Evaluation of a Pre-Vascularized Osteoinductive Polymeric Scaffold for Bone Tissue Regeneration

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Statement of Purpose: Bone loss and skeletal deficiencies due to traumatic injury, abnormal development, or cancer are major problems worldwide. In the U.S., over 3 million orthopedic procedures are performed annually; approximately 500,000 of these are bone-grafting procedures[1]. With the best options (autograft and allografts) having serious problems there is a critical need for a new type of bioactive implant. Bone is a complex tissue composed of 2 basic structures, trabecular bone and cortical bone. Most tissue-engineered (TE) grafts for bone replacement seek to replace only the trabecular bone, which leads undesirable bone mechanical properties. Another limitation of the current TE grafts is a lack of early vascularization *in vivo,* which is necessary for long-term graft viability. To overcome these issues seen with TE grafts, we have developed a composite synthetic bone graft, which mimics native bone architectures and promotes the differentiation of human mesenchymal stem cells (hMSCs) into osteoblasts and vascular endothelial cells simultaneously. We hypothesize that the joining of a porous trabecular scaffold with the addition of hydroxyapatite (HAP), pre-vascularized cortical bone scaffold, and HAP columns will promote differentiation of hMSCs into osteoblasts and vascular endothelial cells in appropriate areas in the scaffolds would be a viable bone replacement with enhanced long-term strength *in vivo.*

Methods: The novel composite scaffold is composed of electrospun FDA approved polymers poly-l-lactide (PLLA) and poly-d-lactide (PDLA), 10 % gelatin (from bovine skin), and inorganic calcium phosphate HAP to promote the differentiation of the hMSCs along the osteoblastic lineage. The trabecular scaffold was fabricated by electrospinning PDLA and PLLA and 15% HAP synthetic powder onto a rotating mandrel with salt crystals. The salt was then leached out yielding a porous structure. We fabricated the biomimetic cortical scaffold by electrospinning PLLA, PDLA and 10% gelatin onto rotating polyethylene oxide (PEO) and PLLA twist until an approximate scaffold diameter of 500µm was reached. PEO is used because it is extremely water-soluble. All scaffolds were crosslinked with the FDA approved enzymatic crosslinking agent transglutaminase (mTG) to increase strength and prevent gelatin leaching. After crosslinking, the salt was leached from the trabecular scaffold and the inner twist was dissolved to form the cortical scaffold to mimic the osteonic structure. Material characterization was performed using scanning electron microscopy (SEM), liquid extrusion porosimeter, and compression mechanical testing. To evaluate the biocompatible and osteogenic inducing capabilities of the trabecular scaffold, hMSCs were seeded on the scaffold and evaluated up to day 28 for alizarin red deposition, osteogenic markers expression and metabolic activity. Human umbilical vein endothelial cells (HUVECs) were seeded onto the cortical scaffold and then decellularized at day 14 using a three-step freeze-thaw method. Metabolic activity and collagen deposition of the HUVECs was evaluated to validate the freeze-thaw methods. hMSCs were then seeded onto the decellularize scaffold and evaluated at day 7 for expression of vascular endothelial markers.

Results: Material characterization confirmed the presence of HAP crystals in the trabecular scaffold with pore ranges necessary for neovascularization (5-10µm) and osteoblast infiltration (100-300µm) (Figure 1). *In vitro* analysis concluded the presence of HAP statistically increase the amount of hMSCs calcium deposition in comparison to the control scaffolds. SEM images of the fabricated osteon yielded an average inner diameter of 0.495±44µm, similar to a native osteon. Confocal images of the cortical scaffold prove the osteonic structure promotes endothelial development circumferentially. The decellularized scaffolds exhibited a 75% decrease in cellular viability but maintained the collagen and non-collagenous angiogenic proteins (Figure 2). Additionally, hMSCs seeded on the decellularized scaffold expressed vascular endothelial growth factor, an early angiogenesis marker, without the presence of any additional growth factors or enhancements (Fig. 3).

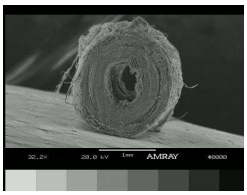

Figure 1. SEM image of cortical scaffold

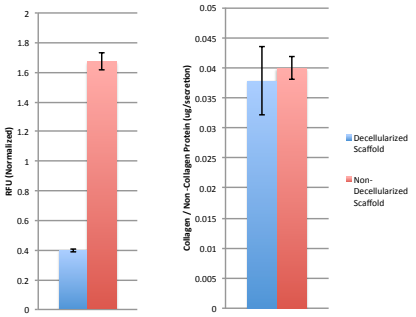

Figure 2. Cellular viability (left) and collagen/non-collagenous protein evaluation of decellularized and non-decellularized scaffolds

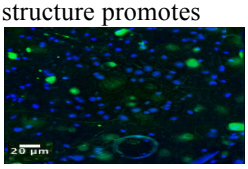

Figure 2: VEGF expression (green) of hMSCs seeded on decellularized cortical scaffold

Conclusion: Studies discussed demonstrate the scaffold ability to promote differentiate hMSCs down the osteoblastic and vasculature lineage. This technology is transformative because it will be the first synthetic bone graft to contain both trabecular and cortical bone structures, designed for vascularized bone growth and load-bearing applications.

References: [1] Bharat M. Desai, MD. Osteobiologics. Am J Orthop. 2007;36 (4 supp):8-11.