Effect of silica doping on microstructral and biological properties of brushite cements

Sahar Vahabzadeh, Mangal Roy, Amit Bandyopadhyay and Susmita Bose (sbose@wsu.edu)
School of Mechanical and Materials Engineering, Washington State University, Pullman, WA 99164

Statement of Purpose: Brushite cements (BrC) have unique characteristics of biocompatibility, bioactivity, self-setting properties, adequate biodegradable rate, moldability and low setting temperature. There are many reports showing that bone cellular activity and in vivo responses of BrC alters in presence of dopants. Zn and Sr stimulate the osteoblast proliferation and collagen type I secretion [1]. However, to produce suitable material for bone regeneration applications, osteoclast activity should be clarified, as both osteoblast (OB) and osteoclast (OC) play significant roles in bone remodeling. In this work, silicon was chosen as a dopant due to its promise in osteoblast proliferation and possible effect on angiogenesis. Four different cements with various Si amount were made and the RAW 264.7 monocyte interaction with them was studied. Our hypothesis is optimal amount of Si in BrC results in better osteoclast activity and biological properties and this would present higher bioactivity in BrC.

Methods: The brushite cement was prepared by mixing β-tricalcium phosphate (β-TCP) and monocalcium phosphate monohydrate (MCPM) at a molar ration of 1:1. 0.5, 0.8 and 1.1 wt% Si doped BrC samples were made by mixing relative amount of SiO_2 with precursors of β-TCP followed by sintering at 1050ºC. Cement paste was obtained by combining the mixed powder with appropriate amount of 2wt% PEG, optimized in previous work [2]. Molded samples were kept in 100% humidity for 1 h followed by 24 h soaking in phosphate buffered saline (PBS). Osteoclastogenesis was studied by seeding human monocyte RAW264.7 (ATCC,USA) on samples followed by receptor activator of nuclear factor κβ ligand (RANKL, Biolegend, CA) addition. Tartrate-resistant acid phosphatase (TRAP) assay and FESEM techniques were used to determine cell proliferation and differentiation.

Results: XRD result showed the formation of β-TCP and dicalcium phosphate dihydrate (DCPD, brushite) after one day of incubation in PBS. No other phase containing Si was formed in doped BrC. Addition of small amount of Si does not stimulate the α-TCP formation. Figure 1 shows the osteoclast cell density after 8 and 14 days of culture. At both time points, doped samples had higher osteoclast cell density compared to pure one. Highest amount belonged to 0.5wt% Si doping. This shows that more Si addition could suppress osteoclast cell differentiation. In addition, cell density was higher after 14 days of culture compared to day 8, for all samples except 1.1 wt% Si-BrC. Cell morphology after 8 days for all samples is presented in figure 2. Big giant cells with defined cell membrane as characteristics of differentiated osteoclast cells were present all over the samples. However, monocyte still proliferated on pure, 0.8, and 1.1 wt% Si doped BrCs, in contrast to 0.5wt% Si doped sample. These results are in accordance to our finding from TRAP assay which showed the highest proliferation of osteoclast on sample with 0.5wt% of Si.

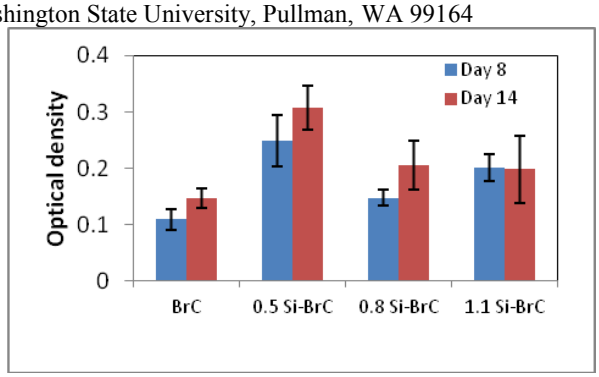

Figure 1. Osteoclast cell density on cements after 8 and 14 days of culture

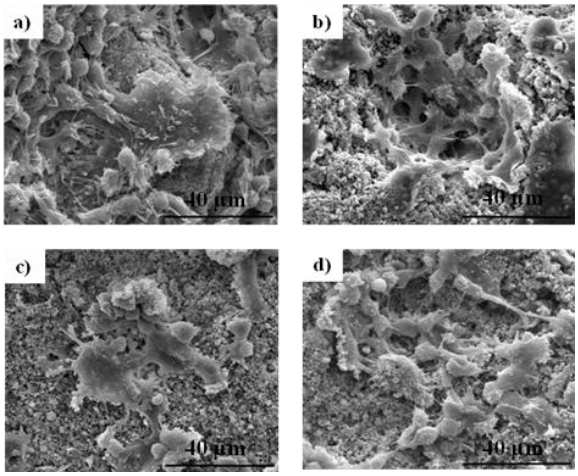

Figure 2. Osteoclast cell morphology on cements after 8 days of culture

Conclusion: Addition of Si did not change the phase composition in brushite cements. However, it affected osteoclast cell differentiatio. Si addition amount, within the range of this study, increased osteoclast differentation at a specific dopant concentration. In addition, among different levels of dopant, the highest level of cell density belonged to 0.5wt% Si amount. This suggested the inhibitory effect of dopant after specific level. This study suggests that there is an optimum level in dopant concentration which can play an important role in osteoclast cell behavior on these brushite cements.

Authors like to acknowledge the financial support from the NIH (NBIB grant # NIH-R01-EB-0073510).

References:

1- Pina S. EUR CELLS MATER. 2010; 20: 162-177.
2- Roy M. MATER SCI ENG C. 2012; 32(8):2145-2152.