S1P Receptor Specific Drug Enhances Mandibular Defect Healing by Modulating Local Inflammation, Enhancing Neovascularization and Increasing Progenitor Cell Recruitment.

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Statement of Purpose: There is a tremendous need for augmentation of bone tissue repair and growth in an array of clinical situations, which include trauma, cancer and congenital defects. Critical size defect (CSD) healing of the mandible presents challeges unique to its location and daily use. S1P is a pleiotropic, autocrine and paracrine-signaling, sphingolipid that is released into the blood upon platelet activation and that binds to a family of five high affinity G-coupled receptors ($S1P_1$-$S1P_5$) to direct a wide range of biological processes triggered during endogenous bone wound healing. We used a rat mandibular defect model and delivered a S1P receptor specific drug, FTY720, in a nanofiber scaffold to accelerate healing. FTY720 been shown to promote neovascularization[1] and osseous tissue in-growth into critical sized defects in both rat cranial and long bone models[2]. We have previously shown that allografts coated with FTY720, accelerates cranial CSD healing by modulating the local inflammation response and enhancing vascularization[3].

Methods: Transwell assays were used to assess the effect of FTY720 on the recruitment of osteoprogenitor cells. FTY720 loaded and unloaded PCL/PLAGA nanofibers were implanted in a dorsal window chamber mouse model and the phenotype of the macrophages recruited to the tissue was evaluated with flow cytometry to examine the effect of FTY720 on local inflammation modulation.

5 mm defects created in WT Sprague Daley rats. Were treated with FTY720 loaded or unloaded PCL/PLAGA nanofibers. Bi-weekly microCT evaluation was done to measure bone growth and density in the defect. Microfil enhanced microCT imaging was done at 3 weeks and 12 weeks post treatment to assess vessel formation. At the end of the study, the defect area was fixed, decalcified, and stained with Mason's trichrome examined for M1 and M2 specific macrophages.

Results: FTY720 loaded nanofibers are more hydrophilic and can maintain a sustained release of the drug for over 4 weeks (data not shown). The local delivery of FTY720 via nanofibers enhances stem cell chemotaxis as evaluated with transwell assays and decreases the ratio of inflammatory $Ly6C^{hi/}$ $CD36^{+}$ to $Ly6C^{lo}/CD206^{+}$ tissue macrophages ($F4/80^{+}$) (data not shown).

We show that bone regeneration in a CSD in a mandible can be improved with FTY720 loaded PCL/PLAGA nanofibers. This treatment resulted in substantial more bone growth than unloaded nanofibers after 3 and 12 weeks as shown by histology and microCT (**Figure#1**). This suggets that the use of appropriate osteoinductive therapies delivered in optimized scaffolds is sufficient for mandibular CSD healing. FTY720 also causes an increase in total volume fraction of blood vessels formed in the defect region, suggesting that it acts as a vascularizing agent (**Figure#2**). Moreover, the presence of FTY720 also alters the local inflammatory profile of the nanofiber implant. Immunohistochemistry shows that the amount of M1 macrophages (CCR7+ cells) is less and the amount of M2 macrophages (CD163+ cells) is higher at the defect site at 12 weeks after FTY720 treatment (data not shown here). This suggests that FTY720 modulates the local inflammatory response and thus, enhances defect site regeneration. It is hypothesized that the nanostructure and porosity of the scaffold plays a critical role in this recruitment.

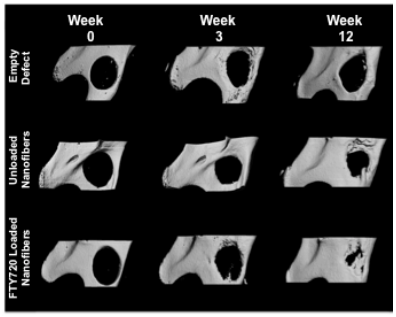

Figure 1: Representative microCT images of the mandibular ramus on the day of surgery and 3 or 12 weeks post-surgery. Defects were left untreated or treated with unloaded nanofibers or FTY720 loaded nanofibers.

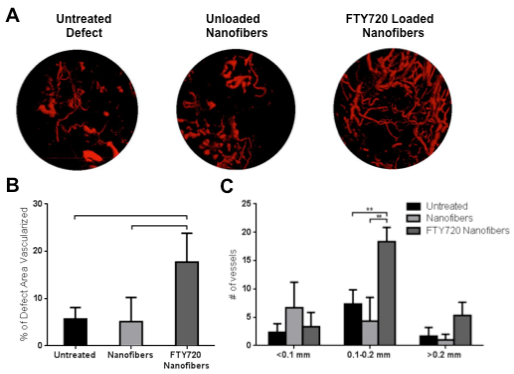

Figure 2: Neovascularization in the defect area visualized with MICROFIL® enhanced microCT. (A) Representative images at week 12 demonstrate vessel perfusion with MICROFIL approximately within the defect area. (B) Two-dimensional quantification of blood vessel area. (C) Number of vessels by vessel diameter within the defect area for the three treatment groups. (*p<0.05*, ***p<0.001*)

Conclusions: These results indicate that the local delivery of FTY720 will significantly accelerate bone regeneration by promoting neovascularization and osteogenesis, and enhancing the recruitment of circulating stem and progenitor cells. Use of immunomodulatory agents such as FTY720 can also enhance MSC allograft survival and eventual bone formation ability through S1P agonism. Such applications can have beneficial outcomes in clinical practice and as recipients may have better response to allograft transplants.

References: [1]Sefcik et al. *Tiss. Eng.A 2010; 17*, 617-629. [2]Petrie et al. *Biomaterials* 2010; 31(25): 6417. [3]Huang et al. *Cell Tiss. Res.* 2010; 347(3):553-66