In Situ Bioprinting of Bone Tissue Constructs

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Abstract: Although relatively nascent, surgical robotic technology has been shown to improve dexterity and facilitate surgeries that were previously unfeasible [1]. In the same vein, 3D bioprinting for biomedical applications allows precise placement and patterning for shear-thinning biomaterials such as hydrogels. *In-situ* bioprinting lies at the crossroads of these two disciplines. Current scaffold development for *in-vivo* bone regeneration applications generally need post-processing (crosslinking, morphological manipulation) in order to achieve biocompatible, structurally stable constructs. Many groups have developed a novel injectable hydrogel to allow the bioprinting, crosslinking at body temperature, and biocompatibility after implantation. Bone morphogenetic proteins (BMPs) have been shown to aid in the development of postnatal bone formation [2]. In this study, new printable bio-ink including BMP2 was developed and bioprinted directly on the critical-size calvarial defect on rats in an operation room using the Multi-Arm Bioprinter [3]. Bone regeneration was then analyzed from micro-CT (µCT) scanning results of the rat skulls at six weeks. From these results, novel printable bio-ink is suitable for *in-situ* bioprinting applications to facilitate increased bone regeneration.

Methods: PEI-pDNA polyplexes for *in-vivo* bio-ink was prepared based on 50 µg plasmid per defect in bio-ink solution. First of all, PEI (2 mg/ml) and pDNA solutions (2 µg/µl) were complexed and freeze dried day before printing process and then combined with β-Glycerophosphate disodium salt hydrate (βGP) mixed with chitosan solution (CS-βGP). Collagen (Coll) (extracted from rat tail [4]) was dissolved in acetic acid to prepare a Coll solution, and then mixed with ratio of 4:1 (v/v) (CS- βGP:Coll). Additionally, collagen sponges were added to the overall solution and mixed for 30 min. at room temperature until a homogenous solution was achieved. Finally, hydroxyapatite (HA) nanoparticles in powder form was added to the final solution and mixed for 15 min.

Results: The scaffold architecture had a circular pattern with 0.3 mm pore size and 0.3 mm filament diameter for *in vivo* study. *In-situ* bioprinting process was occurred after opening 5 mm-diameter 2 mm-thick two defects on rat skulls. Then, the bio-ink was bioprinted into the defect sides. Afer 6 weeks of bioprinting, calvarium tissues were harvested to measure bone volume fractions via µCT scanning (see Fig. 1). Scanned images were imported to Avizo software to calculate bone volume fraction.

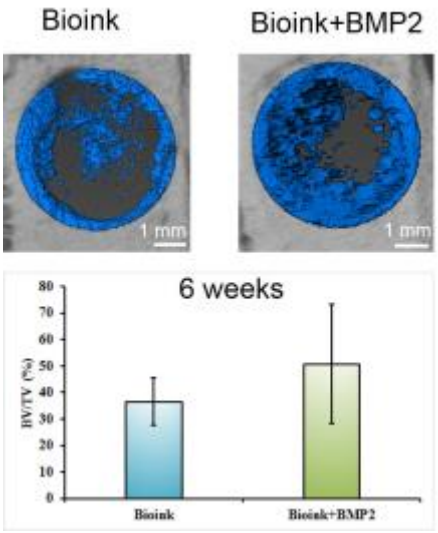

Figure 1. Results of bone volume fraction

Conclusions: New bio-ink including BMP2 was able to bio-print and support bone regeneration over time by providing newly-formed mineralize tissue. BMP2 incorporation was demonstrated increased bone formation compared to the only bio-ink scaffolds.

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