In Vivo Remodeling of 45S5 Bioactive Glass/Polyurethane Biocomposites with Initial Bone-like Mechanical Properties

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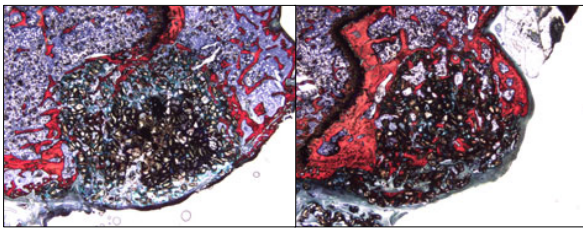
Statement of Purpose: Injectable, settable bone grafts that possess initial mechanical strength exceeding that of host bone and maintain strength comparable to bone while remodeling could improve the clinical management of a number of orthopaedic conditions, such as repair of open tibial plateau fracture, screw augmentation, and vertebroplasty. Injectable polyurethane (PUR) biocomposites are an attractive alternative to calcium phosphate cements due to their tough mechanical properties and active remodeling [1]. 45S5 bioactive glass (BG) has widely been used for bone regeneration purposes due to its osteoconductivity and bioactivity [2, 3]. Discrepancies about its biocompatibility have risen; exposed particles have been shown to induce a negative inflammatory response *in vivo*, while those delivered in a polymer vehicle have not [4, 5]. Previously we determined that surface-modified BG/PUR biocomposites have a compressive and torsion strength of 67.4 MPa and 29.1 MPa, exceeding reported trabecular bone values of 4-12 MPa and 6.1 MPa, respectively. In this study, we investigated the *in vivo* bone remodeling properties of BG/PUR biocomposites. We hypothesized that a BG/PUR biocomposite, with initial mechanical properties exceeding those of trabecular bone, would show balanced bone remodeling when implanted into femoral condyle plug defect model in rats over an 8-week period. The initial bone-like mechanical strength of BG/PUR biocomposites, as well as their balanced remodeling *in vivo,* underscores their potential utility as weight-bearing bone grafts.

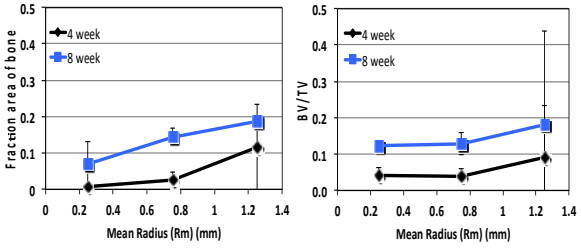
Methods: Prior to reaction with the PUR binder, BG particles were functionalized with the silane-coupling agent 3-aminopropyl-triethoxysilane, as well as surface grafting of polycaprolactone (PCL)[6, 7]. Biocomposites were prepared from a lysine triisocyanate– poly(ethylene glycol) prepolymer, iron (III) acetylacetonate catalyst, PCL triol (Mn ~300 g mol^{-1}), and surface-modified BG (56.7 volume %). Mechanical testing was completed in compression and torsion modes. For the *in vivo* study, a 3 mm diameter x 5 mm length unicortical defect was created in the diaphysis of the rat femur. Fabricated biocomposites were injected into the defect, and allowed to cure *in situ*, followed by wound closure. Rats were sacrificed 4 and 8 weeks after implantation (n=5). After harvesting the defects, X-ray microtomography (μCT) images were taken. Radial analysis, by cylindrical tubes, was conducted for μCT evaluation. After fixation, the specimens were embedded in poly(methyl-methacrylate), ground-sectioned (50 μm), and stained with Sanderson's Rabid Bone Stain in conjunction with Von Gieson Solution. The amount of bone growth in the biocomposite area was quantified, in a radial fashion, in order to evaluate balanced bone remodeling.

Results: As shown in Figure 1A, 4-weeks after implantation there was substantial cellular infiltration into the surface-modified BG/PUR biocomposite and modest new bone formation (red). After 8-weeks (Figure 1B), appositional bone growth was seen near the BG particle and PUR surfaces throughout the entire BG/PUR biocomposite. No signs of prolonged negative inflammatory response that hindered bone growth were observed. As show in Figure 2, radial quantification of fraction area bone and bone volume fraction (BV/TV) by histomorphometry and μCT, respectively, within the biocomposite showed comparable values and an increase in bone present between the 4- and 8-week time points. At a mean radial distance (Rm) of 1.25 mm, the fraction area of bone (and BV/TV) increased from 0.11 (0.13) to 0.19 (0.18) between the 4- and 8-week time points.



A B

Figure 1. Thick (50 μm) decalcified sections of BG/PUR biocomposite in femoral plug defects in rats stained with RBS and von Gieson solution, imaged at 2X magnification. (A) 4- and (B) 8-weeks after implantation.



A B

Figure 2. Radial bone growth quantification in biocomposite region by (A) histomorphometry and (B) μCT analysis. Rm from center of defect.

Conclusions: BG particles delivered via the surface-modified BG/PUR biocomposite did not induce a negative inflammatory response *in vivo*. After 4-weeks, the low porosity (<10%) BG/PUR biocomposite allowed for cellular infiltration via creeping substitution. After 8-weeks, BG/PUR biocomposite supported balanced remodeling within a rat femoral condyle plug defect, as supported by histomorphometric and μCT analysis of bone growth within the biocomposite region.

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