Feinstein Institutes for Medical Research
Northwell Health®

SAWC Spring | WHS
April 30–May 4, 2025
Grapevine, Texas

FIBROBLAST DYSFUNCTION UNDER HYPERGLYCEMIC CONDITIONS: A DOSE DEPENDENT ANALYSIS OF OXIDATIVE STRESS AND MIGRATION

Parmeshar singh[1], Fanjaniaina Andriamifidy Henintsoa[1], Amanda Beneat[1] ,Blanca Espin[1], Alisha Oropallo[1,2,3] Lance Becker[1, 2] ,Daniel Grande[1, 2]

1.Feinstein Institute for Medical Research, Northwell Health, Manhasset, NY, United States.
2.Donald and Barbara Zucker School of Medicine at Hofstra/Northwell, Hempstead, NY, United States.
3.Department of Vascular Surgery, Comprehensive Wound Healing Center, Northwell Health, New Hyde Park, NY, United States.

BACKGROUND

Diabetes delays wound healing due to poor blood circulation, nerve damage, and a weakened immune response. Prolonged hyperglycemia fosters a sustained pro-inflammatory environment and impairs the normal function of skin-resident cells such as human dermal fibroblasts (HDFs). In diabetic conditions, fibroblasts exhibit diminished proliferation and reduced synthesis of essential extracellular matrix (ECM) components. These impairments collectively hinder granulation tissue development, delay wound closure, and increase vulnerability to chronic infections.

OBJECTIVE

In this study, we aim to investigate how poor glycemic control affects HDF behavior in vitro. Specifically, we assess how these alterations in glycemic levels influence the migration of fibroblasts and further evaluate whether increasing glucose concentrations lead to a dose-dependent escalation in oxidative stress.

METHODS

Adult Human dermal fibroblasts (NHDF-c adult Promo cell) were cultured in fibroblast growth media (Promocell) enriched with a growth supplement mix (Promocell). Cells were grown in varying glucose conditions for six days (5 mM, 35 mM, and 50 mM) to evaluate cell migration, while 100 mM glucose was used exclusively for assessing ROS generation. At 95% confluency, cells were seeded onto a Type I bovine collagen(Advanced biomatrix)coated well plate. Scratch wound was created using the Incucyte®WoundMaker tool, and migration dynamics were monitored over the next 24 hours using the Incucyte live imaging system. Data was analyzed using Incucyte analysis software. ROS generation was detected using CellROX™ Deep Red Reagent (Invitrogen), and visualized with a confocal microscope.

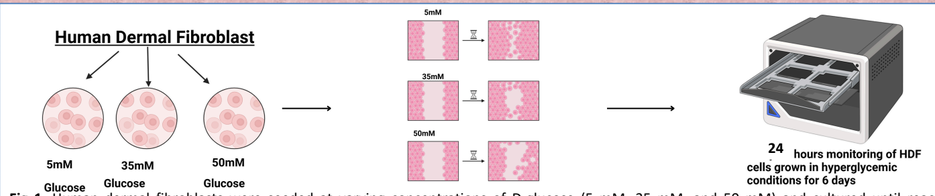

Fig 1. Human dermal fibroblasts were seeded at varying concentrations of D-glucose (5 mM, 35 mM, and 50 mM) and cultured until reaching approximately 95% confluency. A uniform scratch was created using the IncuCyte® WoundMaker tool, and wound closure was monitored over 24 hours using the IncuCyte® Live-Cell Analysis System.

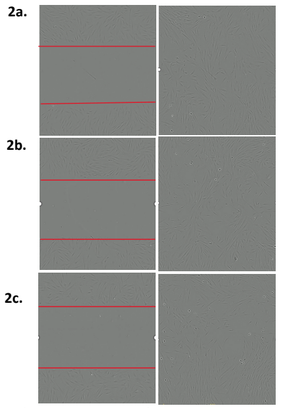

Fig 2 . presents phase-contrast images of HDF,s captured at baseline (left) and after 17 hours (right) for all three concentrations: **2a.** – 5 mM, **2b.** – 35 mM, and **2c.** – 50 mM

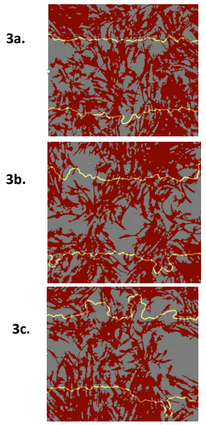

Fig 3. shows HDF migration at 17 hours, with red coloration indicating cell confluence. Panels include: **3a.** – 5 mM, **3b.** – 35 mM, and **3c.** – 50 mM.

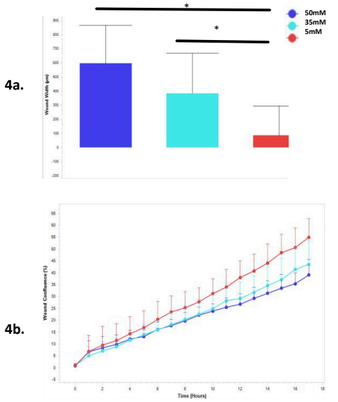

Fig 4. displays analytical images showing wound confluence percentage **(a.)** and confluent wound area **(b.)** after 17 hours.(*p value<0.05)

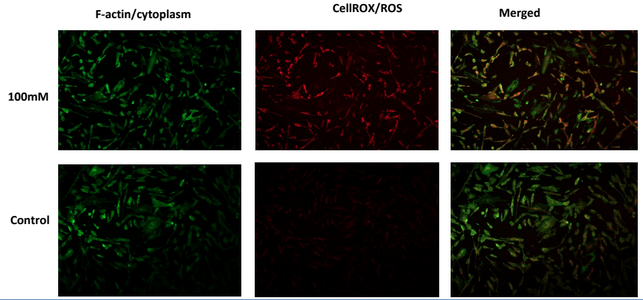

Fig 5. displays representative confocal images of human dermal fibroblasts from two groups: control and those treated with 100 mM glucose.

RESULTS

Elevated glucose concentrations markedly impair cell migration.
Hyperglycemic conditions result in broader wound gaps and reduced wound confluence.
The most significant difference in wound closure was observed at the 17-hour mark.
Wound healing was significantly hindered in the 50 mM glucose group compared to the 5 mM.
ROS (reactive oxygen species) levels were notably higher at 100 mM glucose.

CONCLUSION

Collectively, these findings suggest that a hyperglycemic environment impairs cell migration in a dose-dependent manner and can compromise cellular oxidative balance. Future studies are required to test how hyperglycemia influences other cellular functions involved in normal wound closure.

LIMITATIONS

A key limitation of this study is the use of only a single skin cell line, human dermal fibroblasts (HDFs), without incorporating other relevant skin cell types, such as murine or alternative human skin cells. Additionally, other characteristics of wound healing including cell proliferation, mitochondrial function, and inflammatory responses were not assessed.

About Northwell Health
Northwell Health is New York State's largest health care provider and private employer, with 22 hospitals, over 550 outpatient facilities and nearly 15,000 affiliated physicians. We care for more than two million people annually in the New York metro area and beyond, thanks to philanthropic support from our communities. Our 63,500 employees – 15,000+ nurses and over 4,000 employed doctors, including members of Northwell Health Physician Partners – are working to change health care for the better. We're making breakthroughs in medicine at the Feinstein Institute. We're training the next generation of medical professionals at the visionary Donald and Barbara Zucker School of Medicine at Hofstra/Northwell and the Hofstra Northwell School of Graduate Nursing and Physician Assistant Studies. For information on our more than 100 medical specialties, visit **Northwell.edu**.