Syndecan-4 Proteoliposomes Enhance Cutaneous Wound Healing and Induce Neovascularization in Ischemic Limb in a Diabetic Hyperlipidemic Mouse

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Statement of Purpose: Peripheral arterial disease (PAD) has a prevalence of 12-20% in population aged 65 and older in the US.[1] About 20-30% of diabetics are also affected by PAD.[2] PAD patients have an occlusion of blood flow in lower extremity due to arterial narrowing, which causes ischemia and pain. There is a high incidence of non-healing cutaneous wounds and ulcers in the lower extremities among PAD afflicted population. Previous research has attempted to use growth factors, growth factor genes or stem cells to revascularize ischemic tissues and heal cutaneous wounds, but none of the methods have found success in clinical trials. We have previously shown that co-delivery of syndecan-4 proteoliposomes with FGF-2 enhanced neovascularization of ischemic tissue in a normal healthy rat.[3] We have also demonstrated that, there is significant growth factor resistance in diabetic, diseased tissue which can be overcome by co-delivering the growth factor co-receptors with growth factors.[4] In this study, we are using this optimized co-delivery system, to demonstrate enhanced wound healing and neovascularization in a diabetic, obese and hyperlipidemic mouse model.

Methods: To recapitulate the human diseased condition, we used Ob/Ob mice (leptin deficient, hyperglycemic and insulin resistant) fed with high fat diet for 15 weeks. To examine the wound healing response, we utilized the mouse excisional wound model[5] where we implanted alginate disks containing treatments into the wound. To evaluate the angiogenic potential, we used hind-limb ischemia model[3] and implanted alginate gels with treatments at the site of incision. The treatments used were: *i) PBS (negative control), ii) FGF-2, iii) syndecan-4 proteoliposomes (S4PL) and iv) S4PL + FGF-2.* The mice were sacrificed after 14 days and the tissues harvested for downstream processing. During hind-limb ischemia surgery, we used the Laser Speckle Contrast Imager to monitor the blood perfusion in the limbs non-invasively.

Results: The novelty of our study is that we used a clinically relevant diseased mouse strain and tested our treatments in established wound healing and ischemia protocols. The excisional wound healing experiment (***Fig. 1***) showed that by day 14, syndecan-4 proteoliposomes with FGF-2 healed the wound completely with total wound closure compared to partial wound closure in all the other groups. It was surprising to see that the S4PL group did almost as good as the control group, demonstrating the importance of growth factor delivery with the co-receptor. The quantification and histology of the samples are in progress (*n=10*). The difference in healing response might be attributed to reduction in co-receptor levels in the presence of disease, as we have shown in our previous study.[4] The hind-limb ischemia experiment also yielded promising results. We noticed that (***Fig. 2***) S4PLs with FGF-2 induce a stronger neovascularization compared to FGF-2 only. The flux ratio is relative to the contralateral limb without ischemia. The difference in flux ratio at day 5 and day 7 are statistically significant. Histology of the ischemic and contralateral muscle samples is in progress.

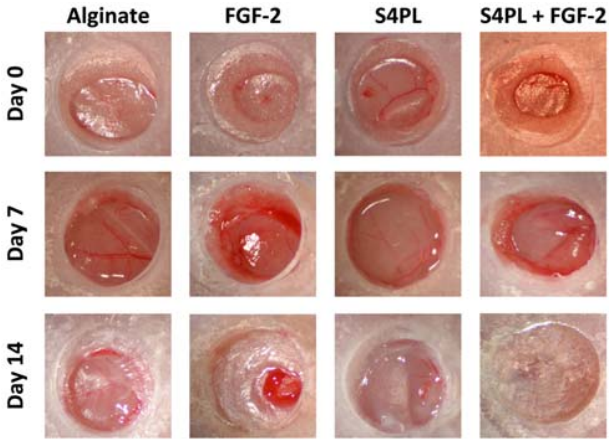

Fig 1. Macroscopic images of the wound with different treatments on days 0, 7 and 14. Inner diameter of circular splint is 5mm. *n=10*

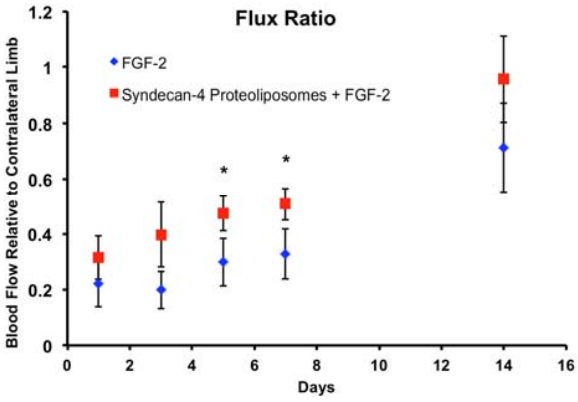

Fig 2. The flux ratios are shown below up to 14 days post surgery using Laser Speckle Imaging of mouse hind limbs post femoral artery ligation surgery. *n=6*

Conclusions: Taken together, these preliminary studies support that co-delivery of syndecan-4 with FGF-2 enhances both wound healing and neovascularization 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. Marso, SP et al. *J.Am.Coll.Cardiol*. 2006; 47: 921-929.
3. Jang E et al. *PNAS*. 2012; 109(5): 1679-1684.
4. Das S et al. *Biomaterials*. 2014; 35(1): 196-205.
5. Wang X et al. *Nat Protocols*. 2013; 8(2): 302-309.