Design and Characterization of 3D Printed Collagen-Calcium Phosphate Composites for Bone Defect Repair

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Introduction: Autografts are the gold standard in bone reconstruction; however, they have critical disadvantages including limited availability and donor-site morbidity. Synthetic alternatives to bone grafting are promising but have yet to be widely adopted clinically. Calcium phosphate cements (CPCs) make up a significant portion of commercially available bone graft substitutes, but have limitations in their current forms. Additive fabrication methods such as 3D inkjet printing have been used to produce CPCs [1] and offer advantages over injection molding, such as complex medical image-guided geometries. Collagen has previously been added into injection-molded CPCs to improve mechanical properties and cellular interaction [2]. In this study, we developed and characterized novel 3D printed collagen-CPC composites for translation to preclinical murine bone reconstruction studies.

Methods: Bone scaffold materials were fabricated by 3D printing with tricalcium phosphate powder that was bound into cement with phosphoric acid delivered by inkjets (ZPrinter450, 3D Systems). The geometrical 3D printing accuracy was optimized by varying the powder particle size from 30-150 μm. Printed samples were imaged by micro-computed tomography to determine geometric accuracy, porosity and pore size. Collagen was directly integrated into the cement during the 3D printing process via the inkjets. The materials were loaded in 3-point flexure to determine whether collagen affected the mechanical properties. Biocompatibility was assessed by culturing C3H/10T1/2 cells on the printed materials and measuring cell viability with a live/dead assay (MultiTox-Fluor, Promega) after 24 and 72 hours.

Results: The optimal particle size range for the TCP powder was 30-70 μm based on minimizing the volumetric error of printed samples compared to the ideal computer image. This particle size range results in a volumetric porosity of 27% ± 1.3% and a mean pore size of 46 ± 2 μm in the 3D printed CPCs. Direct incorporation of collagen during the 3D printing process significantly increased the material flexural strength as a linear function of collagen concentration (Fig. 2A). C3H/10T1/2 mesenchymal stem cells maintained significantly higher viability on collagen-CPC composites compared to CPC alone (Fig. 2B). Further, we demonstrated the feasibility of 3D printing scaffolds that represent a murine femoral mid-shaft with a 500 μm diameter intramedullary canal. The bone regeneration potential of these scaffolds is currently being studied in a critically sized murine femoral defect model.

Discussion: Volumetric integration of collagen in 3D printed CPCs has important implications for enhancing efficacy as bone graft substitutes. Collagen incorporation into hand mixed CPCs has previously been shown to improve cellular attachment, viability, proliferation, and activity as well as mechanical properties [3-5], but a 3D printed CPC-collagen composite has not been attempted to our knowledge. Our data demonstrate superior cellular and mechanical performance with collagen printing via the cement binder solution. The inherent porosity of these materials is critical for fluid flux, cellular infiltration and complete replacement of the biodegradable scaffold with new bone. Studies are currently underway to assess the efficacy of 3D printed CPCs with or without collagen in regenerative repair of 2 mm murine femoral defects. Early, longitudinal x-ray based observations suggest similar mineralized callus formation and bony bridging compared to devitalized allografts after 5 weeks of healing, which supports that these materials may be a good alternative to bone grafts.

Significance: Inkjet printing of collagen solutions with high resolution is technically challenging and has not been previously utilized in 3D printing of calcium phosphate cements. This study demonstrates the feasibility of these processes, the in vitro benefits of volumetric collagen inclusion, and the potential for improved synthetic alternatives to bone allografts.

Acknowledgments:

Figure 1. Scanning electron micrographs demonstrate a 3D printed mouse femoral scaffold (A) and a cell attached to a collagen-CPC composite surface (B).

Figure 2. Addition of collagen in 3D printed CPCs significantly improves strength and biocompatibility. A) 3-point flexural strength increases linearly with collagen concentration according to a regression analysis. * denotes $p<0.05$ by ANOVA with Tukey's post-hoc test. B) Cell viability (live/dead signal) is significantly higher on CPCs with collagen (CPC+Col) as analyzed by ANCOVA with culture time as the covariate (# $p<0.05$).

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