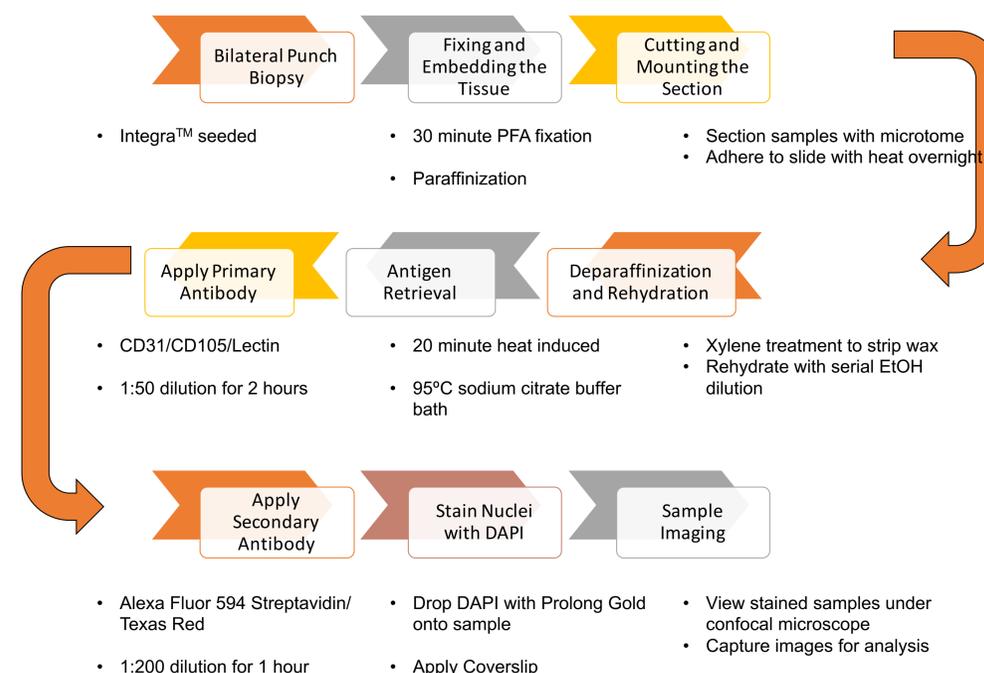


Regeneration of Blood Vessels Within Diabetic Wounds After Treatment with Mesenchymal Stem Cells

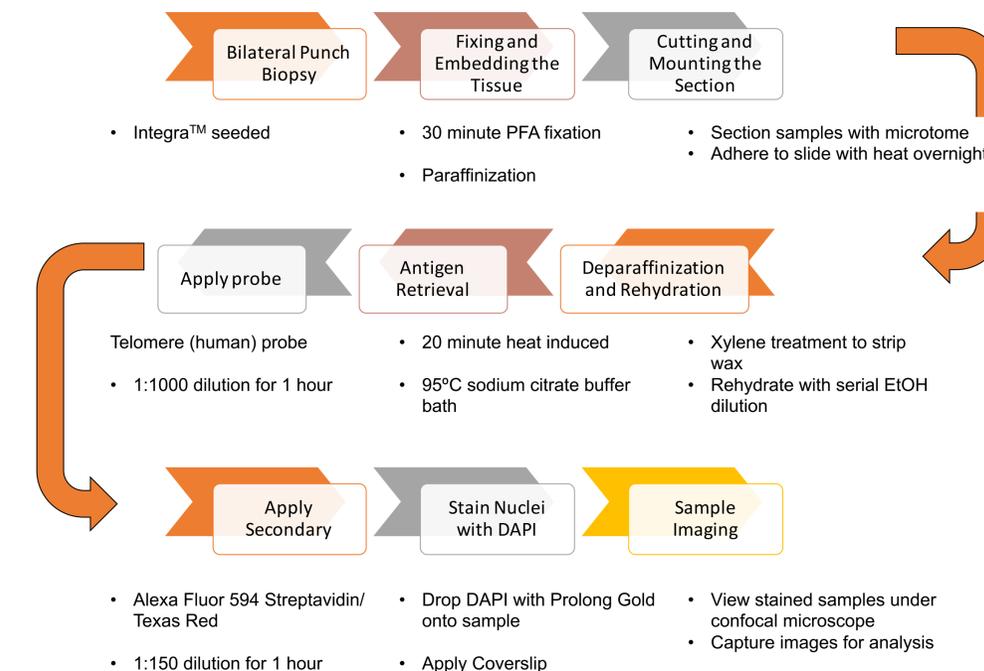
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Materials and Methods –IHC-



Materials and Methods –FISH-



Results –IHC-

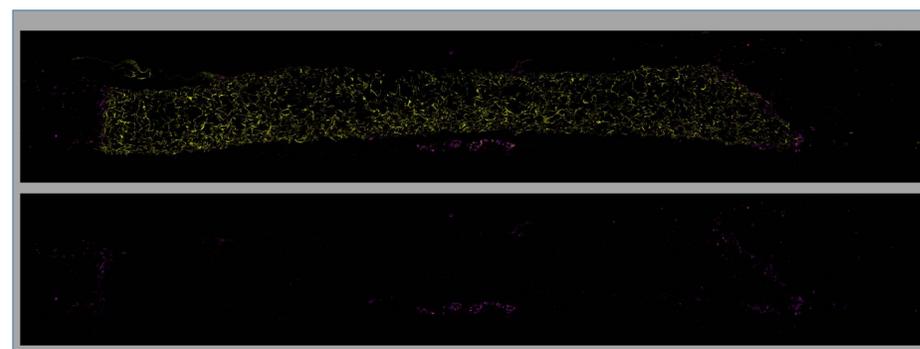


Figure 1: 100x images of CD31. Yellow represents collagen. Magenta represents Texas Red staining CD31

Results –IHC- Cont.

