

Proposal title: **Establishing a Human *Ex vivo* Wound Model and Whole-Mount Staining Approach to Accurately Evaluate Skin Repair**

Introduction: Aging is the strongest risk factor for chronic, non-healing wounds, with older adults accounting for ~85% of all cases. Despite advances in regenerative medicine, most mechanistic studies rely on murine models that heal primarily by contraction and incompletely recapitulate human wound repair. To enhance translational relevance and align with NIH priorities for New Approach Methodologies (NAMs), this project will establish a human *ex vivo* skin wound model to study age-associated impairments in healing.

Problem Description and Significance: Age-related changes in skin architecture, stem cell function, and estrogen signaling impair re-epithelialization and tissue repair, yet these mechanisms are difficult to study directly in humans. Human-based *ex vivo* models provide a powerful alternative to animal studies, enabling controlled, physiologically relevant investigation of wound healing dynamics while reducing reliance on *in vivo* experimentation. Establishing and validating such a model in aged versus young human skin is a critical unmet need.

Hypothesis: *Ex vivo* wounds generated from aged human skin will exhibit delayed and incomplete re-epithelialization compared to young skin, mirroring age-associated impairments observed *in vivo* in humans and mice.

Objective: To establish and validate a reproducible human *ex vivo* partial-thickness wound model capable of quantifying age-related differences in wound closure and cellular responses.

Study Design and Methods: Discarded surgical skin from young (20–35 years) and aged (>65 years) donors will be obtained under IRB approval #IRB202500916. Partial-thickness wounds will be generated using a standardized punch biopsy method adapted from published protocols (from our collaborator, Dr. Mat Hardman). Explants will be cultured at an air–liquid interface for up to 7 days. Wound closure and tissue organization will be assessed using stereomicroscopy, histology (H&E, Masson’s trichrome), and whole-mount immunostaining for keratinocytes (K14), fibroblasts (vimentin), myofibroblasts (SMA), and proliferation (Ki67), followed by confocal imaging. Quantitative wound closure will be calculated as percent re-epithelialization over time and compared between young and aged samples.

Role of the Veterinary Student: The veterinary student will have primary responsibility for this study, including: Processing discarded human surgical skin and generating partial-thickness *ex vivo* wounds using biopsy punches; Maintaining explant cultures and monitoring tissue viability and wound closure; Performing histological processing and whole-mount immunostaining; Acquiring and quantifying wound closure data using ImageJ; Performing statistical analysis comparing young versus aged skin healing kinetics. The student will gain hands-on training in human tissue handling, NAMs methodologies, and quantitative analysis of tissue repair, forming the foundation for future translational research in wound healing.

Citations for methods cited: Wilkson et al.,2021. DOI: [10.3791/62326](https://doi.org/10.3791/62326)