Purpose: Multipotent stromal cells (MSCs) have attracted much attention for their capacity to accelerate wound healing. However, there are no approved MSC based therapies. Exosomes, nanosized extracellular vesicles, may be key to translating MSC therapy to the bedside. We previously found that nuclear-factor-erythroid-2-related -factor-2 (Nrf2) regulates MSC multipotency and promotion of diabetic tissue repair. Here, we explore a novel role of Nrf2 in exosome biogenesis and investigate whether exosome treatment recapitulates the effects MSCs have on diabetic wound healing.

Methods: Whole bone marrow was subcultured from long bones of non-diabetic human donors. Adherent cells were characterized by microscopy, tri-lineage differentiation assays, and flow cytometry. Exosomes were harvested by differential ultracentrifugation of conditioned MSC media. For Nrf2-active exosomes, MSCs were incubated with potent Nrf2 activator, CDDO-Im. Exosomes were characterized by immunoblotting, nanoparticle tracking analysis (NTA), and transmission electron microscopy (TEM). Full-thickness humanized-stented wounds were created on adult Leprdb/db diabetic mice (db/db). Exosomes were injected intradermally and circumferentially to the wound margin 1-day post-excision and photographed regularly until closure. Tissues were harvested at day 10 post-wounding for histological/biomolecular analysis.

Results: MSCs adopt an adherent fibroblast morphology and demonstrate robust differentiation along osteogenic, chondrogenic, and adipogenic lineages in culture. >95% of MSCs express positive markers (CD44, CD73, CD90, and CD105) and less than 5% express negative markers (CD45, CD31, CD14, CD19, or HLA-DR). Immunoblotting of MSC exosomes shows enrichment for positive exosomal markers CD81, CD9 and TSG101 and no detection of negative markers, Calnexin and GM-130. NTA shows a nanoparticle population with mode diameter of 168.0±6.5nm. TEM of exosomes reveals flattened cup-like spheres and confirms the size determined by NTA. NTA demonstrates that Nrf2-activated human MSCs increase exosome secretion by 54%, compared to Nrf2-baseline MSCs (p<0.05). When administered to wounds, both Nrf2-baseline and Nrf2-active exosome treatment significantly reduced closure time to 15.5 and 14 days respectively, compared to 29.8 days for vehicle-treated wounds of diabetic mice (p<0.05 for both treatments when compared to vehicle). Importantly, exosome treatment of diabetic wounds eliminated the delay in healing compared to C57/B6 wounds (16.6 days; p<0.05 compared to exosome-treated db/db wounds). Nrf2-active exosome treatment reduces closure time by 2.6 days compared to untreated C57/B6 wounds, though this benefit is nonsignificant. Histological analysis at day 10 shows that exosome-treated db/db wounds have significant decreases in epithelial gap and expanded granulation compared to vehicle-treated wounds. CD31 Immunoreactivity of 10 day wounds confirms a greater density of blood vessels in the wounds exosome-treated vs vehicle-treated diabetic wounds.

Conclusions: Enhancing Nrf2 function in MSCs multiplies exosome yield. Exosome therapy harnesses MSC ability to promote angiogenesis, a process critical for ensuring swift diabetic wound closure. MSC exosome-based therapies hold tremendous promise to improve chronic wound outcomes for patients with diabetes, and our results demonstrate the need for further investigation for rapid translation.

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Granulation And Genomic Evaluation Of Easy To Use Novel Negative Pressure Wound Therapy Dressings

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Introduction: NPWT with reticulated open cell foam (ROCF) has impacted the practice of healing wounds. New approaches have been contemplated for longer wear dressings. Two novel dressings were evaluated for cellular genomic responses, granulation tissue thickness formation, and average peel force to remove the dressing in a porcine wound healing study.

