histologic analysis during the first week after injury. This was similarly performed for mice treated with ciprofloxacin or vehicle control.

RESULTS: Flow cytometry demonstrated that genetic loss of scleraxis among mesenchymal cells significantly reduced the presence of macrophages (F4/80+) and neutrophils (Cd11b+Ly6G+) at the injury site within 48 hours after injury. The presence of PDGFRa+ mesenchymal cells was also significantly reduced based on both flow cytometry and histologic analysis. These findings were confirmed with ciprofloxacin treatment. Furthermore, genetic loss of Scleraxis and ciprofloxacin both corresponded with a significant reduction in mesenchymal cell proliferation. Ciprofloxacin treatment led to reduced chondrogenic differentiation and aggrecan expression. Genetic loss of Scleraxis reduced ectopic cartilage formation when compared with wild type controls.

CONCLUSION: These findings indicate that Scleraxis is a potent target to prevent mesenchymal cell proliferation and inflammation. Ciprofloxacin, an FDA-approved drug, has therapeutic efficacy as an anti-inflammatory agent with translational potential to prevent pathologic wound healing.

Identification and Therapeutic Targeting of a Central DNA-Based Mechanism Through Which Movement Augments Inflammation

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PURPOSE: Musculoskeletal trauma and surgery poses a unique challenge to surgeons: early mobilization facilitates rehabilitation but also promotes local inflammation and pathologic wound healing. However the mechanism by which extremity movement promotes local inflammation remains poorly characterized. Recently, discrete structural webs composed of DNA and histones, known as neutrophil extracellular traps (NETs), have been identified as key components of the inflammatory cascade elicited by neutrophils. Here we demonstrate that physically or chemically disrupted NETs are responsible for augmenting local inflammation by directly inducing NET formation (NETosis) yielding a therapeutic target to prevent inflammation caused by early movement.

METHODS: Mice received dorsal hindlimb tendon transection +/- cast immobilization with subsequent treatments to destabilize NETs (+/- DNase I), inhibit NETosis (+/- Cl-Amidine), and/or inhibit NET-induced NETosis (+/- ODN-2088). MicroCT imaging was performed to evaluate In vitro experiments were performed to confirm that NETs produced by neutrophils in response to the inducing agent PMA are able to induce neutrophils to form NETs (NET-induced NETosis). Furthermore, in vitro experiments were performed to confirm that Cl-Amidine but not ODN-2088 inhibits PMA-induced NETosis.

RESULTS: In vitro experiments confirmed that NETs are capable of inducing secondary NET formation (NET-induced NETosis). Both Cl-Amidine and the toll-like receptor 7/8/9 inhibitor ODN-2088 reduced the number of NETs formed through NET-induced NETosis (Cl-Amidine: 46.6 v. 9.1, p<0.05; ODN-2088: 46.6 v. 11.0, p<0.05). Cast-immobilization of mice after tendon transection eliminated ectopic cartilage and heterotopic ossification. Flow cytometry showed that cast-immobilization significantly reduced the normalized presence of neutrophils (1.0 v. 0.08, p<0.05) and macrophages (1.0 v. 0.13, p<0.05) 48 hours after injury. However, treatment of cast-immobilized mice with DNase to destabilize NETs led to a rebound increase in inflammation (neutrophils: 1.0 v. 6.39, p<0.05; macrophages: 1.0 v. 3.0, p<0.05) and caused ectopic cartilage and heterotopic ossification. Furthermore, Cl-Amidine and ODN-2088 both reduced early neutrophil presence in mice with the mobile hindlimb (Cl-Amidine: 1.0 v. 0.4, p<0.05; ODN-2088: 1.0 v. 0.27, p<0.05) and in DNase-treated mice with cast-immobilization (Cl-Amidine: 1.0 v. 0.27, p<0.05; ODN-2088: 1.0 v. 0.28, p<0.05).

CONCLUSION: These results elucidate a central mechanism by which movement induces inflammation - NETs produced during the early inflammation are disrupted and further induce NETosis. These experiments identify a class of toll-like receptors such as ODN-2088, which are capable of reducing inflammation caused by movement by targeting NET-induced NETosis. These findings have immense value in preventing wound healing pathology associated with unchecked
inflammation during movement, while allowing the initial inflammatory response to injury to proceed unimpaired.

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Photochemical Tissue Passivation (PTP) Prevents Contracture of Full Thickness Wounds

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PURPOSE: Large surface area wounds, resulting from burns, trauma, or iatrogenic injury, often result in significant scarring and wound contracture. Despite treatment with any of the currently available gold-standard therapies, contracture can still develop as a sequelae of the process of normal wound healing, specifically, myofibroblast activity. Photochemical Tissue Passivation (PTP) is a process that induces collagen cross-linking after a tissue is painted with photosensitizing dye and then exposed to visible light. PTP has also been shown to limit myofibroblast activity in healing surgical wounds. PTP is easy to perform and can be complete in minutes, and has been established as a safe treatment modality in animal and human studies. We hypothesize that PTP treatment to wounds can significantly decrease the morbidities associated with wound contracture by reinforcing the wound bed with collagen cross-linking and limiting the fibrotic response during wound healing.

METHODS: Full-thickness, excisional 1cm x 1cm wounds were created on the dorsum of C57BL/6 mice. Wounds were either left alone to heal as a control group, or were treated with PTP on the day the wounds were created. Wounds beds on animals in the treatment group received PTP treatment at a fluence of 60J/cm². Wound areas were measured with serial photography for three weeks, at which time animals were euthanized and wound skin was harvested for histological processing with H&E and Masson’s Trichrome staining. Histology was then reviewed by an expert dermatopathologist.

RESULTS: PTP visually prevented wound contracture throughout the study. Wound size on serial photography was calculated as a percentage of the original wound area. Sizes of PTP-treated wounds were almost three times greater than controls after one week (68.6±15% vs 25±9%, p=0.004) and almost seven times greater after two weeks (38.8±22% vs 5.6±6%, p=0.05). At the end of the three-week study, while control wounds had visually completely closed, PTP-treated wounds were not yet closed and were over 10 times larger than control wounds (19.3±20% vs 1.8±2%, p=0.17). On histologic review, PTP treatment promoted increased ingrowth and development of dermal cells, increased vascularity, and development of skin appendages compared to control wounds.

CONCLUSION: PTP prevents wound contracture in full-thickness, excisional wounds, and may accelerate the process of wound healing and development. These findings suggest future utility of PTP treatment not only for excisional wounds, but also for wounds with a high incidence of contracture and associated morbidity, including burns and skin-grafts.

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Isolation of CD248 Expressing Adipose Derived Stromal Cells for Targeted Improvement of Wound Healing

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PURPOSE: Wound healing remains a global issue of disability, cost, and health. Addition of cells from the stromal vascular fraction of adipose tissue have been shown to increase the rate and quality of dermal wound healing. The present study aimed to investigate the angiogenic mechanisms of CD248+ stromal vascular fraction cells in the context of full thickness excisional wounds.

METHODS: Single cell transcriptional analysis was used to identify angiogenic gene-expressing ASCs, then correlate with surface marker expression. SVF cells isolated from human lipoaspirate were FACS sorted based on the presence of CD248. Cells were analyzed for gene expression of VEGF and HGF, and subsequently assessed for tubule formation in vitro. Following this, 6mm full thickness dermal wounds were