histologically. To mechanistically understand epidermal Nrf2 function, we isolated WT and cKO wound-associated keratinocytes by flow cytometry and performed RNA-seq, further coupling with chromatin immunoprecipitation (ChIP), immunohistochemistry, qPCR/protein expression analysis, and functional assays.

**Results:** Nrf2 expression analysis in full-excisional wounds reveals nuclear translocation of Nrf2 is defective in wound edge-associated keratinocytes of diabetic mice (WT 97.0±3.6% vs. diabetic 22.5±16.4%; p<0.05). We demonstrate the functional importance of epidermal Nrf2 in wound regeneration, as its induced deletion severely delays wound closure to levels observed in diabetic mice (WT 15.6±1.2days vs. cKO 34±1.7days vs. diabetic 30±0days; p<0.0001). Histologic assessment of the gross wound reveals not only impaired re-epithelialization (p<0.0001), but also reduced neovascularization (p<0.05) and collagen maturation, suggesting its governing role of both keratinocyte and non-keratinocyte autologous regenerative programs.

Transcriptome-wide analysis (±2-fold differential expression; q<0.05) coupled with ChIP enrichment analysis corroborates our finding, showing epidermal Nrf2 directly regulates expression of not only oxidoreductase-related genes, but also those affecting many paracrine signaling-mediated regenerative responses, including inflammation, immune-cell guidance, extracellular structure, and angiogenesis (p<0.05). The significance of epidermal Nrf2-mediated intercellular communication is demonstrated through a prominent defect in monocyte/macrophage trafficking, observed during early (130.3±15.0cells/area vs. 35±2.6cells/area; p<0.05) and later phases of repair (p<0.05). This defect results from downregulated expression of chemokine Ccl2 in cKO wounds, which we find to possess a Nrf2-binding motif that exhibits dynamic Nrf2 binding upon wounding. Induced expression of Nrf2 in primary keratinocytes results in Ccl2 upregulation, and its application is sufficient for restoring physiologic wound regeneration in cKO wounds (Vehicle: 29.7±1.73days vs +Ccl2: 17.3±0.6days; p<0.0001).

**Conclusion:** In-depth analysis of Nrf2 in the wound environment uncovers an indispensable role of epidermal Nrf2 in regulating initiation of the regenerative response that is critical for physiologic wound repair. We find epidermal Nrf2 is necessary for mediating paracrine crosstalk between keratinocytes and monocytes/macrophages, specifically through the direct regulation of Ccl2 which promotes immune cell guidance to the wound edge. Together, our findings provide the basis for continued investigation on the therapeutic value of Nrf2 in restoring diabetic wound regeneration.

**103**

**Cd26 Knockout And Inhibition Promotes Dorsal Wound Healing Via Modulation Of Engrailed-1 Positive Fibroblasts**

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**Purpose:** Engrailed-1 (En1) positive fibroblasts are responsible for scar formation in the dorsal skin of adult mice during the postnatal period. The cell surface marker CD26, also known as dipeptidyl-peptidase-4 (DPP4), is expressed by the vast majority of En1 positive fibroblasts and can be used to isolate this lineage of scar-forming fibroblasts. We have previously shown that inhibition of CD26 with Dipro tin results in decreased cutaneous scarring during wound healing. To further interrogate this biology, we hypothesized that inhibition of CD26 with Dopro tin results in decreased cutaneous scarring during wound healing. To further interrogate this biology, we hypothesized that inhibition of CD26 with a small molecule CD26/DPP4 inhibitor (MK0626) or CD26 knockout mice, might improve the rate of wound healing, decrease scar fibrosis, and achieve a more regenerative phenotype in the context of wound healing.

