To investigate the complex cellular interactions underlying cutaneous wound healing, several humanized mouse models have been proposed. However, current models that utilize full-thickness human adult skin are limited by poor engraftment, and variability of skin samples across age, gender, body site, and sun-exposure. We describe a novel xenograft model using full-thickness human neonatal foreskin to investigate the mechanisms mediating fibrosis in human skin.

**Methods:** Human foreskin was collected from routine neonatal circumcisions, cut into 8 mm circular full-thickness grafts, and transplanted subcutaneously on the dorsum of NOD.CgPrkdcsidIl2rgtm1Wjl (NSG) pups five to seven days after birth. After pup weaning three weeks later, the overlying mouse skin was excised to expose the human foreskin xenograft to air. Xenografts healed over the subsequent 30-60 days. To explore the baseline response to wounding, linear incisions were made in the xenograft. Wounds were followed grossly and harvested on post-operative day (POD) 14. To induce fibrosis, bleomycin was injected daily into the dermis of unwounded xenografts until harvest one week later. To reduce fibrosis, fibroblast growth factor 2 (FGF2) was injected into a xenograft on POD 1, 2, 3 and 4 after wounding and was harvested two weeks later. Harvested tissues were processed for histology, immunofluorescent, and Picro Sirius Red stain.

**Results:** Human foreskin successfully engrafted with mouse dorsal skin. On H&E staining, xenografted human skin was visualized as distinct from mouse skin by a thicker dermis and lack of hair follicles, consistent with the microscopic differences seen between ungrafted foreskin and mouse skin. This was confirmed by mouse-specific CD90 immunofluorescence staining. Wounded xenografted skin at POD14 displayed a higher density of collagen on Trichrome staining and immunostaining with human- and mouse-specific Collagen I. Grossly, unwounded bleomycin-injected skin appeared fibrotic while FGF2-injected wounds appeared more similar to unwounded skin. These findings were confirmed histologically using Trichrome and Picro Sirus Red staining. FGF2-injected wounds also had a marked increase in vascularity as measured by CD31 staining.

**Conclusions:** This is a novel xenograft model of human foreskin that serves as a platform to explore wound healing and fibrosis. Future work will include using the model to further investigate human fibroblast heterogeneity and potential therapeutics to reduce human scarring.

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**SPADE Analysis Reveals The Recruitment Of Rare Immune Cell Subtypes To Site Of Injury Following Treatment With Immunomodulatory Hydrogels**

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**Purpose** - Wound care following surgical procedures involves careful management, reducing risk of infection, and maximizing tissue oxygenation. Even with new strides in biological and synthetic products, achieving adequate wound care remains an important topic of interest. In this study, we develop an injectable hydrogel to promote wound resolution while increasing polarization of immune subsets towards healing phenotypes. These poly(ethylene glycol)-maleimide (PEG-MAL) hydrogels are capable of locally delivering therapeutic doses of the specialized pro-resolving mediator aspirin-triggered resolvin D1 (AT-RvD1) and the immunomodulatory cytokine interleukin 10 (IL-10) to enrich the injured tissue niche. To objectively uncover cellular heterogeneity from flow cytometry data, we utilized Spanning-tree Progression Analysis of Density-normalized Events (SPADE), a computational dimensionality reduction technique. This method highlighted established responses to wound healing, and, more notably, revealed novel aspects of the wound healing cascade in response to immunomodulatory treatment.

**Methods:** The murine dorsal skinfold window chamber model was used to monitor vascularization and wound healing in response to hydrogel treatment. Each animal received an unloaded control hydrogel on the caudal side of the window chamber, and a loaded hydrogel rostrally. Loaded gels contained either IL-10, AT-RvD1, or both. At days 1, 3, and 7, dorsal tissue was excised and digested for flow cytometry. SPADE analysis was performed on manually gated single cell events to identify proportions of cells from the innate and adaptive immune system.

**Results:** We show that AT-RvD1 and IL-10 alone are able to modulate the recruitment of various pro-inflammatory
and pro-regenerative immune cells, but dual delivery of these factors enhances the recruitment of pro-regenerative immune cells, including M2 macrophages and tolerogenic dendritic cells, suggesting a synergistic interplay. Moreover, novel computational methods revealed the recruitment of rare immune cell subtypes, particularly from the adaptive immune system, to the site of injury following immunomodulatory hydrogel treatment. These findings suggest a promising method to target pro-regenerative cells from different branches of the immune system, and this treatment has the potential to enhance tissue regeneration and prevent wound healing complications after skin tissue injury.

Conclusions: Biomaterial implants to deliver cells or molecules capable of recruiting and promoting the host immune response after injury can be instrumental to the restoration of tissue homeostasis and the promotion of wound healing. This dual-delivery system has the potential to improve therapeutic healing outcomes via synergy of cellular recruitment and polarization processes. Moreover, dimensionality reduction techniques, such as SPADE, provide novel and objective analytical approaches for analyzing high-dimensional data. We applied SPADE to mouse flow cytometry data and demonstrated that SPADE can be used to identify functional changes in response to treatment. Interestingly, SPADE enabled the identification of unexpected immune cell populations. This allows for the future development of immunomodulatory treatments that tailor the immune response and enhance the process of healing.

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Dehydrated Amniotic Membrane Allograft Promotes Healing Of Complex Wounds

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Purpose: Human Dehydrated Amniotic Membrane Allograft* (DAMA) has successfully been used to regenerate tissue following injury. The objective of this study was to determine the efficacy of amniotic membrane in promoting healing and tissue growth in chronic wounds.

Methods: Sixty-two wounds in thirty-eight consecutive patients (males n=19, 50%; females n=19, 50%) with an average age of 69 (range: 39-99) were treated in a multi-disciplinary setting with amniotic allograft for complex wounds throughout of body. Patients were evaluated weekly for an average period of 6 weeks (range: 1-71) in accordance with standard of care (SOC). Treatments consisted of wound debridements, offloading (if necessary) and appropriate wound dressings. Wound size was measured and amniotic membrane was applied with regard to product protocol. Results were measured against a historical control of wounds treated with SOC only.

Results: Demographic variables that were considered include: body mass index (BMI), hypertension, immune suppressors or steroid use, and the presence of diabetes, cardiac, renal, and vascular diseases. Wound locations treated included: upper extremity (2), back and sacral (8), abdomen (3), ischium (1), thigh (1), and lower extremity (47). All wounds had adequate vascular supply as verified by Doppler ultrasound and all infected wounds were treated with a full course of antibiotics as determined by the infectious diseases specialist. Initially, the wounds measured an average of 43 cm² (range: 1-441). 15 wounds in 11 patients went on to fully heal. Remaining open wounds had improved and measured an average of 40 cm² (range: 1-360). Seven patients had negative pressure wound therapy used as an adjunct to assist with the allograft in conforming to the irregular depths of the wounds. All patients with healed wounds regained pre-injury functional status.

Conclusion: Human dehydrated amniotic membrane allograft is useful as an adjunct in wound closure techniques in assisting the formation of granulation tissue and healing of complex wounds in all body sites. It can help decrease healing time, and in some cases, eliminate the need for skin grafts or flap reconstruction. It can also help in cases of limb salvage when all other efforts have failed.

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Immunomodulation Of Acellular Dermal Matrix Through Strategic Cytokine Incorporation Enhances Biointegration

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