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

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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 evaluate the wound tissue.

FISH

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

Materials and Methods –IHC-

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<th>Cutting and Mounting the Section</th>
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Materials and Methods –FISH-

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Results –IHC-

- **Integra™ seeded**
  - 30 minute PFA fixation
  - Paraffinization
  - Section samples with microtome
  - Adhere to slide with heat overnight

Results FISH

- **Telomere (human) probe**
  - 1:1000 dilution for 1 hour
  - 20 minute heat induced
  - 95°C sodium citrate buffer bath
  - Xylene treatment to strip wax
  - Rehydrate with serial EtOH dilution

- **Alexa Fluor 594 Streptavidin/Texas Red**
  - 1:200 dilution for 1 hour
  - Drop DAPI with Probing Gold onto sample
  - Apply Coverslip
  - View stained samples under confocal microscope
  - Capture images for analysis

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

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.

Figure 3: 60x images of hMSCs and mouse 3T3 keratinocytes. hMSCs showing specificity of FISH LNA probe for hMSCs (red signal). 3T3 keratinocytes 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.

Acknowledgements

This material is based upon work supported by the National Science Foundation through the Robert Noyce Teacher Scholarship Program under Grant #1136419. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation. The research was also made possible by the California State University STEM Teacher and Researcher Program, in partnership with Chevron (www.chevron.com), the National Marine Sanctuary Foundation (www.marinesanctuary.org), and Sacramento State University. I would also like to thank Dr. Peavy for his support and guidance throughout this project.