distinct transcriptional trajectories, one characterized by mechanical activation (*En-1* lineage-positive, “fibrotic” trajectory) and the other characterized by developmental and regenerative pathways (*En-1* lineage-negative; Rspo1, Dkk2/3, Trps1). Cross-platform data integration confirmed that fibroblasts in the fibrotic trajectory correlated with myofibroblast proteomic signatures (Col1a1/2, Fn1, etc.) and fibrotic/scar ECM features. In contrast, fibroblasts in the regenerative trajectory negatively correlated with myofibroblast markers and were associated with a “basket-weave” ECM pattern quantitatively indistinguishable from that of unwounded skin.

Our integrated dataset suggested an important role for Wnt pathway proteins in ENF-mediated skin regeneration, so we compared POD 14 scars and regenerated wounds by multiplexed *in situ* hybridization (RNA-Scope) for Rspo1 (Wnt agonist), Trps1 (master hair follicle regulator), Ank1 (YAP target gene), and Dpp4 (EPF marker). Quantification of RNA granules across thousands of cells using a custom image analysis pipeline revealed that ENF-mediated healing (low Dpp4) in YAP-inhibited (low Ank1) wounds yielded regeneration of functional hair follicles through Wnt-mediated pathway activation (high Rpos1, Trps1). These data suggest that YAP inhibition unlocks wound regeneration via Wnt-active, *En-1* lineage-negative fibroblasts.

**Conclusion:** By studying regenerating (verteporfin-treated) versus scarring wounds across multiple healing timepoints and high-dimensional data modalities, we were able to profile fibrotic versus regenerative healing at unprecedented depth. Our integrated analysis revealed that dermal fibroblasts in these two wound settings exhibit distinct molecular trajectories defined by divergent transcriptomic, proteomic, and ultrastructural properties. Further, we found that wound regeneration in the context of verteporfin treatment is associated with suppression of mechanical signaling and activation of key Wnt pathway members including Trps1 (a gene with known hair follicle developmental roles). These results could have important implications for both the fundamental study of wound healing and potential anti-scarring therapeutic avenues.

4

**Prolonged Tourniquet Use Following Blast Related Lower Extremity Injuries Increase Heterotopic Ossification in a Pre-clinical Model**

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**Purpose:** Traumatic heterotopic ossification (tHO) has become a signature pathology affecting wounded military personnel who have sustained blast-associated traumatic amputations during the recent conflicts in Iraq and Afghanistan. Heterotopic ossification is characterized by the abnormal development of mature bone deposits in extra-skeletal sites such as muscle, tendon, and soft tissues, leading to impaired wound healing, pain, reduced range of motion, and limited use of prostheses. While many factors influence the formation of tHO, the extended use of tourniquets to limit catastrophic hemorrhage during prolonged field care (PFC) has not been explored. Herein, we investigate the impact of tourniquet use following blast-related injury on ectopic bone formation.

**Methods:** Utilizing an established pre-clinical model of blast-associated complex lower limb injury and traumatic amputation, we evaluated the effects of extended tourniquet use on tHO formation. Male rats (11-12-week-old) were subjected to blast overpressure exposure, femur fracture, and soft tissue crush injury. A pneumatic tourniquet (250-300mmHg) was applied to the injured limbs for either 90- and 150-minutes followed by trans-femoral amputation. Limbs were assessed for HO formation using microCT. Analysis of muscle/soft-tissue osteogenesis-related gene transcripts and multiple serum inflammatory mediators were measured by using qRT-PCR and Luminex multiplex assays, respectively.

**Results:** At 12 weeks, volumetric analysis with microCT imaging revealed an 70% increase in total bone formation (P=0.007, n=11) near the site of injury in rats subjected to 150-minutes of tourniquet time compared to rats with no tourniquet time in the setting of blast-injuries. Rats subjected to 150-minute tourniquet usage and blast injury had increased expression of osteochondrogenic genes including Bmp2 (5.4-fold increase, P=0.01) as early as 6 hours.
post-injury while Hif1α (4.2-fold increase, P<0.01), Sox9 (2.8-fold increase, P<0.01), Runx2 (8.2-fold increase), and Bmp2 (7.7-fold increase, P<0.02) remained elevated for 7 days. Analysis of cytokines and chemokines in the serum demonstrated increased expression of key analytes in the tourniquet group above that induced by traumatic amputation alone in the control group in factors including IL-1 (22-44%, P<0.005) and IL-6 (13-69% p<0.03) between 6 hrs and POD7.

Conclusions: These findings suggest that extended tourniquet time leads to both significant increases in key transcription factors associated with early endochondral bone formation, as well as increased systemic inflammatory mediators. Increased expression of Hif1α with prolonged tourniquet use also demonstrates the importance of tissue hypoxia and Hif1α signaling in combat applicable tHO and the potential development of targets for therapeutic inhibition. This data supports mechanisms by which extended tourniquet times under PFC conditions could result in increased local neuromuscular dysfunction and systemic inflammation, resulting in increased local tissue injury and potential further functional loss secondary to tHO in wounded military personnel.

5

3D Transglutaminase Fibronectin Hydrogel Therapy Improves the Healing of Chronic Irradiated Porcine Skin Wounds

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Purpose: Chronic irradiated wounds are characterized by a delayed and incomplete healing course. Currently, there are no therapies directed at the deficient or dysfunctional biology associated with cutaneous radiation injury. We have previously demonstrated that fibronectin is a key extracellular matrix glycoprotein known to be significantly down-regulated in radiation-damaged skin. We further identified that an enzymatically crosslinked hydrogel is a suitable construct for incremental fibronectin release in vitro and in murine wound models. Our present objective was to investigate the design of this fibronectin hydrogel dressing for the treatment of irradiated wounds in the clinically relevant porcine irradiated wound model.

Methods: We created a chronic irradiation skin injury model in female Yucatan minipigs. Two 1-month-old minipigs underwent irradiation of the right dorsolateral neck region for 5 consecutive days in 5.5 Gy fractionated doses for a total of 27.5 Gy. Following irradiation, the minipigs were allowed 6 weeks of recovery to enable chronic irradiation skin changes to develop. After recovery, nine 1 cm x 1 cm full-thickness wounds were created in the irradiated fields. After wound creation, 100 μl of fibronectin hydrogel was topically applied on experimental wounds and 100 μl of phosphate-buffered saline (PBS) hydrogel was applied on control wounds. Standardized wound photographs were taken at weekly time intervals to calculate the percentage of wound closure relative to original wound size. Tissues isolated from the wound areas were evaluated histologically for wound healing quality and analyzed for gene and protein levels of radiation injury mediators with quantitative RT-PCR and ELISA.

Results: Wounds treated with fibronectin hydrogel demonstrated significantly faster wound closure and decreased scarring than wounds treated with PBS hydrogel. On postoperative day 21, the mean percentage of wound area relative to original wound size was significantly higher in the control wounds (21.3% ± 2.8%) than in the fibronectin-treated wounds (4.7 ± 1.0%). By the experimental endpoint on postoperative day 28, the mean percentage of control wound area was 6.8% ± 2.9% while all fibronectin-treated wounds were fully healed. Picrosirius red staining demonstrated that the fibronectin-treated wounds had decreased total scar area (9.9 ± 3.0 mm2) compared to control wounds (38.1 ± 3.6 mm2). In addition, fibronectin hydrogel treatment was associated with decreased levels of radiation-induced inflammatory mediators. RT-qPCR of samples from fibronectin-treated wounds had significantly lower mRNA levels of TGF-β1 (0.45 ± 0.09) compared to levels in control wounds.