Sphingosine 1-phosphate receptor three regulates implant arteriogenesis by recruitment and localization of anti-inflammatory monocytes to surrounding microvessels

Anthony O. Awojoodu[1], Lauren S. Anderson[2], Kyle Martin[3], Shayn M. Peirce-Cottler[2] Edward A. Botchwey[1]
[1]Department of Biomedical Engineering – Georgia Institute of Technology, Atlanta, GA
[2]Department of Chemical and Biomolecular Engineering – Lafayette University, Eaton, PA
[3]Department of Biomedical Engineering – The University of Virginia, Charlottesville, VA

Statement of Purpose: Arteriogenesis is the process by which new arterioles form and existing arterioles remodel, increasing the number and diameter of resistance vessels in a tissue. The development of materials that stimulate arteriogenesis is a critically important clinical need with potential to dramatically improve healing outcomes and long-term functionality of tissue-engineered implants. Implantation is followed by recruitment of inflammatory cells that often leads to fibrous encapsulation. Two distinct sets of monocytes contribute to this response: classically activated inflammatory monocytes (IM) and alternatively activated anti-inflammatory monocytes (AM), which potently induce blood vessel growth[1]. Implants that specifically recruit these regenerative cells to remodeling vessels can maximize blood vessel maturation and enhance overall healing outcomes. Sphingosine 1-phosphate (S1P) is a pleiotropic, autocrine and paracrine signaling molecule that binds to a family of 5 high affinity G-coupled receptors ($S1P_1$-$S1P_5$) to direct a wide range of biological processes. We have shown that activation of S1P receptors 1 and 3 enhances microvascular remodeling[2]. The objective of this study was to interrogate the affects of S1P receptor signaling on the regulation of inflammation and implant arteriogenesis.

Methods: FTY720 ($S1P_1$/$S1P_3$ agonist) was encapsulated in 50:50 poly(lactic-co-glycolic acid) (PLAGA) and delivered in the murine dorsal skinfold window chamber (backpack) or spinotrapezius ligation model to CX3CR1-eGFP or WT C57Bl/6 mice. Image J and MATLAB were used to quantify vessel density, tortuosity and cell proximity in remodeling vessels. Flow cytometry for antibodies against CD45, CD11b and Ly6C were used to identify monocytes in tissue. Luminex based cytokine bead assays were performed to detect concentrations of cytokines from biological specimens.

Results: 7 days after spinotrapezius ligation $CX3CR1^{high}$ AM, were recruited to remodeling vessels in eGFP-CX3CR1 mice treated with FTY720 (Fig. 1a) and were often perivascular (Fig. 1a insert). FTY720 significantly enhanced the tortuosity of vessels, a classic sign of arteriogenesis, as well as $CX3CR1^{+}$ AM associated with them (Fig. 1a). Flow cytometry was used to quantify the proportion of AM and IM in tissue surrounding implants and FTY720 decreased the proportion of IM in tissue (12%) compared to PLAGA (19%) but increased the proportion of AM (10% compared to 7%) relative to unloaded implants. Furthermore, many inflammatory cytokines were significantly elevated in tissues surrounding polymer implants in dorsal tissue above sham but FTY720 significantly decreased the expression of inflammatory chemokines (TNF-a, MIP-1, MCP-1, IL-6, IL-1B) known to attract IM (Fig. 1b). These remodeling results were dependent on $S1P_3$ on marrow-derived and local cells as the day 3 FTY720-induced vascular length density in $S1P_3^{-/-}$ bone marrow mice (17%) and $S1P_3^{-/-}$ mice with WT marrow (28%) were significantly less than in WT mice (114%) (Fig. 1c).

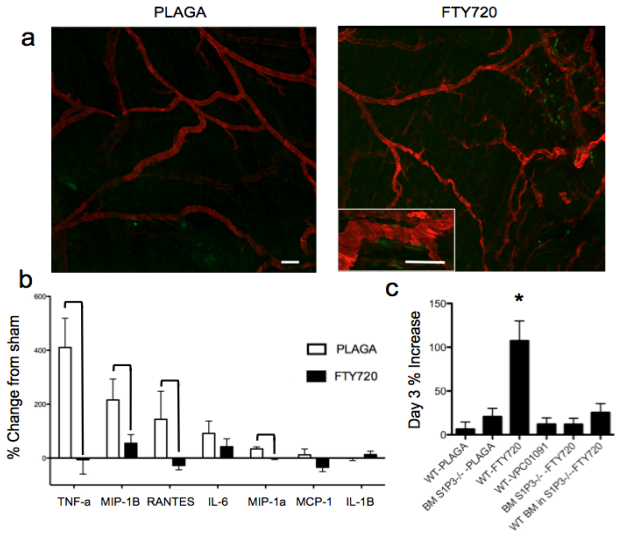

Figure 1. a) FTY720 enhances recruitment of CX3CR1+ monocytes in spinotrapezius muscle. b) FTY720 significantly reduced inflammatory cytokines in tissue. c) $S1P_3$ activation enhances microvascular length density

Conclusions: Arteriogenesis can be enhanced by local $S1P_3$ activation which significantly decreases the secretion of inflammatory cytokines leading to enhanced AM, and reduced IM, recruitment to remodeling vessels in muscle and soft tissue. This regulation of inflammation promotes significant vessel expansion and maturation in inflamed and ischemic microvascular networks. Novel strategies, like this, to promote tissue regeneration through regulating inflammation can enhance healing outcomes mediated by biomaterial implants.

References:

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2) Sefcik LS et al. *Tissue Eng Pt A*, 2011, 17, 617-629

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