Effect of Calcium Phosphate Coatings and Bone Morphogenetic Protein (BMP)-2 on In Vivo Bone Regeneration using 3-dimensional Poly (propylene fumarate) Scaffolds in Rabbit Calvarial Model

Mahrokh Dadsetan[1], M. Brett Runge[1], Teja Guda[2], Pamela R. Brown Baer[2], Dindo Mijares[3], David T. Silliman[2], Lichun Lu[1], Racquel Z. LeGeros[3], Joseph C. Wenke[2], Michael J. Yaszemski[1]

[1]Biomaterials and Tissue Engineering Laboratory, Mayo Clinic, Rochester MN, [2]United States Army Institute of Surgical Research, Fort Sam Houston, TX, [3]Department of Biomaterials & Biomimetics, New York University, New York, NY

Statement of Purpose: Hydroxyapatite and phosphate-containing coatings have been studied extensively to better integrate biomaterial implants with bone for applications such as hip replacement, dental implants and screws for fracture fixation. These coatings provide a bone-like mineral matrix that stimulates the in vivo bone environment. In this work, we have investigated the effect of calcium phosphate coatings on surface property of poly (propylene fumarate) (PPF) scaffolds and the effect of surface coatings on release kinetics of recombinant human bone morphogentic protein-2 (rhBMP-2) has been evaluated. In the present study, calcium coated scaffolds loaded with rhBMP-2 were implanted in critical size rabbit calvarial defects to assess bone regeneration in vivo.

Methods: PPF with a number average molecular weight (Mn) of 2000 was used for fabrication of 3D scaffolds via stereo-lithography technique. The 3D PPF scaffolds with the dimension of 15.2 mm x 2.5 mm were surface coated with three different coating materials: carbonate hydroxyapatite (synthetic bone mineral, SBM), magnesium substituted β- tricalcium phosphate (β-TCMP), and biphasic calcium phosphate (BCP), a mixture of hydroxyapatite (HA) and β- tricalcium phosphate (β-TCP). The coated scaffolds were characterized using scanning electron microscopy (SEM), energy disperse diffraction analysis (EDXA), and X-ray photoelectron spectroscopy (XPS). RhBMP-2 at the concentration of 0, 50, and 100μg was then loaded on scaffold surfaces using bovine collagen solution (3mg/mL) and implanted in rabbit calvarial defects.

Rabbit Surgery: To assess the healing potential of the calcium coated PPF scaffolds, critical-sized defects were created in adult female New Zealand White Rabbits according to a previously published protocol. PPF scaffolds with the three different calcium phosphate coatings and the three rhBMP-2 levels were implanted in 90 rabbit calvarial defects (n=10 scaffolds per group for each of the nine groups). After 6 weeks, scaffolds were harvested and scanned using micro-computed tomography (micro-CT) and processed for histology.

Results: SEM pictures reveal that surface coating alters surface topography (Fig. 1, upper panel). Surface analysis of scaffolds after coating using EDXA confirmed the presence of calcium and phosphate on surfaces. All scaffolds revealed an initial burst release of rhBMP2 in the first three days (about 90%) followed by slow release up to 42 days. The initial burst release was greatest on scaffolds with BCP coating compared to other tested scaffolds. Micro-CT images after 6 weeks implantation show an increased bone formation on scaffolds with 50 and 100 μg rhBMP2 (Fig. 1, lower panel). Figure 2 shows the percent bone volume in the entire defect normalized to the void volume of the porous PPF scaffolds. The data in this figure show that scaffolds without BMP have 36% bone volume. The addition of 50 and 100 μg of BMP to the scaffolds increased the bone volume to 69% of the void space available for bone growth. The bone volume was similar on scaffolds coated with SBM and β-TCMP. No significant difference was observed between the coatings and BMP dosages on these scaffolds. The bone volume was slightly less on scaffolds coated with the BCP.

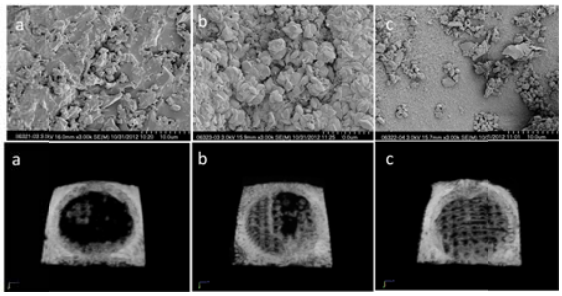

Figure 1. SEM images of coated scaffolds (upper panel): a) SBM, b) β-TCMP, c) BCP. 3D micro-CT images of scaffolds with SBM coating after 6 weeks implantation in the presence of varying amounts of rhBMP2 (lower panel): a) 0 μg, b) 50μg, c) 100μg.

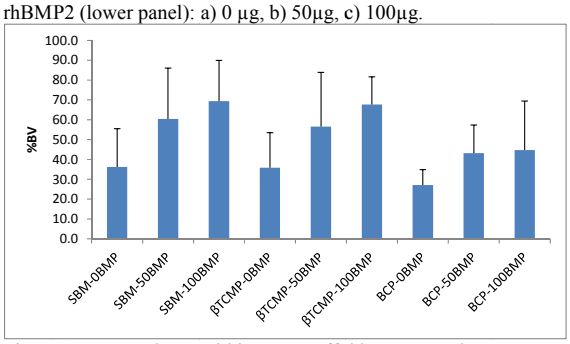

Figure 2. Bone volume within PPF scaffolds, expressed as percentage of available volume in scaffold. Data present average ±standard deviation.

Conclusions: The calcium phosphate coated PPF scaffolds fabricated via stereolithography have interconnected pores, appropriate mechanical properties for the treatment of segmental skeletal defects, and are promising candidates for further scaffold development. Our findings in this work demonstrate that a combination of calcium phosphate coating and BMP controlled delivery improves the in vivo performance of 3D porous PPF scaffolds.

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