wounds. Delayed wound closure may be a result of neo-vascularization deficits from dysfunctional endothelial cells. Restoration of Nrf2 signaling in diabetic wounds decreases wound closure time and increases neovascularization of the wound bed. The membrane protein CXCR4 and its ligand, SDF-1, have been implicated in modulating the neo-angiogenesis required to revascularize wounded tissue. Here, we identify a possible role of Nrf2 dysregulation in endothelial cells impairing angiogenesis in wound neovascularization.

**Methods:** To study the functional role of endothelial Nrf2, we crossed Cadherin5 (Cdh5)CreER and Nrf2fl/fl mice to generate Cdh5-CreER;Nrf2fl/fl double transgenic mice and administered tamoxifen to induce conditional deletion of Nrf2 in cadherin 5 expressing endothelial cells (KO). Ten mm diameter full-thickness excisional wounds were created on the dorsum of 6-8-week-old wildtype (WT), heterozygous, and KO mice. 10 days post wounding, wound endothelial cells were isolated using flow cytometry and angiogenesis assay was performed. In order to elucidate a relationship between Nrf2 and the CXCR4/SDF-1 axis, C166 mouse endothelial cell line was transfected with Nrf2 siRNA to create Nrf2 knockdown (KD) cells. After transfection, cells were then placed in a 5% oxygen hypoxic incubator for 48 hours to model a hypoxic wound environment. QT-PCR for Nrf2, CXCR4, SDF-1, and NQO1 was performed.

**Results:** In the Nrf2 KO mice, time to wound closure was delayed compared to wildtype mice (KO 33.0±1.73 days vs. WT 14.0±0 days) and was similar to diabetic mice (KO 33.0±1.73 days vs. Diabetic 31.0±1.41 days). Primary endothelial cells from the wounds of KO mice demonstrated significantly decreased angiogenic functionality when compared to heterozygous controls. KO endothelial cells formed significantly fewer branches (KO 64.75±14.81 branches/cm² vs. control 282.8±46.7 branches/cm², p=0.014) and fewer branching points (KO 39.0±5.18 branching points/cm² vs. Control 150.8±15.34 branching points/cm², p=0.0031). We found that selective Nrf2 KO in endothelial cells reduced their capacity to form tubular networks, indicating impaired angiogenic activity. QT-PCR of the C166 endothelial cells after transfection and incubation in a hypoxic environment demonstrated reduced transcription of Nrf2 compared to control of 76.5% (p=0.001) and NQO1, a downstream target of Nrf2, decreased 66.4% (p=0.02). Expression of CXCR4 and SDF-1 decreased 61.7% (p=0.001) and 47.6% (p=0.02) respectively.

**Conclusions:** Nrf2 expression in endothelial cells is necessary for physiologic wound healing. Nrf2 knockdown results in increased time to wound closure and decreased angiogenic function. Decreased Nrf2 expression also results in decreased transcription of the neo-vascularization related proteins CXCR4 and SDF-1 in a hypoxic environment. These findings suggest that the Nrf2 dysregulation associated with diabetes may contribute to the impaired angiogenesis and decreased revascularization present in chronic diabetic wounds.

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**Epidermal Nrf2 Orchestrates Tissue Regeneration Through Regulation Of Ccl2**

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**Purpose:** Type 2 diabetic (T2D) chronic wounds affect more than 4.5M people in the US and remain the leading cause of non-traumatic amputations. While many signaling pathways are implicated as responders to tissue damage, our work has demonstrated that poor diabetic wound healing is partly due to mismanagement of intracellular oxidative stress that results from dysfunction in the Nrf2/Keap1 signaling pathway. This study focuses on uncovering the molecular mechanisms orchestrated by epidermal Nrf2 that are critical for initiating a regenerative response, and are defective in diabetic wounds.

**Methods:** T2D mice (Leprdb/db) were used to describe dysfunctional cellular and molecular programs that impair wound healing. To investigate epidermal Nrf2 function in the context of tissue regeneration in vivo, we generated inducible transgenic mice to conditionally ablate Nrf2 expression in K14 basal keratinocytes (K14 CreER; Nrf2flo/flox, cKO) upon administration of tamoxifen. Ten-millimeter diameter, stented, full-thickness excisional wounds were created on the dorsum of 6-8-week-old WT, diabetic, and cKO mice to examine wound closure kinetics and to analyze
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.

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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 Diproton 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 En1Cre;R26IntG CD26 knockout mouse (CD26−/−;En1Cre;R26IntG). A