ready availability and lack of immunogenicity in allogeneic use. We investigated the potential of human amniotic fluid-derived multipotent stromal cells (AFSCs) to promote wound healing in a preclinical type II diabetic model.

**Methods:** Amniotic fluid was collected with informed consent from full-term gestational patients at caesarian sections, approved by NYU Institutional Review Board. AFSCs were differentiated in adipogenic, osteogenic, chondrogenic media. For therapy, multiple donor AFSCs were cultured together and passage 3 cells used. Ten mm diameter full-thickness stented wounds were created on dorsum of adult LepR<sup>db/db</sup> mice (blood glucose≥400mg/dL). 5x10<sup>4</sup> AFSCs in 10uL culture media were topically applied to the wound bed immediately after excision. Culture media, or no treatment were controls. For in vivo imaging, AFSCs were stained with DiI prior to topical application, and wounds were imaged using an In Vivo Imaging System at 560/620nm. Wounds were photographed to monitor wound closure. Wound tissues were harvested post-operatively for histological analysis. Statistical significance was determined by comparing experimental and control groups using a one-way ANOVA.

**Results:** Minimum 1x10<sup>6</sup> AFSCs were harvested per 1mL full-term amniotic fluid, determined by plastic adherence. AFSCs were multipotent and differentiated along adipogenic, osteogenic and chondrogenic lineages. Dil-labeled AFSCs were detectable in the diabetic wound bed up to 120hours post-treatment. AFSCs resulted in significantly accelerated diabetic wound closure by 19±1.6 days, compared to vehicle or untreated wounds at 25±1.6 and 26.4±0.98 days, respectively (p<0.05). Single topical application of AFSCs reduced pathologic wound healing time by 76% and 78.7% compared to vehicle and untreated diabetic wounds, respectively. Pathologic wound burden with AFSC treatment also significantly decreased by 56% and 44.2%, compared to vehicle and untreated diabetic wounds, respectively (p<0.05 for both). Vehicle and untreated diabetic wounds did not demonstrate any significant differences in any parameter, indicating that the cell culture media does not affect wound healing. AFSC administration also accelerated rate of wound healing for the diabetic wounds, compared to vehicle or untreated diabetic wounds, p<0.05 for both. There was no significant difference between wound healing rates of vehicle-treated and untreated diabetic wounds. Tissue sections from post-op day 7 confirmed lower gap between epithelial wound edges with AFSC-treatment. Immunoreactivity showed similar quantities of F4/80-expressing macrophages in the wounds tissues at post-op days 1-3, in all AFSC-treated, vehicle and untreated diabetic wounds. By post-op day 7, higher numbers of arginase1-positive M2-macrophages are present in AFSC-treated diabetic wounds compared to vehicle-treated ones. The results indicate that AFSCs support the critical M2 macrophage subsets to contribute towards closure of diabetic cutaneous wounds.

**Conclusions:** With approximately 600mL per gestation, amniotic fluid provides a feasible and reliable source of multipotent stromal cells. Application of AFSCs successfully changed the immunophenotype of the diabetic wound and reduced wound healing time. Our results support further investigation to translate AFSCs into a readily available and viable therapy for chronic diabetic wounds.

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**6**

**Topical Antibiotic Elution In A Collagen-rich Hydrogel: Limiting Systemic Exposure To Antibiotics For Healing Of Infected Wounds**

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**Purpose:** Chronic wounds have become a prominent concern in the face of rising rates of obesity, peripheral vascular disease, and diabetes. Biofilms have been implicated in delayed wound closure. A novel collagen-rich hydrogel derived from human extracellular matrix presents an avenue for treating chronic wounds by providing appropriate extracellular proteins for healing and promoting neovascularization. Using the hydrogel as a delivery system for localized secretion of therapeutic dosage of antibiotics presents an attractive means of maximizing delivery while minimizing systemic side-effects.

**Methods:** The authors investigated the elution of antibiotics from the collagen-rich hydrogel, and the efficacy of the treatment in disrupting biofilm in multiple models using *Pseudomonas Aeruginosa*. Growth inhibition, biofilm disruption, and mammalian cell cytotoxicity were quantified.

