Supporting information for

Effects of Silicon on Osteoclast Cell Mediated Degradation, *In Vivo* Osteogenesis and Vasculogenesis of Brushite Cement

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1. Materials and Methods

1.1. Cement characterization

1.1.1 Phase analysis, microstructure and compressive strength measurements

Phase analysis of cements before and after immersion in DMEM media for 14 days was carried out by X-ray diffraction (XRD) method using Siemens D-500 Diffractometer system operating at 35.0 kV and 30.0 mA, which used a Cu Kα radiation with a Ni filter at a step size of 0.1^{o} and a count time of 1 sec per step. The surface morphology of the pure and doped BrCs was characterized using a field emission scanning electron microscope (FESEM) (FEI Inc., OR, USA) at 30 kV. Compressive strength of BrCs were measured using a screw-driven universal testing machine (AG-IS, Shimadzu, Japan) with a constant crosshead speed of 0.33 mm/min. Compressive strength was calculated using the maximum recorded load and the sample dimensions. Compressive strength was tested on at least seven samples for each composition.

1.1.2 Setting time

According to ASTM C266, initial and final setting times of the BrCs were measured using Gillmore needle. A needle of 113.4g weight and 2.12 mm diameter was placed on the cement sample and initial setting time was recorded as the time the needle could not leave an impact on the surface of the cement paste. Similarly, a needle of 453.6g weight and 1.06 mm diameter was used to determine the final setting time.

1.2. In vitro Osteoclastogenesis

1.2.1 *Osteoclast cell morphology*

Field emission scanning electron microscope (FESEM, FEI 200F, FEI, OR) was used to assess the morphology of cells. BrCs were removed from culture medium after 5 and 8 days of

experiment and fixed with 2 % paraformaldehyde/ 2 % glutaraldehyde in 0.1 M phosphate buffer overnight at 4 ºC. Following three times of rinsing in 0.1 M phosphate buffer, samples were post-fixed in 2 % osmium tetroxide (OsO_4) over night at 4 ºC. Fixed samples were then dehydrated in an ethanol series (30 %, 50 %, 70 %, 95 %, and 100 % three times), followed by hexamethyl-disililane (HMDS) drying over night. Dried samples were gold-coated and observed under FESEM.

1.2.2. Immunofluorescence and confocal laser-microscopy

The osteoclast-like-cells were fixed in 4.0 % paraformaldehyde in 0.1 M phosphate buffer overnight at 4 ºC after 8 and 14 days of culture. After washing 3 times with PBS (10 min each), the cells were permeabilized with 0.1 % Triton X-100 (in PBS) at room temperature for 5 min. Samples were then washed with PBS and incubated in a Tris-buffered saline with 1% bovine serum albumin (TBST–BSA, pH 8.3) blocking solution at room temperature for 1 h. Actin staining was performed by incubating the samples in 1:40 rhodamine– phalloidine (Molecular Probes, Invitrogen, Germany):PBS for 30 min in the dark. Samples were then rinsed with PBS and incubated in 1:50 primary antibody (mouse anti-vitronectin, Abcam,MA, USA):TBST for 2 h and then kept at 4 °C overnight. After incubation, samples were rinsed with TBST–BSA 3 times. The diluted secondary antibody (Alex Fluor 488 antimouse) was used to incubate the cells. After 1 h of incubation, samples were washed with TBST–BSA followed by PBS, and mounted on glass coverslips with Vectashield mounting medium (Vector Labs, Burlingame, CA). 4′,6-diamidino-2-phenonylindole (DAPI) was also added and samples were kept at 4 °C for future imaging. A Zeiss 510 laser scanning microscope (LSM 510 META, Carl Zeiss Micro-Imaging, Inc., NY, USA) was used for confocal imaging.

1.3. In vivo study

1.3.1. Histomorphology

All bone-implant BrCs were fixed in 10 % buffered formalin solution for 72 h prior to histological analysis. The specimens were dehydrated for undecalcified tissue sections preparation, and further used for Masson Goldner's trichrome staining.

