Biofunctionalizing Devitalized Bone Allografts through Polymer-Mediated Growth Factor Delivery

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Statement of Purpose: Structural bone allograft tissue accounts for over 40% of bone grafting cases **[1]** but has a history of poor integration with host bone and subsequent failure. Indeed as many as 30%-60% of allograft implants encounter some complication when evaluated at the 10-year mark **[2]**. Multiple surgical indications exist, however, that depend on the use and successful integration of structural allograft tissue. The purpose of this study was to impart bioactivity and therefore enhance functionality of the allograft by applying a thin polymeric coating capable of delivering bone morphogenetic protein-2 with both quantitative and temporal precision. This has the potential to enhance the utility of allografts in healing large scale bone defects.

Methods: *Bone Harvest:* Tibial and femoral bone samples were harvested from male and female Sprague-Dawley retired breeder rats (300-600g). The samples were cleaned of soft tissue and bone marrow, and were rinsed in chloroform for 12 hrs. The allografts were then autoclaved at 121°C and 15 PSI. *Allograft Coating:* Polymer coating of allografts was based on previous studies **[3]**. Briefly, 50:50 poly (lactide-*co*-glycolide) (PLGA) (Lakeshore Biomaterials, Inc. Birmingham LA) was dissolved in a 1:12 (w/v) concentration with methylene chloride. The allografts were rinsed with the polymer solution using a 3cc syringe, then submerged vertically in the polymer solution for 2 minutes. The samples were then placed in tubes filled with1 ml of polymer solution and stored at -20°C for 12 hours followed by lyophilization. *Microcomputed Tomography Imaging:* MicroCT images were taken of 5 mm segments of coated allograft and rendered into 3-dimensional images. Volume of polymer coating on the periosteal and intramedullary surfaces of the allograft was computed from microCT images. *Protein loading:* Recombinant human bone morphogenetic protein-2 (BMP-2) (Creative BioMart, NY) was loaded by surface adsorption onto coated allografts by placing them into the concentrated factor solution, frozen at -20°C for 12 hours, lyophilized for 24 hours, and then stored at -20°C. Mass of allograft samples was measured before and after polymer coating, and loading. *Release Study:* The coated allografts were placed in phosphate buffered saline (PBS) at 37°C under agitation. Samples of buffer were taken at 1, 2, 4, 6, 8, and 24 hours, and at 3, 5, 7, 10 days. The release of the protein was analyzed using the Quantikine human BMP-2 Immunoassay kit (R &D systems, Minneapolis, MN). Two experimental groups were evaluated over 10 days; coated allograft with BMP-2 loaded, and coated but unloaded allograft. *Statistics:* Data was analyzed using one-way analysis of variance ($p<0.05$).

Results: MicroCT imaging revealed a continuous coating of polymer on both the periosteal and intramedullary surfaces of the allograft segment ranging from 25-100µm thick (figure 1). Analysis of MicroCT imaging indicated that the distribution of polymer coating, while trending toward more on the periosteal portion of the allograft, was not statistically different between the two surfaces (figure 2). Analysis of BMP-2 release from the surface of the coated allograft indicated an early peak in factor delivery,

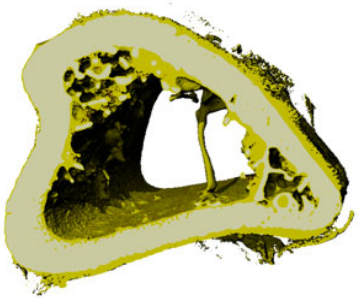

Figure 1. MicroCT rendered images showing polymer coating on inner and outer surfaces of allograft (darker yellow color). Coating thickness ranges from 25-100µm in thick-ness.

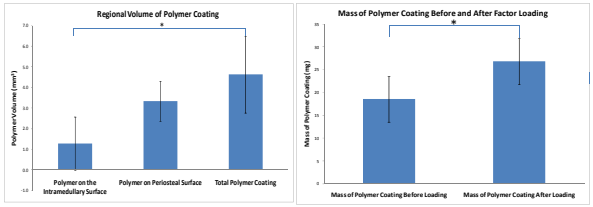

Figure 2. Distribution of polymer coating between periosteal and intramedullary surfaces indicates no significant differences in volume (left). Addition of growth factor to coating increased the mass of coating on the allograft (right).

but sustained release over the first 10 days of incubation (figure 3).

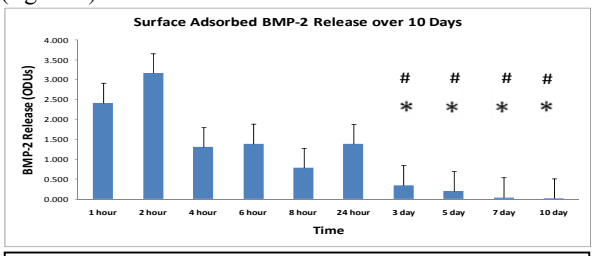

Figure 3. Release of surface adsorbed BMP-2 over 10 days shows an early peak and gradual decrease in quantity. (#) and (*) indicate statistical significance between 1 hour and 2 hour time point and those labeled, respectively.

Conclusions: This study has demonstrated the feasibility of imparting biofunctionality to devitalized allograft through the addition of a thin factor-loaded polymer coating while maintaining the inherent structure of the allograft. Future studies will evaluate this potential by delivering separate growth factor systems via surface adsorption and encapsulation, and in vivo potential of this approach to enhance allograft incorporation with host bone.

References:[1]Editor. Orthop Network news. 1999;10:10-17. [2]Wheeler DL. Clin Orthop Rel Res. 2005:435:36-42 [3]Petrie-Aronin C. Biomaterials. 2010:31:6417-24.