Methods: Eleven domestic swine were used to obtain samples from full-thickness surgical wounds treated with either NPWT ROCF®, NPWT novel dressing 1®, or NPWT novel dressing 2®. Biopsy samples were taken at study termination on either Day 7 or Day 13. Porcine polymerase chain reaction (PCR) wound healing arrays (Qiagen, Valencia, CA) were performed to determine differences in gene expression (>2 fold difference; p<0.05). Histopathology evaluations and morphometry measurements assessed granulation tissue quality and thickness, respectively. The peel force required to remove the dressings was obtained using a custom test device.
Results: Granulation: In the day 13 group, there was a significant difference (p<0.0441) in granulation tissue thickness between ROCF + interfacial layer (ROCF + IFL) (5.33 mm ± 0.14) as compared to CPUF (7.43 mm ± 0.33). No significant difference were seen between ROCF + IFL (5.33 mm ± 0.14) and GM (5.80 mm ± 0.22). Moreover, each treatment group (ROCF + IFL, CPUF, and GM) demonstrated a significant increase in granulation tissue deposition over time (p<0.04).

Genomics: The novel GM dressing at Day 13, relative to Day 7, demonstrated greater upregulation in cell binding and catalytic activity which included Matrix metalloproteinase 1 (6.58), Matrix metalloproteinase 3 (8.83) and Decorin (2.15). These are important epithelial markers and known for collagen binding. Novel CPUF dressing at Day 13, as compared at Day 7, demonstrated similar results. There was an increase in MMP1 (10.30) and MMP3 (13.46), both of which are important in keratinocyte migration and endothelial cell activity. There was an increase in Catenin (2.29), which may be responsible for the inhibition signal that causes cells to stop dividing once the epithelial sheet is complete. In addition, cell adhesion genes (Integrins: ITGA2, ITGB3 and ITGB6) were all up regulated (2.01, 2.62, 6.90 respectively).

Peel Force: Regardless of group or timepoint, ROCF required significantly greater average force for removal. A dressing change at day 4 did not statistically affect the amount of peel force required to remove ROCF for the groups with a day 7 end of in-life/termination. The use of the interfacial layer with GF brought the force required for removal as low as the other novel dressings.

Conclusions: This preclinical study illustrates that the tested novel dressings induced more granulation tissue formation with less peel force, and interesting genomic responses than the controls. Following 13 days of treatment in a porcine model, the novel NPWT dressings have been shown to increase genes involved in epithelization while increasing granulation tissue and decreasing dressing removal force, which might lead to better wound healing outcomes.

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#GM dressing (NPWT novel dressing 1)

*CPUF dressing (NPWT novel dressing 2)

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Effect Of Nitric Oxide Releasing Gel On Excisional Wound Healing

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Purpose: Nitric Oxide (NO) plays a pivotal role as a messenger molecule that signals to cells during wound repair. The amount of NO secreted changes during the three classic stages of wound healing: inflammation, proliferation and regeneration. NO levels normally increase rapidly after skin injury in the inflammatory phase and gradually decrease as wound healing proceeds toward the proliferation and regeneration. However, although NO is known to impact proliferation, differentiation and migration of keratinocytes, the molecular mechanisms of how increased NO concentration affects wound healing and leads to re-epithelialization and wound closure is far from completely understood. Here we reveal that local continuous administration of NO-releasing gel on excisional wound healing accelerates overall wound healing despite an initial delay in wound closure.

Methods: Murine excisional wound model was studied to investigate the effects of NO releasing gel on angiogenesis and re-epithelialization. NO gel was created by adding cellulose derivatives to sodium nitrate solution. 1) Mouse model: 15-week-old C57BL/6 mice were randomized into treatment groups: Nitric Oxide or PBS control (N=5 mice per group). A full-thickness wound was excised using a sterile 6-mm punch biopsy tool on each side of the dorsal midline. An NO- releasing gel was locally applied to the wound site twice daily until wound closure on D21. 2) Tissue analyses: Wounds were harvested on day 2 and 7 after wounding and upon closure. The presence of epidermis, dermal integrity, vasculature and inflammatory cells were visualized by histology and immunofluorescent techniques.

Results: In the murine excisional model, NO-treated wounds healed completely four days earlier than PBS-treated wounds (Wound closure Day 15.6±0.7 vs. Day 19.4±0.5). However, initially NO-treated wounds closure was slower than PBS-treated wounds. Wounds harvester from the NO treatment group exhibited a more robust dermal layer, collagen deposition, and neovascularization. Neovascularization...