post-injury while $\text{Hif1}\alpha$ (4.2-fold increase, $P<0.01$), $\text{Sox9}$ (2.8-fold increase, $P<0.01$), $\text{Runx2}$ (8.2-fold increase), and $\text{Bmp2}$ (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 $\text{IL-1}$ (22-44%, $P<0.005$) and $\text{IL-6}$ (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 $\text{Hif1}\alpha$ with prolonged tourniquet use also demonstrates the importance of tissue hypoxia and $\text{Hif1}\alpha$ 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 downregulated 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 (1 ± 0.13). Similarly, RT-qPCR data revealed that relative mRNA levels of SMAD3 were significantly lower in fibronectin-treated wounds (0.34 ± 0.11) than in control wounds (1 ± 0.43). Lastly, protein level correlation with ELISA identified significantly lower TGF-β1 concentrations in fibronectin-treated wounds (2682 ± 515.83 pg/mL) compared to control wounds (5244.5 ± 700.08 pg/mL).

Conclusion: Hydrogel-facilitated delivery of fibronectin significantly improved the rate and quality of wound healing in a porcine chronic irradiation wound model. Thus, this novel mechanism of fibronectin supplementation demonstrates potential for treating these otherwise nonhealing wounds.

6

Biomimetic Microtissue Keloid Scar System Using Keloid-derived Fibroblasts and Macrophages

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Purpose: Keloid is a disease that affects millions of patients and has relatively few effective treatment options. Unfortunately, traditional 2D monolayer culture of keloid derived fibroblasts show little resemblance to the pathological process in vivo. Additionally, keloid is notably a pathology largely specific to humans, without good in vivo models available. To fill this gap, we have developed a 3D in vitro microtissue keloid scar model with human keloid derived fibroblasts and peripheral blood derived macrophages.

Methods: Under IRB approval, keloid tissue was from patients and used to develop a human keloid derived fibroblast line. Keloid spheroids were fabricated from these keloid derived fibroblasts and human peripheral blood derived macrophages. Commercial human skin-derived fibroblast and 2D monolayers were used as controls. Quantitative PCR with fibrosis genes (collagen-1, aSMA, TNF, IL1β, IL6 and TGFβ) and immunofluorescent staining with (collagen-1, aSMA, CD68 and pSTAT3 were performed to validate the keloid spheroids as a keloid tissue regarding gene expression level.

Results: Spheroids had significantly higher expression levels of all fibrosis related genes compared to the 2D monolayer control. Among the spheroid groups, keloid spheroids had much higher gene expression levels of collagen-1 and aSMA, which was confirmed by the immunofluorescent staining with the same correspondence proteins. Interestingly, keloid spheroids showed lower gene expression levels of common fibrosis related cytokines (TNF, IL1β, IL6 and TGFβ). However, IF of pSTAT3 was upregulated in keloid spheroid, which is consistent with previous literature of keloid research. Lastly, qPCR fibrosis array and human comprehensive gene expression assay validated the result of qPCR and indicated that macrophages in the keloid spheroids showed signs of polarization in both M1 and M2 directions.

Conclusions: We have developed a keloid mimicking spheroid microtissue as a more physiologically relevant in vitro keloid model for drug development and research exploring gene and protein expression pathways. This platform recapitulates important features of keloid behavior not seen in 2D culture. Future work will include screening of keloid spheroid responses to potential therapeutic treatments.

QUICK SHOTS

QS1
Creeping Fat Adipocytes Drive Intestinal Fibrosis Through Adipocyte-to-fibroblast Conversion in a Novel Model of Inflammatory Bowel Disease

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Purpose: Crohn’s disease (CD) is a subtype of inflammatory bowel disease (IBD) characterized by patchy, transmural inflammation throughout the digestive tract and creeping