3D Polymer Reinforced Calcium Phosphate Cement Scaffolds for Bone Tissue Engineering

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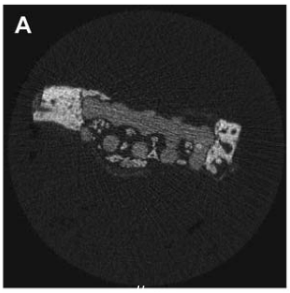
Statement of Purpose: Although promising results have been achieved in bone tissue engineering, the search for better scaffold materials continues. Calcium phosphate cements are a particularly interesting option, as they possess many advantages (e.g. compositional similarity to bone, osteoconductivity, resorbability, injectability). Importantly, due to their injectable nature, calcium phosphate cements are amenable to rapid prototyping methods of scaffold fabrication (e.g. indirect casting), which allows for precise control over the 3D scaffold architecture and, potentially, customization. While the utility of calcium phosphate cements as scaffold materials has previously been hampered by their poor mechanical properties, we recently developed a novel method of 3D scaffold reinforcement by polymer infiltration and *in situ* curing [1]. Our reinforcement method significantly enhances calcium phosphate cement mechanical properties, and is the only polymer reinforcement method suitable for augmenting the mechanical properties of 3D calcium phosphate cement scaffolds prepared by indirect casting. To further evaluate the utility of our scaffold fabrication method, the purpose of this study was to investigate the ability of a degradable 3D polymer reinforced calcium phosphate cement scaffold to facilitate bone regeneration *in vivo*.

Methods: 3D polymer reinforced calcium phosphate cement scaffolds (diameter = 9 mm; height = 3 mm) consisting of orthogonally intersecting cylindrical beams (diameter = 1 mm; x-y spacing = 750 μm; z spacing = 500 μm) were prepared by indirect casting followed by polymer infiltration and *in situ* curing, as we previously described [1]. The cement component of the scaffold consisted of dicalcium phosphate dihydrate, which is a highly resorbable calcium phosphate. The reinforcing polymer was poly(propylene fumarate), a strong, slowly degrading polyester, crosslinked with N-vinyl pyrrolidinone. The reinforced scaffolds were evaluated *in vivo* using a rabbit calvarial defect model. Briefly, 10 mm diameter circular defects were created in the parietal bones of 6 adult male New Zealand white rabbits using a trephine bur. Each animal received one scaffold seeded with allogeneic rabbit bone marrow derived mesenchymal stem cells (MSC) using a type I collagen gel carrier, and one control scaffold without MSC. Bone formation was evaluated after 6 weeks by microCT and quantitative histomorphometry. Statistical analysis was performed by ANOVA ($\alpha = 0.05$) and post hoc Tukey comparisons. A balanced block design was used to account for variability between experimental animals.

Results: The presence of the scaffold within the defect was still noted after 6 weeks. The scaffold was easily distinguished from bone by microCT based on its lower density. Notably, although a foreign body reaction against the scaffold was observed, significant amounts of bone were observed to be present within the scaffold pores by both microCT (Figure 1A) and histology (Figure 1B). MicroCT analysis revealed that the addition of MSC did not significantly affect the extent of bone formation, as the thresholded volume within the defect (i.e. scaffold + new bone) for MSC seeded and control scaffolds was calculated to be 113.31 ± 22.87 mm^3 and 127.00 ± 17.32 mm^3, respectively. This result was corroborated by histomorphometry. Nevertheless, comparison of histomorphometric measurements of sections taken at different regions in the scaffold revealed that significantly more bone was formed in the outer regions of the scaffold. Only 5-10% of the available defect area was filled with bone in the center of the scaffold, whereas roughly 20% of the available area was filled with bone in peripheral regions. This result suggests that in-growth from the surrounding tissue was the primary mechanism of bone formation.



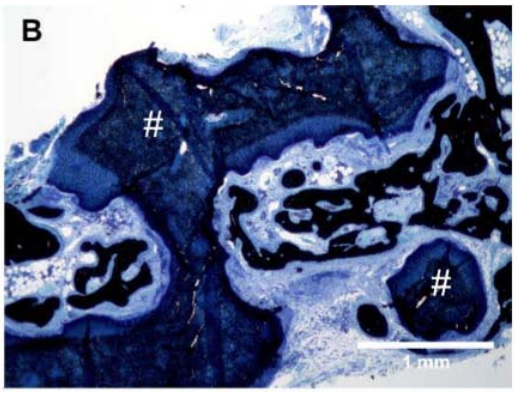


Figure 1. MicroCT (A) and histology (B) of scaffolds implanted for 6 weeks. The scaffold is marked with #. Bone, which was more dense than the scaffold in the microCT and stained black in the histology, was observed within the scaffold pores.

Conclusions: This study describes the application of a 3D polymer reinforced calcium phosphate cement scaffold for bone tissue engineering. Although the addition of MSC did not significantly enhance the amount of bone formation, large amounts of bone were observed to grow into the scaffold from the surrounding tissue after just 6 weeks. This study provides an important foundation for future research on 3D polymer reinforced calcium phosphate cement scaffolds.

References: 1. Alge DL, Chu T-M. J Biomed Mater Res Part A. 2010; 94(2):547-55.