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THE UNIVERSITY OF ARIZONA COLLEGE OF MEDICINE TUCSON Surgery

Dermal Fibroblasts Contribute to Oxidative Stress in Diabetes

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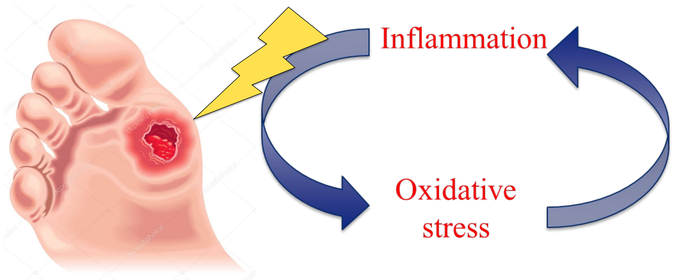
BACKGROUND

- Diabetes is a common medical condition with numerous comorbidities including chronic wounds, which cost the US healthcare system upwards of $13 billion annually
- Hyperglycemia in diabetes leads to increased production of reactive oxygen species (ROS) that lead to oxidative stress with associated tissue damage and impaired wound healing

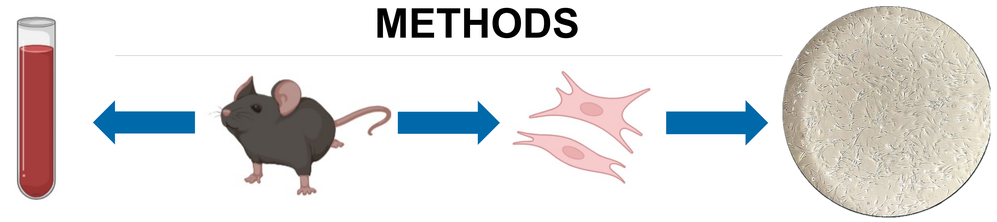


- The understanding of the cellular mechanisms of oxidative stress in diabetes and impaired wound healing requires further elucidation
- Fibroblasts are a key cellular component of wound healing that play a role in extracellular matrix formation, inflammation, and angiogenesis, but their role in oxidative stress has not been defined

HYPOTHESIS

- **Diabetic mice will demonstrate a systemic increase of ROS production consistent with increased oxidative stress compared to controls**
- **Dermal fibroblasts isolated from diabetic mice play a role in increased ROS production**

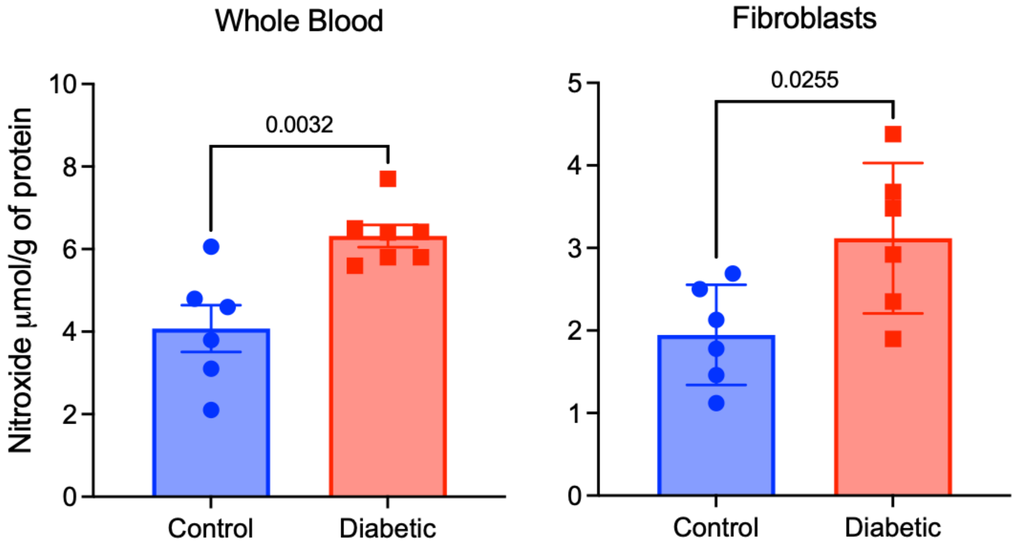
METHODS



- Whole blood was extracted via cardiac puncture from 12-week-old female diabetic (db/db) mice and heterozygous control (db/+) mice (N=7 per group)
- Fibroblasts were isolated from skin of 12-week-old female diabetic and heterozygous control mice and cultured in low-glucose Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS) and 1% antibiotic-antimycotic in tissue culture flasks, passed into 6-well plates (N=6 per group), and cultured until 80% confluence was reached
- Production of ROS was evaluated with electron paramagnetic resonance (EPR) spectroscopy using a hydroxylamine probe (cyclic hydroxylamine 1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine, or CMH) that reacts with ROS, generating a nitroxide radical that can be detected by EPR. Nitroxide levels generated from both whole blood and cultured fibroblasts treated with the EPR probe and normalized to protein concentration were compared between diabetic mice and heterozygous controls

RESULTS

ROS Production Measured by CMH

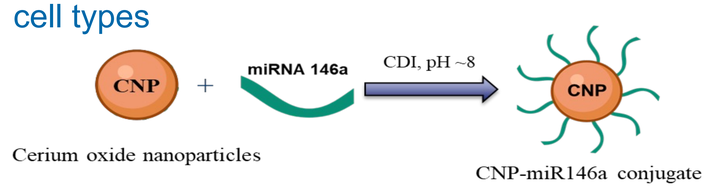


CONCLUSIONS

- **Whole blood of diabetic mice demonstrated upregulation of ROS compared to controls, consistent with systemic upregulation of oxidative stress**
- **ROS production was significantly elevated in dermal fibroblasts of diabetic mice compared to controls**
- **Fibroblasts may play a role in the production of ROS that leads to oxidative stress and may serve as a target for potential therapeutics**

FUTURE DIRECTIONS

- We are currently evaluating the effects of wounding on the production of ROS, performing EPR at 3- and 7-day timepoints after the creation of 8-mm full-thickness dorsal cutaneous wounds on diabetic and heterozygous mice
- We are also exploring other cell types that may contribute to the production of ROS, such as neutrophils
- As we further characterize the cellular mechanisms of diabetic wound healing, we aim to explore how a novel therapeutic conjugating cerium oxide nanoparticles (CNP) to the anti-inflammatory microRNA(miR)-146a affects specific cell types



DISCLOSURES

Dr. Liechty - President of Ceria Therapeutics, INC.
Dr. Zgheib - Chief Scientific Officer of Ceria Therapeutics, INC.