Osteoblast Interactions on Electrically Polarized Biphasic Calcium Phosphates

Mohammad Solaiman Tarafder, Amit Bandyopadhyay, Susmita Bose.
W. M. Keck Biomedical Materials Lab, School of Mechanical and Materials Engineering, Materials Science Program
Washington State University, Pullman, WA 99164-2920, USA. E-mail: sbose@wsu.edu

Introduction: Hydroxyapatite (HAp) and β-tricalcium phosphate (β-TCP) based calcium phosphates (CP) are widely used for hard tissue repair and augmentation in orthopedics and dentistry due to their compositional similarity with human bone. Biphasic calcium phosphate (BCP) consisted of HAp and β-TCP has combined advantages of bioactivity and bioresorbability. The **objective** of this research is to study the effects of electrical polarization and grain size on surface properties, *in vitro* and *in vivo* biological properties of CP based ceramics. Our **hypothesis** is electrical polarization, chemistry and microstructure in HAp ceramics can modify bioactivity and *in vivo* tissue integration abilities. The **rationale** is that once we delineate the fundamental information on bioactivity of HAp ceramics and identify optimal material composition, microstructure and surface charge we should be able to design and tailor better bone grafts *based on application needs such as osteoporosis and Paget's disease, rickets disease and bone cancer*. In this study electrical polarization of biphasic TCP-HAp composites (BCP) was used where, hydroxyl groups in HAp crystals was used to pole the BCP composites.

Methods: Three different BCPs with different hydroxyapatite (HAP) / β-tricalcium phosphate (β-TCP) wt.% ratio were made. The compositions of the BCPs were β-TCP75%+HAp25%, β-TCP50%+HAp50%, and β-TCP25%+HAp75% respectively. 1 wt% MgO and 0.3 wt% ZnO were added to attain better sintering density. All disk samples [12 mm (ϕ) X 1 mm (h)] were then sintered at 1200^0C for 2h at 1^0C/min heating rate. Phase analysis was done by XRD. The composites were then electrically poled at 400^0C for 1h with a dc electric field of 5KV/cm [1]. Stored static current in the composites as a result of polarization was determined from the thermally stimulated depolarization current (TSDC) measurement. Contact angles on the un-poled (U-poled), negatively-poled (N-poled) and positively-poled (P- poled) surfaces of the composites were measured using the sessile drop method with a face contact angle set-up equipped with a camera (VCA Optima, AST Products INC., MA, USA). Deionized distilled water and cell culture media (Dulbecco's modified eagles medium/nutrient mixture F-12 Ham , DMEM at pH 7.4) were used to investigate the wettability. *In vitro* bone cell interactions on the U-poled, N-poled and P-poled surfaces of the three composites were investigated by culturing human fetal osteoblast cell (hFOB) for 3, 7, and 11 days. The MTT assay (Sigma, ST. Louis, MO) was performed to assess hFOB cell proliferation on the poled vs. unpoled surfaces of these composites. Cell morphology was assessed by SEM.

Results: More than 95% of theoretical density was obtained for almost all composites and phase purity of the BCP composites was confirmed by X-ray diffraction pattern. Stored charge in the BCP composites was gradually increased with the increase in HAp percentage. 2.38 $\mu C/cm^2$, 1.59 $\mu C/cm^2$, and 1.11 $\mu C/cm^2$ stored charge were achieved for composites containing HAp75%, HAp50%, and HAp25% respectively. Increased wettability of the composites after polarization was confirmed by lowering of the contact angles on the poled surfaces. MTT assay (Fig. 1) confirms rapid cell proliferation on the N-poled surfaces compared to U-poled and P-poled surfaces in the first few days. Initial cell proliferation was not preferred by the P-poled surfaces as much as preferred by U-poled and N-poled

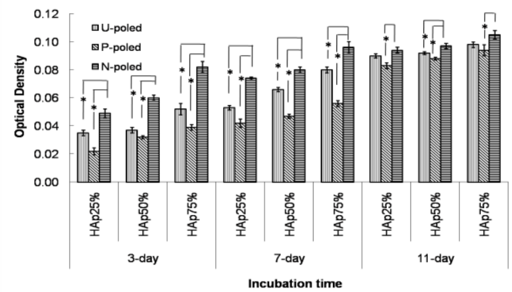

Figure 1. MTT assay of hFOB on the U-poled, P-poled and, N-poled surfaces of BCP cpmposites after 3, 7, and 11 days (*$p < 0.05$, n = 3).

surfaces. After 11-day culture, there was not very large difference in cell density among the U-poled, P-poled, and N-poled surfaces although a significant difference (*$p < 0.05$) in cell density was always observed between P-poled and N-poled surfaces. SEM morphologies of the

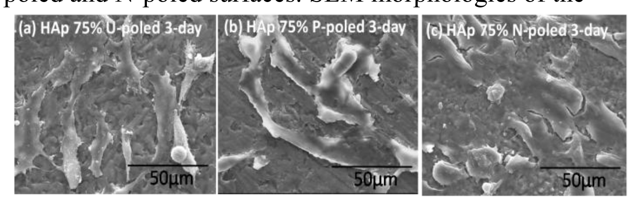

Figure 2. SEM micrographs of hFOB cells showing the cell adhesion and proliferation on U-poled, P-poled, and N-poled surfaces of the HAp 75% BCP composite after 3 days of culture.

hFOB cells showed an early stage cell adhesion, proliferation, and mineralization on the N-poled surfaces. Increase in osteoblast attachment was observed with the increase in HAp percentage in the composite.

Conclusions: N-poled surface of electrically polarized pure HAp showed better cell-material interactions and early stage mineralization. We observed that the surface property of BCP composites, can also be tailored to enhance early stage osteoblast adhesion, proliferation, and mineralization. Though a better osteoblast interactions was observed with the increasing HAp percentage in the BCP composites, N-poled surfaces showed early stage osteoblast adhesion, proliferation, differentiation and mineralization. This interaction is enhanced as the stored charge or HAp percentage is increased in the BCP composites.

Authors like to acknowledge the financial support from the NIH (grant # R01 EB 007351).

References: 1. Bodhak S. Acta Biomat. 2009;5:2178-2188.