

Tricalcium phosphate based resorbable ceramics for bone tissue engineering: Influence of SiO₂ and ZnO dopants

Susmita Bose, Scott Rewinkel, Mohammad Tarafder, Neal Davies and Amit Bandyopadhyay

W. M. Keck Biomedical Materials Research Laboratory

School of Mechanical and Materials Engineering, Washington State University

Pullman, WA 99164-2920, USA. E-mail: sbose@wsu.edu

Statement of Purpose: Resorbable calcium phosphates (CaPs) have gained much attention, as resorbable scaffolds can degrade in the body allowing bone in-growth and eventually replacing the scaffold with natural tissue. **Objective** of this work is to understand the effects of dopants on mechanical, *in vitro* and *in vivo* biological properties of β -tricalcium phosphate (TCP) to achieve controlled degradation kinetics. Our **hypothesis** is the presence of dopants, e.g. SiO₂ and ZnO, can influence the biodegradation and biocompatibility of resorbable CaPs. The **rationale** is, once we identify optimal composition/s for different degradation behavior, we should be able to develop scaffolds for specific applications such as spinal fusion and craniomaxillofacial replacements.

Methods: β -TCP powder (Berkley Advanced Biomaterials Inc. Berkeley, CA, 550 nm) with three compositions (i) pure β -TCP, (ii) β -TCP with 0.5wt% SiO₂, (iii) β -TCP with 0.5wt% ZnO and 0.5wt% SiO₂ were prepared by solid state method. Compact samples were prepared by uniaxially pressing and then sintering at 1250°C for 2 h. Phase analyses were performed using X-ray diffraction (Philips PW 3040/00 X'pert MPD).

Surface morphologies were analyzed by scanning electron microscope (SEM, FEI Inc., OR, USA). The infrared spectra were recorded by a FTIR spectrometer (Nicolet 6700, ThermoFisher, Madison, WI). Strength degradation studies were performed for 16 weeks in simulated body fluid (SBF). TCP based 3D scaffolds were made by solid freeform fabrication (SFF) method. In this layer by layer manufacturing process, 3D scaffolds were printed by printing a chemical binder onto powdered TCP. 3D interconnected porous scaffolds with pre-set dimension [7 mm (ϕ) X 10.5 mm (h)] and pore size (500 μ m, 750 μ m, and 1000 μ m) were made using 550 nm β -TCP by ExOne 3D ceramic printing machine (Imagen LLC – An Ex One Company, Irwin, PA). Scaffolds were sintered at 1250°C for 2h at 1°C/min heating rate.

Compressive strengths of these scaffolds and compacts were measured with an universal testing machine (Autograph, Shimadzu, Japan) with a constant crosshead speed of 0.33mm/min. *In vitro* bone cell interactions on the 3D porous scaffolds were investigated by culturing human fetal osteoblast cell (hFOB). Cell morphology was assessed by SEM observation. *In vivo* study was performed for 16 weeks into rat distal femur model with both pure and doped TCPs.

Results: XRD analysis revealed that β -TCP was the major phase in all compositions (JCPDS No 09-169). The average relative density of pure β -TCP, β -TCP-Si and β -TCP-Si/Zn was above 90%. SEM micrographs of surface revealed highly dense structure. The grain size of pure and doped β -TCP samples were between 2.8 and 3.1 μ m under SEM. Addition of dopants increased the grain size of β -TCP. Compressive strengths of all sintered samples

and strengths after 16 weeks degradation in SBF are presented in Figure 1. Initial compressive strengths of pure and doped β -TCP were between 300 and 400 MPa. The strength degradation study revealed that doped β -TCP samples maintained higher compression strength than pure β -TCP in SBF over 16 weeks. However, pure β -TCP showed a drop of 62% in compressive strength where as β -TCP-SiO₂ and β -TCP-SiO₂-ZnO showed drop of 41% and 11%, respectively. Samples of all compositions showed increase in weight over 16 weeks in SBF which was due to hydroxy-carbonate apatite (HCA) formation, as revealed by SEM, XRD and FTIR.

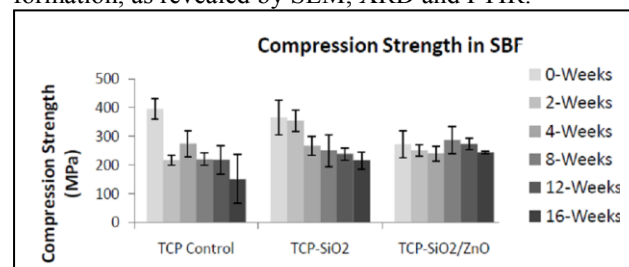


Figure 1. Compression strength of β -TCP samples after 0, 2, 4, 8, 12, and 16 weeks in SBF.

Bulk density of TCP based scaffolds with pore size between 500 μ m and 1000 μ m, and volume fraction porosity > 50% had compressive strengths between 3 and 7 MPa. SEM morphologies, showed good cell adhesion, as shown in Figure 2.

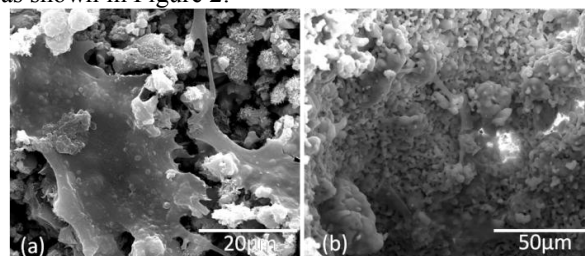


Figure 2. SEM micrographs showing cell adhesion and proliferation of hFOB cells on 3D scaffolds: (a) on surface and (b) inside the pore wall.

In vivo results revealed that osteocalcin and collagen I concentration in blood serum was higher in rats implanted with β -TCP-SiO₂/ZnO samples compared to pure β -TCP control. Push out tests using these TCP based ceramics structures showed good implant – tissue integration.

Conclusions: β -TCP compacts and scaffolds were made with oxide dopants. Pure and doped β -TCP samples showed compressive strengths between 300 and 400 MPa. All samples, both compacts and scaffolds, demonstrated good bioactivity in SBF as well as in *in vitro* cell culture. *In vivo* study showed higher osteocalcin and collagen I concentration for doped implant confirming good bone regeneration for doped β -TCP. These results suggested that properties of bioresorbable doped TCP could be tailored for specific tissue engineering applications. Authors like to acknowledge the financial support from the NIH (grant # R01 EB 007351) for this work.