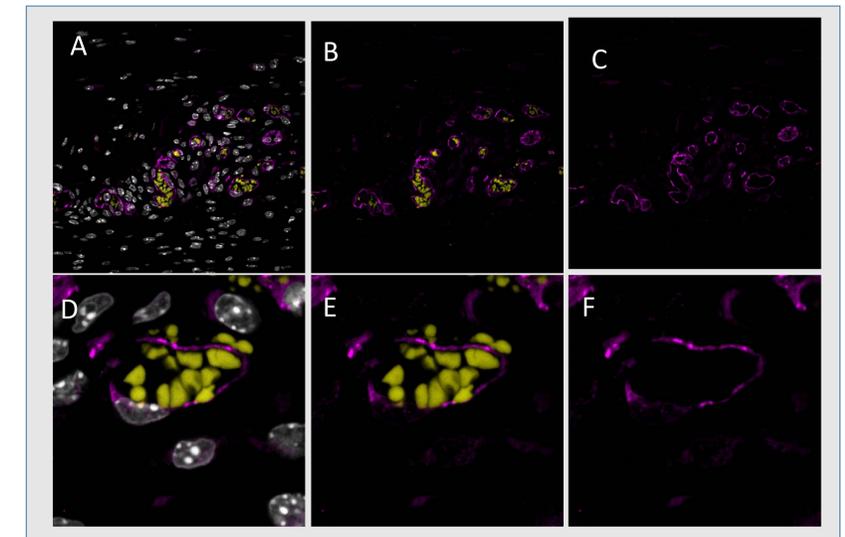


Figure 2: 100x (A, B, C) and 600x (D, E, F) images of CD31. Yellow represents red blood vessels. White represents DAPI nuclear staining. Magenta represents Texas Red staining CD31

Results FISH

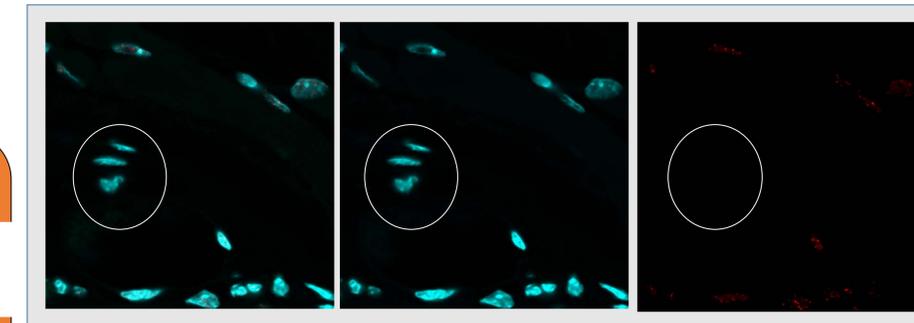


Figure 3: 600x images of hMSCs and mouse 3t3 keratinocytes. hMSCs showing specificity of FISH LNA probe for hMSCs (red signal). 3t3 keratinocytes no showing specificity of FISH LNA probe for hMSCs (circle).

Conclusion

From the quantification of Fluorescent imaging data, our results indicate that wound tissue treated with MSCs and timolol had the highest blood vessel regeneration and it was statistically significant when compared to control levels.

A Fluorescent in situ Hybridization (FISH) protocol to identify human chromosomes was successfully implemented using positive and negative control slides so that human MSCs can be identified when delivered to mouse wound tissue.

Future experiments will examine how long the MSCs persist and whether they migrate outside the wound tissue bed.

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Introduction

Diabetes is a chronic disease that affects more than 30 million Americans. This disorder leads to a variety of acute and chronic complications, including diabetic ulcers (chronic wounds). Particularly, diabetic individuals are prone to damage in their peripheral tissues which leads to a high prevalence of ulcers in their extremities, often leading to limb amputations.

Objective

The objective is to improve healing outcomes for diabetics through the use of mesenchymal stem cells (MSCs) to stimulate healing, in which vasculogenesis is an important aspect.

Background

In this study, healing rates of type II diabetic mice wounds were evaluated when human MSCs were delivered within a collagen scaffold (Integra™) and treated with timolol, a beta blocker that inhibits the effects of epinephrine.

The experiment

We examined wounded mice after 7 days that had received either no MSCs (control), MSCs, or MSCs treated with timolol for blood vessel development using immunohistochemical staining and confocal fluorescence microscopy.

Biomarkers

Blood vessel biomarkers GSL-I Isolectin B₄ and CD31 were used to stain the wound tissue and

FISH

Fluorescent in situ Hybridization (FISH) was performed to human MSCs when delivered to mouse wound tissue.