The Essential Role of Sphingosine 1-Phosphate/S1P(3) Receptor in Enhanced Neovascularization and Mechanical Integrity of Massive Allografts: Pharmacological Manipulation to Improve Graft Incorporation

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Statement of Purpose: Massive structural allografts commonly used for limb salvage can exhibit challenging complications such as non-union or fracture. Growing evidence suggests the largest barrier to successful allograft incorporation and sustained mechanical integrity is delayed or absent vascularization. To address this limitation, we designed a novel continuous polymer coating system to provide sustained localized delivery of pharmacological agent, FTY720, a selective agonist for sphingosine 1-phosphate (S1P) receptors, within massive tibial defects. 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 ($S1P_{1\text{-}5}$). Previous studies showed sustained release of FTY720 from 2D biodegradable polymer films in the mouse dorsal skinfold window chamber promotes formation of new arterioles and structural enlargement of existing arterioles (Wamhoff BR. Arterioscler Thromb Vasc Biol. 2008;28:1454-1461). In this study, we evaluate 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: To evaluate PLAGA polymer's ability to coat all surfaces of the bone while maintaining an adequate pore structure, Sprague Dawley rat tibia were coated with various concentrations of PLAGA (polymer:solvent) and analyzed using the quantitative micro-computed tomography (microCT). Once the optimal polymer coating was determined, three different types of allografts were implanted into rat tibial defect model: uncoated allograft (U), 1:12 PLAGA-coated (C), and 1:12 PLAGA-coated loaded with 1:200 FTY720 (w/w) (C/L). Osseous remodeling and host bone-allograft integration was monitored using microCT at week 0, 2, 4, and 6. After 6 weeks post-op, tibia were excised and tested under compression in an Instron 4511 machine to determine the elastic modulus and ultimate compressive strength. To quantify vascular remodeling response to FTY720, tibia samples were stained for mature vessel lumens (SMA) and monocyte recruitment (CD45).

Results: Mechanical evaluation following six weeks of healing suggest significant enhancement of mechanical stability at the host bone-allograft interface in FTY720 treatment groups compared with unloaded controls (Fig. 1). Furthermore, significant enhancement in smooth muscle cell investment was evident in FTY720 treated groups compared with untreated groups. FTY720 significantly enhances the number of proliferating cells found on microvessels, suggestive of an arteriogenic effect (Fig. 2).

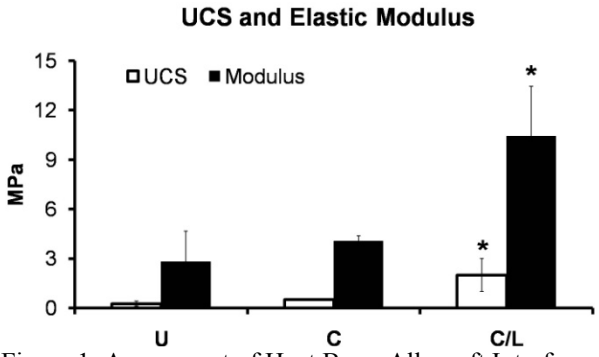

Figure 1. Assessment of Host Bone-Allograft Interface Mechanical Integrity. Results from Instron 4511 demonstrate that the 1:12 PLAGA coated and 1:200 FTY720-loaded (C/L) group had a significantly higher UCS and elastic modulus in comparison to the uncoated (U) and 1:12 PLAGA coated group (C) groups ($p < 0.05$ for modulus, $p = 0.081$ for UCS).

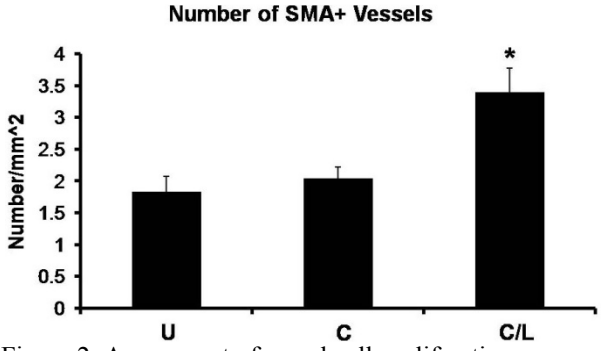

Figure 2. Assessment of mural cell proliferation on expanding microvessels. Immunohistochemical results demonstrate that the C/L group had a significantly higher number of SMαA+ cells compared U and C groups ($p < 0.05$).

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, significantly enhances the number of smooth-muscle-invested vessels within allograft tissue sections and increases elastic modulus and ultimate compressive strength host-graft constructs. These results support the use of FTY720 delivery for promoting angiogenesis and improving the healing outcomes of bone tissue-engineered therapies.