Functionalizing Intact Allografts to Enhance Allograft Remodeling and New Bone Formation

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Statement of Purpose: Structural bone allograft accounts for 40% of bone grafting procedures [1] but suffer from limited remodeling and new bone formation, which is associated with a clinical failure due to non-unions and late graft fractures. The purpose of this study was to enhance functionality of inert allograft by modifying the surface of the graft material with vascular endothelial growth factor (VEGF) and bone morphogenetic protein-2 (BMP-2), encouraging remodeling, host-allograft integration and overall wound healing. The rapid release of VEGF stimulates angiogenesis and, we hypothesize, osteoclastogenesis. Sustained release of BMP-2 enhances osteogenesis.

Methods: Femoral bone samples were harvested from Sprague-Dawley rats, cleaned of bone marrow, and autoclaved. 50:50 poly (lactide-*co*-glycolide) (PLGA) (Lakeshore Biomaterials, Inc. USA) was dissolved in a 1:8 (w/v) concentration with tetrahydrofuran [3]. The allografts were rinsed with and subsequently placed in polymer solution and stored at -20°C, followed by lyophilization. MicroCT images were taken of coated allograft and rendered into 3-dimensional images. VEGF (Sigma Aldrich, Inc.) was loaded by surface adsorption onto coated allografts and recombinant human BMP-2 (Genscript, NJ) was encapsulated within the coating by adding a concentrated factor solution to the polymer solution prior to allograft coating. The efficacy of VEGF (when combined with RANKL) to enhance osteoclastogenesis was evaluated by osteoclast formation and resorption using the RAW264.7, monocytic cell line. At day 14, cells were fixed and stained for the osteoclast marker tartrate-resistant acid phosphatase (TRAP) using a Sigma TRAP kit (Sigma, USA). Osteoclast resorption pits were imaged in hydroxyapatite-coated Corning Osteo-Assay Surface 24-well plates (Corning Life Sciences, USA) and quantified using the Von Kossa staining method [3]. Images were captured with a Zeiss observer Z.1 microscope (Carl Zeiss, USA) and analyzed using NIH ImageJ software. A pilot *in vivo* study was performed to qualitatively assess the bioactivity of the encapsulated BMP-2 by evaluating healing in a critical size femoral defect over 4 and 8 weeks. *Statistics:* Osteoclast quantification data was analyzed using one-way analysis of variance ($p<0.05$).

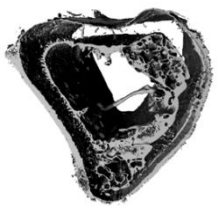

Figure 1. MicroCT image of allograft coating alone, with allograft removed from image showing thin, continuous polymer coating on both the endosteal and periosteal surfaces.

Results: MicroCT imaging revealed a continuous coating of polymer on both the periosteal and intramedullary surfaces of the allograft (fig.1). Release of VEGF was concentrated between days 3 to 14 with 96.8% of VEGF released during this time while release of encapsulated BMP-2 was more sustained over full 42-day release period. The *in vitro* data confirmed a statistically significant and dose dependent increase in TRAP positive multinucleated osteoclast cells in the presence of VEGF + RANKL vs. RANKL alone (fig.3a). The functionality of the osteoclasts differentiated from RAW 264.7 cells was confirmed by Von Kossa staining in which the white areas shown in figure 2 (e, f & g) are the resorption lacunae created by multinucleated osteoclasts. Percent surface resorbed area was quantified (not shown) and the group with VEGF released from the allografts revealed a higher percent of resorption area than both positive and negative control (RANKL alone and no RANKL, respectively). *In vivo* data revealed evidence of mineralized callus by day-14 radiographs of allografts loaded with BMP-2 and continued to develop over the 8-week period (fig. 3).

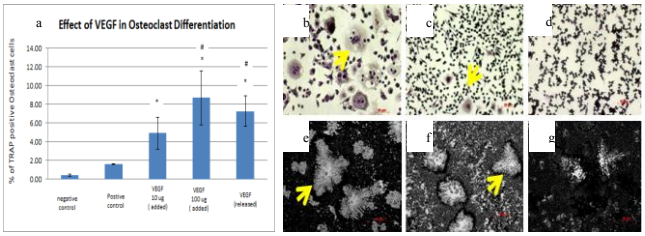

Figure 2. (a) Percentage of TRAP positive multinucleated cells in different groups. (#) and (*) indicate statistical significance between positive and negative control and those labeled, respectively. RAW264.7 cells were observed in the presence of VEGF surface adsorbed coated allograft placed in the transwell and RANKL solution (50 ng/ml) in the media (b and e), RANKL (50 ng/ml) in media (c and f) and media alone (d and g). The images were captured after 14 days of culture for TRAP staining (b,c,d) and after 21 days for Von Kossa staining (e,f,g).

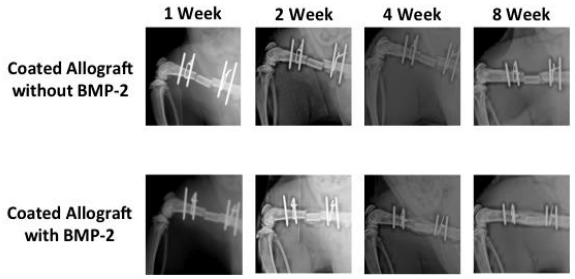

Figure 3. Radiographs of the rat femoral segmental defect at 1, 2, 4, and 8 weeks. Continuing formation of the mineralized callus observed in the BMP-2 loaded coated allograft confirm bone union at the host site.

Conclusions: This study has demonstrated the feasibility of using polymer coated allograft as a carrier for local controlled supply of growth factors that are imperative to enhance bone remodeling toward complete allograft incorporation. VEGF release is anticipated to encourage early vascularization, but our data also suggests it may also facilitate allograft resorption during healing. BMP-2 release has resulted in robust bone formation compared to naked allograft. Future studies will evaluate remodeling ability of functionalized allografts *in vivo* utilizing large scale segmental femoral defects in rats with simultaneous delivery of VEGF and BMP-2.

References: [1] Marino, J. Orthop Clin 2010: 41(1):15-26. [2]Petrie-Aronin C. Biomaterials. 2010:31:6417-24. [3] Kartner, N. J. Biol. Chem. 2010: 285:37476-90.