**Results:** The antibiotic-loaded hydrogel showed sustained release of antibiotics for up to 24 hours at therapeutic levels. The treatment inhibited bacteria growth and disrupted biofilm at multiple time points. The hydrogel was capable...
of accommodating various classes of antibiotics and did not result in cytotoxicity in mammalian fibroblasts or adipose stem cells.

**Conclusions:** An antibiotic-loaded collagen-rich hydrogel is capable of controlled antibiotic release without local cell death. A human-derived hydrogel possessing essential proteins for wound repair that is capable of eluting therapeutic levels of antibiotic is an exciting prospect in the field of chronic wound healing.

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Nerve Growth Factor Derives From Pericytes And Smooth Muscle Cells After Extremity Trauma

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**Purpose:** Neurotrophic factors like nerve growth factor (NGF) have been described in the literature as a crucial regulator in developmental biology and inflammation of musculoskeletal tissues. While extremity trauma has been shown to elicit re-engagement of developmental programs like NGF upregulation and invasion of new sensory nerve endings in a mature organism, the source of NGF has remained largely unknown. In a validated extremity trauma mouse model, we have previously demonstrated a significant attenuation of injury site re-innervation by sensory fibers following inhibiting of afferent neural signaling and NGF binding of its receptor TrkA. Given this significant impact of NGF on sensory reinnervation of injured soft tissues and downstream bone formation, we examined the source of NGF after extremity trauma using single cell transcriptomic and reporter proteomic technologies.

**Methods:** A 30% dorsal burn and Achilles transection was performed. The tendon site tissues were harvested from baseline (t0) and day 3, 7, and 21 (n=3-4/group) after induction. Samples were prepared for library generation on a 10x Genomics Chromium Controller, sequenced on an Illumina HiSeq 4000, and analyzed with Cell Ranger Software for pre-processing and alignment to the mm10 genome. Downstream analyses including unsupervised clustering and canonical correlation analyses were performed with Seurat. Immunofluorescent (IF) labeling of cellular markers including αSMA was performed using NGF-eGFP reporter mice at baseline, and at 1,3 and 9 weeks after injury (n=2-3/group).

**Results:** To localize cell specific Ngf expression from injured soft tissue, 9 cell clusters were defined across all timepoints: mesodermal (Prx1) populations including Acta2\(^+\) pericyte/vascular smooth muscle (SMC) and Pdgfra\(^-\) mesenchymal cells, two Pecam1\(^+\) endothelium, and four inflammatory cell populations (mixed, B cell, T cell, and neutrophil). Ngf was found uniquely enriched in the pericyte/SMC cluster in a composite view. These pericytes/SMCs were found in increasing number and Ngf expression across timepoints, peaking at day 21. To further characterize this joint cluster (1273 cells), the pericyte/SMC cluster was isolated and blindly re-clustered to produce new sub-clusters, distinguishing Pdgfrb (platelet derived growth factor receptor beta)\(^{high}\) Prx1\(^{high}\) Abcc9\(^+\) pericytes from Acta2\(^{high}\) Pln\(^-\) Myh1\(^{high}\) SMCs; an uncharacterized cluster with 24 cells was discarded from analysis. Of these two sub-clusters, vascular SMCs demonstrated the highest Ngf expression at baseline (mean normalized Ngf expression of 0.52 [in 41% of cells] vs 0.28 [in 23% of cells]). IF of NGF-eGFP reporter mice show robust NGF co-localization with αSMA\(^+\) SMCs.

**Conclusions:** This is the first work characterizing the pericyte and vascular SMC as a major contributor to NGF signaling at an extremity injury site. The fine resolution defining the cellular source of NGF provides insight into potential mechanisms correlating nascent nerve and vascular growth given the intersection of these pathways at the level of pericytes and myofibroblastic SMCs that will inform future candidate therapeutics to improve extremity trauma healing and re-innervation.