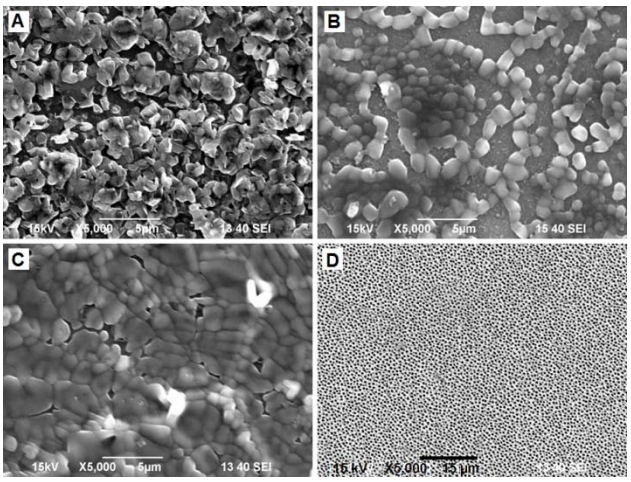

Figure 1. Apatite formation after 21 days on 20 nm porous Al_2O_3 with (A) 1X SBF, (B) 2X mSBF, and (C) 4X mSBF. No apatite formation observed on (D) 100 nm porous Al_2O_3.

Conclusions: SEM analysis showed that surface nanotopography and modification to SBF formulation can influence apatite layer deposition. Ongoing work involves assessing effects of SBF on the total amount and phase of hydroxyapatite deposited on these surfaces. The study will lead to more consistent test methods and data for assessment of "bioactivity" for materials, and therefore is of value to the regulatory review of bone void filler products for orthopedic, dental and craniofacial applications

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