1.3.2. Masson Goldner's trichrome staining

After fixation in buffered formalin solution, samples were dehydrated in a graduated ethanol (70 %, 95 %, and 100 %), ethanol-acetone (1:1), and 100 % acetone series for undecalcified tissue section preparation. Samples were then embedded in Spurr's resin and blocks were sectioned perpendicular to the implant axis using a diamond saw. The sections were polished, stained by Masson Goldner's trichrome stain. Imaging on tissue sections were performed using light microscope [Olympus BH-2, Olympus America Inc., USA].

2. *Results*

2.1. Surface morphology of cements

Figure 1S shows the microstructure of undoped and Si doped BrCs after 1 day incubation in PBS. In all compositions, granules of TCP and needle/plate like structure, resembling DCPD were found.

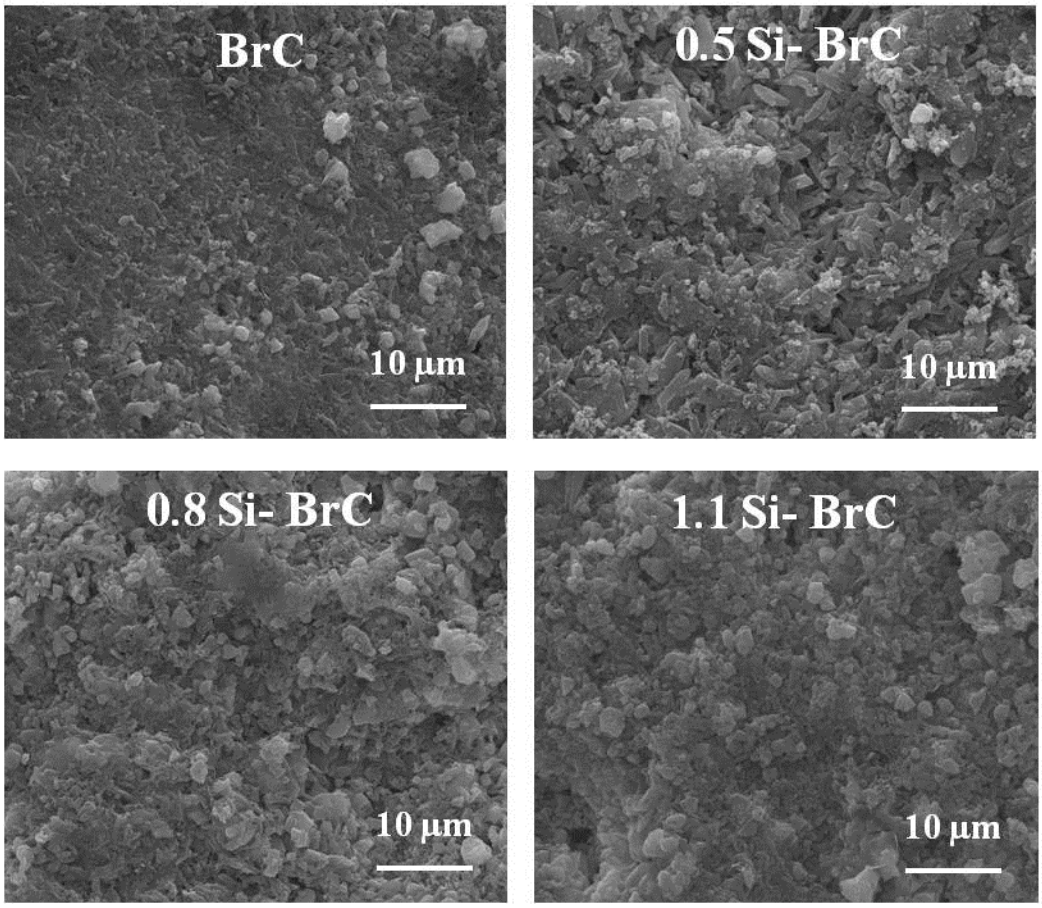

Figure 1S- FESEM micrographs of undoped and doped BrCs after 1 day incubation in PBS.

