Enhancing Bone Allograft Integration Through Local Delivery of Sphingosine 1-phosphate Receptor Targeted Drugs

Cynthia S. Huang,[1] Sunil S. Tholpady,[2] Edward A. Botchwey.[1,3]
[1] Department of Biomedical Engineering, [2] Department of Plastic Surgery, [3] Department of Orthopaedic Surgery; University of Virginia, Charlottesville, VA.

Statement of Purpose: Massive craniofacial and tibial allografts commonly can exhibit challenging complications. A crucial aspect to explore is the cleaning and processing of allografts to maximize osteoinductivity, biocompatibility, and structural integrity. Growing evidence suggests the largest barrier to successful allograft incorporation and sustained mechanical integrity is delayed or absent vascularization. Another concern is excessive immune response hindering the regenerative process or leading to rejection. To address this limitation, we designed a novel continuous polymer coating system to provide sustained localized delivery of FTY720, a selective agonist for sphingosine 1-phosphate (S1P) receptors, from calvarial allografts. S1P is an autocrine and paracrine signaling small molecule that impacts proliferation, survival and migration of endothelial cells, mural cells, osteoblasts, and osteoblastic precursors through a family of G protein-coupled receptors ($S1P_{1\text{-}5}$). In this study, we evaluate two cleaning methods as well as the ability of FTY720, locally released from thin biomaterial surfaces, to improve allograft vascularization, mechanical integrity, osseous remodeling, and incorporation at the host-graft interface.

Methods: Allografts were cleaned and processed with one of two methods: (1) detergent, hydrogen peroxide, and 70% ethanol washes (DePaula CA. Cell Tissue Bank. 2005; 6:287-298.) or (2) Allowash XG® with LifeNet Health. Three types of cleaned Sprague Dawley calvaria pieces (~5x5x1 mm were implanted into in rat sub-periosteum: uncoated allograft , 1:12 PLAGA-coated, and 1:12 PLAGA-coated loaded with 1:200 FTY720 (w/w). Initial FTY720 release *in vitro* was quantified with a sphingosine kinase 2 assay. Osseous remodeling and host bone-allograft integration was monitored using microCT at week 0, 1, 2, 3, and 4. After 4 weeks post-op, calvarial grafts were excised for uCT and histological analysis. To quantify vascular remodeling response to FTY720, samples were stained for mature vessel lumens (α-SMA, lectin).

Results: Cumulative *in vitro* FTY720 release from a coated/loaded allograft in simulated body fluid with 4% FAF-BSA after a day reached biologically significant levels and had continuous gradual release. MicroCT evaluation (Fig 1.) and histology (Fig. 2) following three and four weeks of healing suggest significant enhancement of interfacial bone growth at the host bone-allograft interface in FTY720 treatment groups compared with unloaded controls, as well as increased vascularization suggestive of an angiogenic effect. Studies of human flat bone processed with method 1 or 2 implanted sub-periosteally are currently being conducted.

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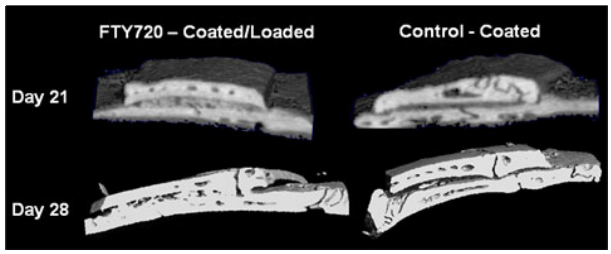

Figure 1. Assessment of Host Bone-Allograft Interface. Results from MicroCT demonstrate that the 1:12 PLAGA coated and 1:200 FTY720-loaded group had more bone growth at the interfacial surface at day 21 and continuing to day 28 compared to the 1:12 PLAGA coated group.

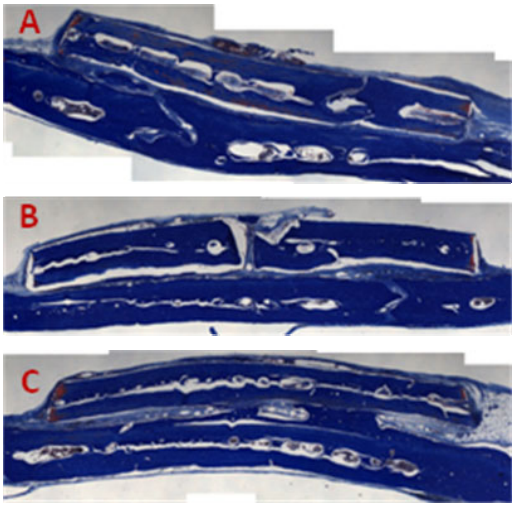

Figure 2. Masson's Trichrome of Allograft and Host Calvaria. FTY720 coated/loaded (C) showed higher interfacial bone growth than either the uncoated (A) or coated (B) allografts at day 28.

Conclusions: Poor vascularization coupled with inferior mechanical stability is the hallmark feature predicting long-term complication and poor functional outcome of massive bone allografts. Our studies confirm that local delivery of $S1P_1$/$S1P_3$ agonist, FTY720, enhances bone growth and vascularization. These results support the use of FTY720 delivery for promoting angiogenesis and improving the healing outcomes of bone tissue-engineered therapies.

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