DR. PHILLIP FROST DEPARTMENT OF DERMATOLOGY AND CUTANEOUS SURGERY

Jozic Lab

Mitochondrial Rejuvenation Accelerates Wound Healing in Aged Skin: Mito-Targeted Therapies in Preclinical Models

Sarah M. Antonevich[1], Kate M. Miller[1], Monica Rodriguez Silva, PhD[2], Sara Danker, MD[3], Lilian Tose, PhD[4], Ryan Lee[1], Ana Monzant[1], Emmy Dugi[1], Christian Alcalde-Pretel, MD/PhD[1], Carlos Moraes, PhD[2], Fotios Andreopoulos, PhD[1], Stephen C. Davis[1], Francisco Fernandez Lima, PhD[4], Ivan Jozic, PhD[1]

[1]Dr. Phillip Frost Department of Dermatology and Cutaneous Surgery, University of Miami School of Medicine; [2]Department of Neurology, University of Miami School of Medicine; [3]Division of Plastic and Reconstructive Surgery, University of Miami School of Medicine.[4]Florida International University.

UHealth UNIVERSITY OF MIAMI HEALTH SYSTEM

ABSTRACT

INTRODUCTION: Impaired wound healing in aged skin is a major challenge in military and veteran healthcare. Mitochondrial dysfunction, including mitochondrial DNA (mtDNA) damage and electron transport chain (ETC) decline, contributes to age-related healing deficits. We hypothesized that restoring mitochondrial function in aged or chronically injured skin could enhance repair and offer a novel therapeutic strategy for older wounded warfighters and veterans.

METHODS: Transcriptomic profiling of young (8-week) vs. aged (72-week) murine skin revealed downregulation of oxidative phosphorylation genes and antioxidant defenses, correlating with delayed re-epithelialization. To establish causality, we used two inducible mouse models: (1) mtDNA damage (mitoPst1^{OE}) via doxycycline-regulated endonuclease, and (2) ETC Complex III disruption (Rieske knockout or Rieske^{KO}) via tamoxifen-inducible Cre. Wound closure and epithelial migration were quantified. In parallel, primary human keratinocyte scratch assays and ex-vivo human skin wound models (young and aged donors) were treated with mitochondrial-targeted compounds: SIRT1 activators, antioxidants (Sonlicromanol, Vatiquinone), and Elamipretide. An ETC inhibitor simulated mitochondrial dysfunction. To translate findings, hydrogels embedding top-performing mitochondrial therapies were evaluated in a chronic-like porcine wound model. Healing outcomes included re-epithelialization, granulation, and inflammation resolution.

RESULTS: Aged mouse skin showed suppressed mitochondrial gene expression and impaired Day-5 wound closure (p<0.01). MitoPst1^{OE} and Rieske^{KO} mice exhibited alopecia, skin atrophy, and delayed healing (p<0.05-0.01), mirroring geriatric deficits. Mitochondrial therapies improved healing across models. In keratinocytes, SIRT1 activators and antioxidants increased migration from ~57% to ~80-90% (p<0.001); ETC inhibition reduced it to 15-35%. Ex vivo aged skin treated with mitochondrial therapies showed re-epithelialization improving from ~37% to >80% (p<0.01). In the porcine model, mitochondrial-targeted hydrogels accelerated closure, enhanced granulation, and reduced inflammation.

CONCLUSION: Mitochondrial dysfunction is a key driver of impaired healing in aging. Mitochondrial-targeted therapies restored repair in aged and chronic wound models. These findings support their translational potential as gerotherapeutics for chronic wounds in military and veteran populations.

BACKGROUND

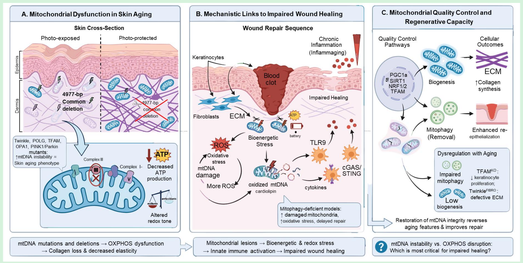

Figure 1: Mitochondrial dysfunction in skin aging and wound repair. A) Aging and photo-exposure promote mtDNA instability, common deletion accumulation, impaired OXPHOS, reduced ATP production, and altered redox balance in skin. B) Mitochondrial injury drives ROS generation, innate immune activation, and chronic inflammatory signaling that disrupt wound healing. C) Mitochondrial biogenesis and mitophagy support regenerative function, whereas their dysregulation with aging reduces extracellular matrix production and re-epithelialization.

