

Isolation and Characterization of Fibroblasts from Diabetic Patients with Chronic Vascular Disease

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Introduction

Successful regenerative medicine therapies will often require stimulation of a response in individuals with degenerative, inflammatory, or neoplastic conditions. Diabetes is a serious, costly, and increasingly common chronic disease that causes devastating complications. Of 7% of the population that suffers from diabetes, 15% will develop lower extremity ulcers that fail to heal and are often associated with inflammation.¹ Design of regenerative therapies for these patients requires understanding at the cellular level, fundamental differences between diabetic and healthy individuals responses to therapeutic interventions. The objective of this study was to isolate fibroblasts from the amputated limbs of diabetic patients and then compare their growth characteristics and response to soluble fibroblast growth factor 1 (FGF-1) to fibroblasts isolated from healthy individuals.

Materials and Methods

Fibroblast isolation. Full thickness skin was harvested from the limbs of diabetic patients following amputation. Tissue was placed in a sterile PBS and gentamicin (200 g/mL) solution for transfer to the laboratory. Each specimen was washed 3X with sterile HBSS (10 mM) in Petri dishes and then turned with the skin-side to the glass bottom for careful dissection of subcutaneous fat. The remaining fat cells were removed by scratching them off from the underlying connective tissue. Small pieces of dermal connective tissue were harvested from the tissue. The pieces were rinsed with HBSS and transferred to Petri dishes (65 x 15 mm), 4-5 pieces per dish. A smaller Petri dish (35 x 10 mm) was placed on the tissue pieces to keep the tissue in place during medium changes. Wells were filled with pre-warmed DMEM (ATCC) containing 10% (v/v) FBS (Fisher), 200 g/mL gentamicin (Invitrogen), 1.25 g/mL fungizone (Invitrogen), and 2 mM glutamine (Gibco) and incubated at 37 C, 5% CO₂. After 2 d of culturing, the medium was replaced DMEM containing 10% (v/v) FBS, 50 g/mL gentamicin, and 2 mM glutamine. Regular medium changes were performed every 2 d. Cell growth was monitored with an inverted microscope. Images of isolated diabetic human dermal fibroblasts (DHDF) and normal human dermal fibroblasts (NHDF) were taken at 10X magnification and baseline growth curves are being performed.

Fibroblast soluble proliferation assay. DHDF from two patients and NHDF were plated 7,000 cells/well into a 96 well plate in 200 µl of growth media. Three days after plating, growth medium was removed and replaced with 200 µl of serum-free medium, DMEM containing, 50 g/mL gentamicin, and 2 mM glutamine. Twenty-four hours later, 50 µl of cytokines (1, 10, and 100 ng/ml of FGF-1 5 U/ml of heparin), diluted in PBS, were added to the quiescent medium. Twenty-four after adding cytokines, medium and cytokines were removed and cell number quantified using the MTS cell proliferation assay. Absorbance was recorded

using an Elisa plate reader at wavelength of 490 nm. Results were normalized by a positive control (20% FBS) and compared using ANOVA with a Tukey-Kramer post-test. $p < 0.05$ was considered statistically significant.

Results and Discussion

Fibroblast isolation. Images of NDHF and DHDF revealed a difference in morphology between the two patient populations. NHDF displayed a spindle-like morphology while DHDF spread multi-directionally (**Figure 1**).

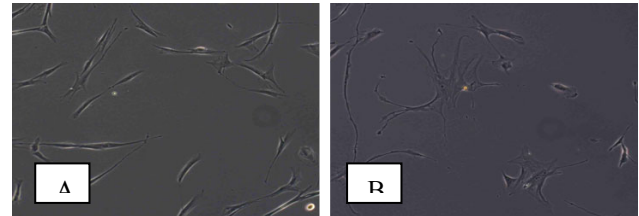


Figure 1: A. NHDFs. Cells maintain normal spindle-like morphology. B. DHDF. Cells appear to lose spindle-like morphology (10X).

Fibroblast Proliferation Assay. Both DHDF (**Figure 2**) and NHDF (not shown) display dose dependent response to soluble FGF-1. Replicate trials are being performed verify that DHDF exhibit a heparin-dependency like that of NHDF.

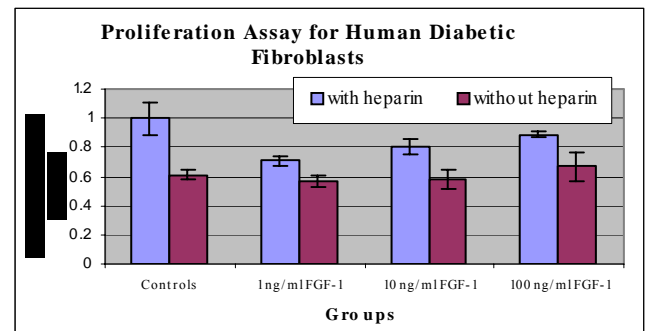


Figure 2: Effect of FGF-1 on DHDF proliferation with and without heparin. FBS was used as the positive control and PBS was the negative control. Groups were compared using the percent of positive control \pm SD.

Conclusions

Fibroblasts were successfully isolated from the amputated limbs of diabetic patients. These cells are taken from the adverse healing environment (i.e. diabetic, chronic vascular disease) of target for many regenerative medicine therapies targeting ulcers, wound healing, and ischemic limb regeneration. Cells isolated from diabetic tissues have an altered morphology compared to healthy dermal fibroblasts. Proliferation assays showed no differences in response to FGF-1. Growth assays are currently being performed and migration assays will be performed to better understand DHDF growth and response to FGF-1.

References

1. Eldor, R. New and experimental approaches to treatment of diabetic foot ulcers: a comprehensive review of emerging treatment strategies. *Diabetic Medicine*. 21:1161-1173, 2004.

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