Bone Regenerative Capacity of 3D Printed Bioactive Ceramic Scaffolds Coated with Bioactive Molecule: Dipyridamole

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INTRODUCTION: Extensive defects of the upper extremity cause significant patient burden, including disability and social stigma. For example, 500,000 bone defects are reconstructed annually in the United States alone at a cost of ~$2.5 billion, due to factors including donor site harvest and lengthy operative times. Bone defects >5cm are usually reconstructed with autologous vascularized bone transfer (bone from another region of the patient's body is harvested to replace the defect) (1, 2), but limitations include donor site morbidity, infection, and delayed healing. These limitations drive innovation of biomaterial applications, but a tissue engineered approach to reconstruction remains elusive (3). The objective of this study was to assess the efficacy of 3D printed bioactive ceramic (3DPBC) scaffolds augmented with dipyridamole (DIPY), an indirect $A_{2A}R$ agonist known to enhance bone formation (4), to stimulate bone regeneration of a critical-sized defect of the radius in an in vivo translational model.

METHODS: A three-dimensionally (3D) printed bioactive ceramic (3DPBC) scaffold was utilized to repair critical sized long bone defects *in vivo*. In this study, 3DPBC scaffolds were fabricated in a two-piece system (**Fig. 1a**). Following IACUC approval (NYU IACUC #: IA16-01391) critical-sized full thickness (~7xm x full thickness) defects were created in the tibia diaphysis in *Ovis aries* (sheep) (N=8). The 3DPBC scaffold composed of β tri-calcium phosphate (β-TCP) was placed into the defect site, along with an intramedullary rod (**Fig. 1b**) and animals were euthanized 24 weeks post-operatively; the tibia were retrieved, *en bloc*, for micro-CT, histological and mechanical analysis. Bone growth was assessed exclusively within scaffold pores and evaluated by microCT and advanced reconstruction software. Biomechanical properties were evaluated utilizing nanoindentation to assess the newly regenerated bone for elastic modulus (E) and hardness (H).

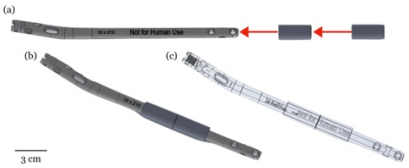

Figure 1: (a) CAD rendering of the intramedullary rod and two-piece scaffold. (b and c) schematic of the placement of the scaffold around the IM nail during the surgical procedure

RESULTS: MicroCT reconstructions illustrated bone in-growth throughout the scaffold, with an increase in bone volume dependent on the Dipyridamole dosage (**Fig. 2**). Qualitative evaluation of the histological micrographs indicated directional bone ingrowth of bone, with an increase in bone formation toward the native bone morphology. Extensive bone formation with signs that scaffold has significantly resorbed, presenting areas of extensive structural discontinuity resorption was observed at both low and high magnifications. Histological micrographs (**Fig. 2**) high magnification to better appreciate the features of the newly regenerated bone within the scaffold. Furthermore, qualitative evaluation did not yield any exuberant bone growth and the newly regenerated bone was limited to the defect and the scaffold regions

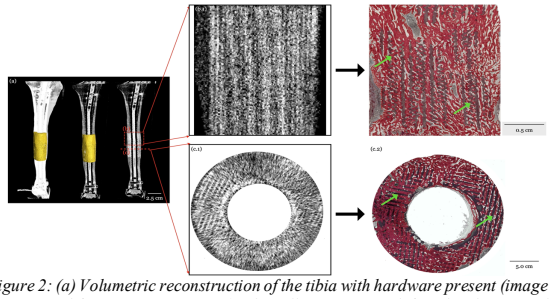

Figure 2: (a) Volumetric reconstruction of the tibia with hardware present (image re-purposed from previous reports). The yellow portion in left and right in panel (a) represent the newly regenerated bone; (Center) radiographic longitudinal and transverse cross-section taken from CT scans, with complementary histological slices, far right. provided in (b.2) (the brighter white regions are the remaining scaffold, while in the dark/black regions in histological micrographs are the scaffold)

DISCUSSION: Our previous studies using a smaller preclinical model, rabbit, yielded favorable results, where with the implantation of a custom-fit 3D printed resorbable bioactive ceramic scaffolds into critical size radius defects resulted in bone morphology that remarkably resembled the original bone segment with a haversian cortical shell presenting cortical-like mechanical properties and associated marrow space. The application of this β-TCP scaffold has the potential for successful treatment outcomes while potentially minimizing the amount of surgery and less time spent in hospitals for individuals that would not fully recover from injury though current technology and treatment strategies available.

SIGNIFICANCE/CLINICAL RELEVANCE: Custom engineered, biocompatible and resorbable, β-TCP scaffolds treated with DIPY demonstrated to have an increased bone regeneration qualitatively and quantitatively. The custom approach and expedited healing has the potential to positively benefit the patient in terms of lowering health care costs and patient's quality of life, as well as returning to form and function.

REFERENCES: [1] Houdek+Semin Plast Surg, 2015; [2] Wood+Mayo Clin Proc, 1985; [3] Kinoshita+ScientificWorld Journal, 2013; [4] Mediero+FASEB J, 2015

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