MITOCHONDRIAL FUNCTIONING IS PERUTRBED IN AGING SKIN

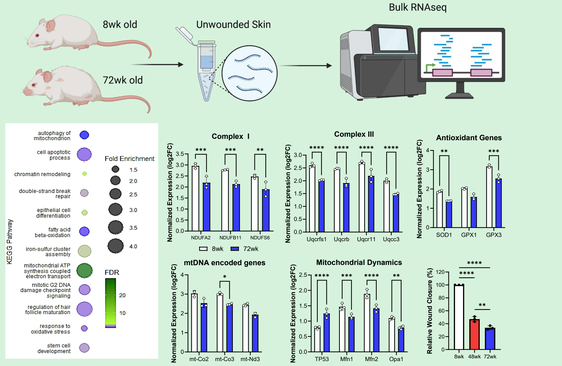

Figure 2: Intrinsically aged murine skin exhibits perturbation in several pathways associated with mitochondrial function. A) KEGG plot demonstrating major biological functions associated with intrinsically aged mouse skin. RT-qPCR validation of bulk RNAseq identified genes involved in Complex I (B), Complex III (C), antioxidant genes (D), mtDNA encoded genes (E), as well as those regulating mitochondrial dynamics (F). As expected, young (8wk-old) animals heal faster than their 48wk and 72wk counterparts (G) [n=3 animals per group, Two-way ANOVA followed by Sidak's multiple comparison test, **p<0.01, ***p<0.001, ****p<0.0001].

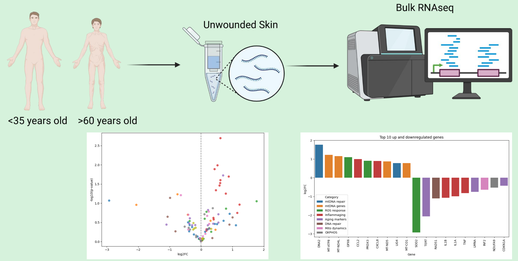

Figure 3: Deregulation of age-associated mitochondrial components of human skin. Volcano plot and pathway analysis demonstrating changes in gene expression of major biological processes associated with mitochondrial function (n=10, 5 younger and 5 older skin donors).

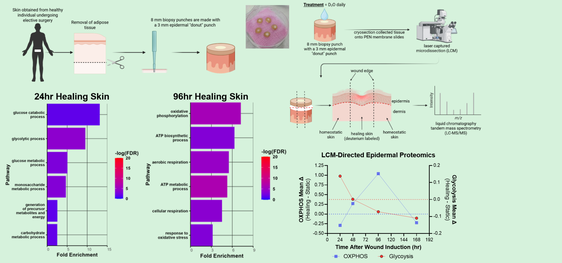

Figure 4: Laser-capture microdissection directed proteomic profiling of acute wounds points to a metabolic shift from anaerobic to mitochondria-driven oxidative program. A) Skin explants from reduction surgeries were topically treated with D_2O daily and wounds harvested at 24h, 48hr, 96h and 7d post wounding (n=3 biological donors), prior to laser captured microdissection of healing and homeostatic skin followed by subsequent proteomic profiling by PASEF TIMS-TOF. B) Pathway enrichment analysis from differentially regulated proteins demonstrated a metabolic shift from glycolysis at 24h to OXPHOS at 48hr, with mean difference plotted over time of wound collection (C).

SKIN-SPECIFIC OF MT-DNA DELETION DELAYS WOUND HEALING

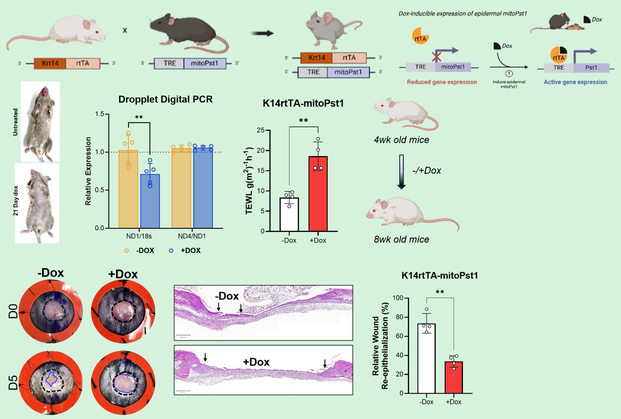

Figure 5: Skin-specific MitoPST1 overexpression induces mtDNA deletion and delays wound closure. A) KEGG plot demonstrating major biological functions associated with intrinsically aged mouse skin. RT-qPCR validation of bulk RNAseq identified genes involved in Complex I (B), Complex III (C), antioxidant genes (D), mtDNA encoded genes (E), as well as those regulating mitochondrial dynamics (F). As expected, young (8wk-old) animals heal faster than their 48wk and 72wk counterparts (G) [n=3 animals per group, Two-way ANOVA followed by Sidak's multiple comparison test, **p<0.01, ***p<0.001, ****p<0.0001].