**Methods:** The effects of MK0626 were initially evaluated on NIH 3T3 fibroblasts in vitro. Migration, measured by scratch assay, and proliferation were assessed on treatment and control specimens at 12 and 24 hours. Results were analyzed using ImageJ. To test the effects of MK0262 in vivo, bilateral full thickness wounds were created in the dorsal dermis of 10-week old C57Bl/6 (wild-type) mice. Equivalent wounds were also made in the dorsal dermis of En1tm1G CD26 knockout mouse (CD26−/−;En1Cre;R26tm1G). A
stented-wound healing model developed in our laboratory, which better mimics human wound healing kinetics, was used. The wild-type mice received oral MK0626 at a high dose (30 mg/kg), low dose (3 mg/kg), or saline control every other day over the course of wound healing. Wounds were harvested at post-operative day 14.

**Results:** *In vitro* scratch assay showed significantly increased fibroblast migration and increased proliferation with treatment of MK0626 compared with vehicle control. *In vivo*, orally-administered MK0626 significantly improved the rate of wound closure in wild-type mice. Dermal scar thickness (as well as En-1 expression) was decreased in En1<sup>hTERT</sup> CD26 knockout mice when compared with En1<sup>hTERT</sup> control mice.

**Conclusions:** Modulation of CD26 expression shows therapeutic potential to encourage regenerative wound healing. Specifically, our *in vivo* experiments show that with oral CD26 inhibition or CD26 knockout, the wound closure rate is increased and the dermal scar is thinner, the latter of which correlates with decreased presence of the En1-lineage-positive fibroblasts. Accelerated wound closure with decreased scarring is immensely beneficial from a clinical perspective.

104

**Bio-functional Collagen Matrix Scaffold Composition Differentially Promotes Paracrine Activity In Human Induced Pluripotent Stem Cell Derived Vascular Smooth Muscle Cells**

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**Abstract:**

**Purpose:** Induced pluripotent stem cell-derived vascular smooth muscle cells (iPSC-VSMC) have the potential to treat chronic wounds by secreting proangiogenic factor vascular endothelial growth factor (VEGF) (1). However, little is known how the extracellular matrix (ECM) composition may impact the cells’ paracrine secretion profile. In this study, our objective was to understand the effects of ECM density and functionalities on the secretory profile of human iPSC-VSMCs.

**Methods:** Type-I collagen was used as the material for the scaffolds and were incorporated with functional biomolecules: hyaluronic acid (HA), fibronectin, and laminin. This allowed fabrication of collagen scaffolds with different functionalities. The functionalities were used in combination with three different densities of type-I collagen (1.25mg/ml, 2.5mg/ml, and 5mg/ml) to study iPSC-VSMC viability and paracrine secretion profile. Several pro-angiogenetic factors included VEGF, SDF, PDGF, bFGF, angiopoietin 1, IL-8, and TGF-beta were investigated. The anti-inflammatory factor IL-10 was also evaluated.

**Results:** Human iPSC-VSMCs embedded in collagen scaffolds containing functional biomolecules showed an increase in cell viability across all the collagen densities compared to the collagen scaffolds without functional biomolecules. The greatest significant level of cell proliferation was display by the 1.25mg/ml scaffolds (P-value=0.0001). Enhanced VEGF was observed in all three of the functionalized scaffolds at 1.25mg/ml collagen density (P value=0.0086) and also in the 2.5mg/ml collagen density scaffold containing HA (p value=0.0026), whereas no significant difference in VEGF level was found among 5mg/ml functionalized scaffolds. Interestingly, fibronectin-functionalized 5mg/ml collagen scaffold exhibited substantially elevated bFGF secretion (P value=0.0049). There was also a positive correlation between increasing amounts of fibronectin with increasing bFGF paracrine secretion (P value=0.0001). Furthermore, our data showed respective upregulation of IL-8 and IL-10 secretion in fibronectin-functionalized collagen scaffolds at the 1.25mg/ml and 2.5mg/ml collagen densities.

**Conclusion:** These results suggested that functionalization along with density differentially regulate paracrine function of human iPSC-VSMCs. Future studies in elucidating the underlying signaling pathways and mouse model experiments will further deepen our understanding of the interaction between ECM and human iPSC-VSMCs. This will ultimately optimize human iPSC-VSMCs as a viable therapeutic agent for chronic wound healing.

**Reference:**

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