2.2. Masson Goldner's trichrome staining

To assess the effects of Si on osteogenesis behavior of BrC, histological analysis was performed after 4, 8, and 12 weeks (**Figure 2S**). Initiation of new bone, osteoid, at the interface of cement-host bone as well as inside the cement site was observed after 4 and 8 weeks. Prominent mineralizarion of newly formed bone was observed in all doped BrCs after 8 weeks compared to undoped BrC. 1.1 Si-BrC showed a complete degradation over the 8 weeks and there was no distinct cement-host bone interface at this time point. Mineralization of osteoid continued until 12 weeks and 0.5 Si-BrC had the highest mineralized bone formation.

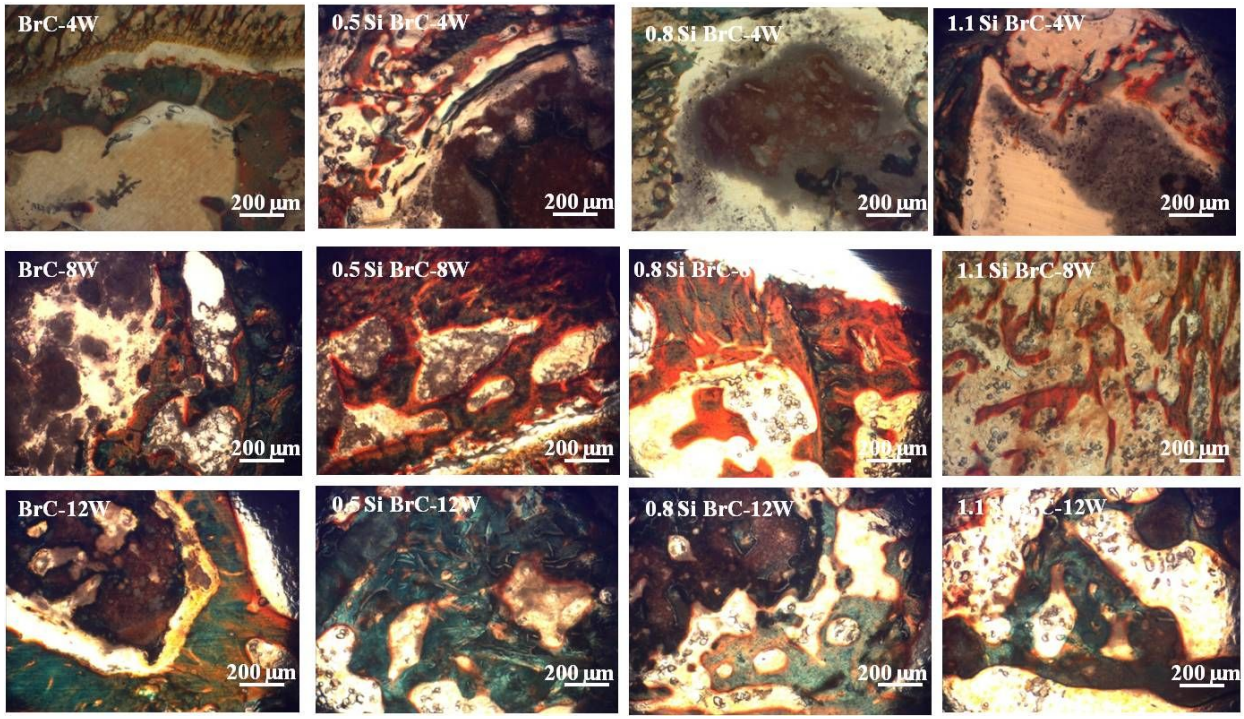

Figure 2S. Photomicrograph of the cements showing the development of new bone formation after 4, 8, and 12 weeks implantation in the rat femur. From left to right: BrC, 0.5 Si-BrC, 0.8 Si-BrC, 1.1 Si-BrC. Top to bottom: 4, 8, 12 weeks.