SKIN-SPECIFIC ETC DISRUPTION DELAYS WOUND HEALING

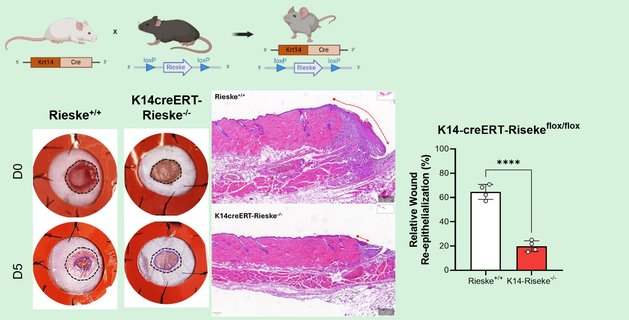

Figure 6: Skin-specific Rieske Iron Sulfur Complex deletion delays healing in vivo. A) KEGG plot demonstrating major biological functions associated with intrinsically aged mouse skin. RT-qPCR validation of bulk RNAseq identified genes involved in Complex I (B), Complex III (C), antioxidant genes (D), mtDNA encoded genes (E), as well as those regulating mitochondrial dynamics (F). As expected, young (8wk-old) animals heal faster than their 48wk and 72wk counterparts (G) [n=3 animals per group, Two-way ANOVA followed by Sidak's multiple comparison test, **p<0.01, ***p<0.001, ****p<0.0001].

ACKNOWLEDGMENTS

We would like to thank our funding sources NIH NIAMS, Stanley J. Glaser Foundation and University of Miami EDR Team Science Aging Award.

CONTACT INFORMATION

Ivan Jozic, Ph.D. / Research Associate Professor
Dr. Phillip Frost Department of Dermatology and Cutaneous Surgery
University of Miami Miller School of Medicine
I.jozic@med.miami.edu
Ph: 305.689.7067

Jozic Lab

MITO-GELS ACCELERATE CELL MIGRATION

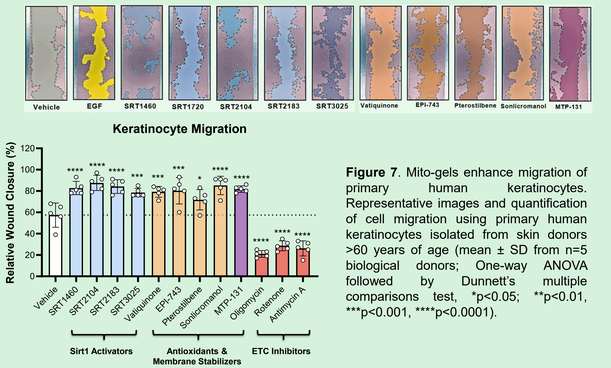

Figure 7. Mito-gels enhance migration of primary human keratinocytes. Representative images and quantification of cell migration using primary human keratinocytes isolated from skin donors >60 years of age (mean ± SD from n=5 biological donors; One-way ANOVA followed by Dunnett's multiple comparisons test, *p<0.05; **p<0.01, ***p<0.001, ****p<0.0001).

MITO-GELS ACCELERATE WOUND HEALING EX VIVO (HUMAN)

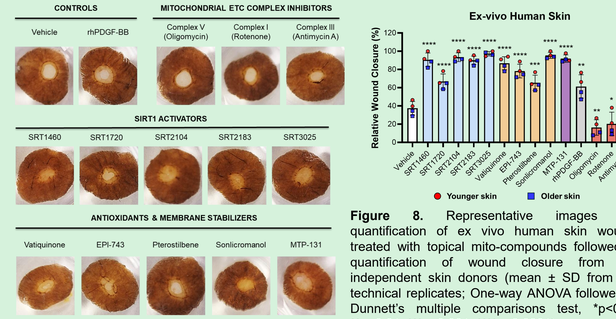

Figure 8. Representative images and quantification of ex vivo human skin wounds treated with topical mito-compounds followed by quantification of wound closure from n=4 independent skin donors (mean ± SD from n=5 technical replicates; One-way ANOVA followed by Dunnett's multiple comparisons test, *p<0.05; **p<0.01, ***p<0.001, ****p<0.0001).

MITO-GELS ACCELERATE WOUND HEALING IN VIVO (PORCINE)

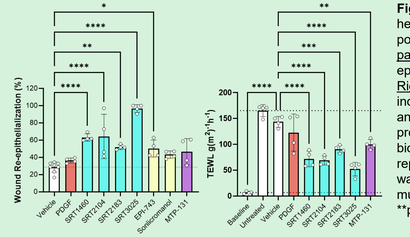

Figure 9. Functional assessment of wound healing in a full-thickness excisional porcine chronic-like wound model. Left panel: Quantification of wound re-epithelialization across treatment groups. Right panel: Measurement of TEWL as an indicator of epidermal barrier restoration and wound closure quality. Data are presented as mean ± SEM with individual biological replicates shown as dots (n=4 replicates/treatment from 2 animals). One-way ANOVA, followed by Dunnett's multiple comparison test, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

TAKEAWAY MESSAGES

- Mitochondrial dysfunction is a fundamental driver of age-associated impaired wound healing.
- Restoration of mitochondrial function significantly improves tissue repair across multiple experimental systems.
- Mitochondrial-targeted compounds enhance re-epithelialization & cell migration.
- Hydrogel-based delivery of mitochondrial therapeutics accelerated healing and improved barrier restoration in a clinically relevant porcine wound model.
- Mito-gels represent a promising translational strategy to improve healing outcomes in chronic and age-related wounds.