Syndesomes Microencapsulated in Alginate for Revascularization in Peripheral Ischemia

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Statement of Purpose: Peripheral arterial disease (PAD) has a prevalence of 12-20% in the population aged 65 and older in the US.[1] Previous research has attempted to use growth factor proteins and genes to revascularize ischemic tissues but none of the methods have found success in clinical trials. In our previous study we demonstrated the loss of co-receptors like syndecan-4 due to long-term diseased state.[2] We have also shown that co-delivery of syndesomes (syndecan-4 proteoliposomes) with FGF-2 enhanced neovascularization of ischemic tissue in normal healthy rats.[3] In this study, we used syndesomes to enhance revascularization in the ischemic hind limb of a growth factor resistant mouse model with diabetes, obesity and hyperlipidemia.

Methods: We used Ob/Ob mice fed with high fat diet for 10 weeks. We induced ischemia through femoral artery ligation and implanted alginate beads containing treatments into the surgical site. We treated the mice with alginate beads that contained FGF-2 or FGF-2 with syndesomes (S4PL). The recovery of perfusion in the mice was tracked using laser speckle imaging. After 14 days, the mice were sacrificed and tissues harvested for histological analysis.

Results: After 14 days, the ischemic limbs of the mice treated with FGF-2 with syndesomes had significantly higher perfusion compared to the FGF-2 alone group (89% vs. 61%; Fig. 1A, B). Morphometric analysis on sections from the calf and thigh muscle of the mice demonstrated increased ischemic changes in the ischemic limbs of the FGF-2 alone group (Data not shown). Immunostaining for von Willebrand factor (vWF) revealed increased neovascularization corresponding with the increase perfusion measured in the FGF-2 with syndesomes group (Fig. 1C, D). Immunostaining for pro-inflammatory M1 macrophage marker CD86 showed similar levels in both treatment groups (Fig. 1E, F). However, immunostaining for pro-wound healing M2 macrophage marker revealed significantly higher levels in the syndesome with FGF-2 treatment group (Fig. 1G, H).

Conclusions: Taken together, these preliminary studies support that co-delivery of syndecan-4 with FGF-2 significantly enhances revascularization in the ischemic hind limb in a clinically relevant diseased mouse model. Our treatment restores the signaling pathway components that are lost due to diseased state causing tissues to become resistant to growth factor therapies.

References:

1. Selvin E et al. *Diabetes Care.* 2006; 29: 877-882.
2. Das S et al. *Biomaterials*. 2014; 35(1): 196-205.
3. Jang E et al. *PNAS*. 2012; 109(5): 1679-1684.

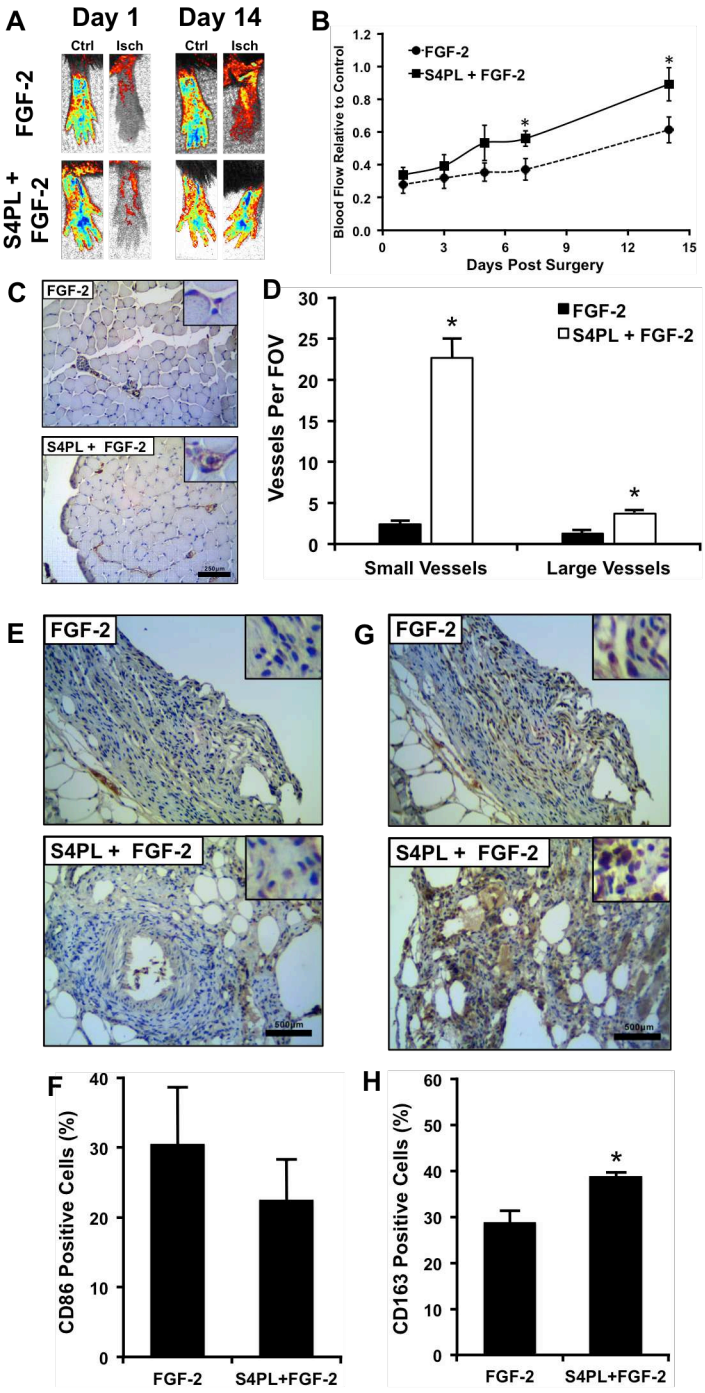

Figure 1. (**A**) Laser speckle contrast images of the hind limbs of mice at days 1 and 14. Left image is the contralateral control limb and right image is the ischemic limb. (**B**) Quantification of blood flow relative to contralateral limb. (**C, D**) Immunostained images for vWF at day 14 and quantification of vessels. (**E, G**) Immunostaining for CD86 and CD163 respectively. (**F, H**) Quantification of the percentage of CD86 and CD163 positive cells respectively. *$p < 0.05$ versus FGF-2 group (n=10).