Xen29 *S. aureus* biofilms. These disks were implanted in the flanks of hairless, immunocompetent CrI:SKH1-hrBR mice, with each mouse receiving with 1 silicone disk on the left flank and 1 nanocomposite disk on the right flank. After 24 hours, the mice were imaged using IVIS to evaluate the infection using the bioluminescent signature from the Xen 29. Mice were treated with either 200µL 2mg/mL Gentamicin or IP Saline, with or without laser (3W, CW, 800nm, 25s) applied directly over the flank 1 hour after injection. Mice were imaged using IVIS 24 hours after treatment. The disks and surrounding infected tissue were extracted and homogenized. The homogenate was diluted and plated in triplicate to calculate the CFU /g per disk. The change in IVIS luminescence before and after treatment was compared. A total of 14 mice were implanted and treated. 2 mice received treatment of IP Gentamicin only, 4 mice received IP saline and laser therapy, and 8 mice received IP Gentamicin and laser therapy.

**Results:** Only mice treated with Gentamicin and laser therapy demonstrated a decrease in luminescence on IVIS after treatment, representing a decrease in bacterial burden. These mice also demonstrated the greatest percent decrease (-65.8%) of CFU/g on the nanocomposite disk relative to the silicone disk. This was also the only treatment group which saw a significant decrease in bacterial burden on the nanocomposite disk relative to the silicone disk.

**Conclusion:** Mice treated with the combined Gentamicin and laser therapy had the largest percent reduction in biofilm burden on the BSe-Si disk relative to the Si disk. This supports the hypothesis that joint heat and antibiotic therapy can be effective at reducing biofilms in vivo. Ultimately, this technology may have clinical utility towards treating biofilms on indwelling medical devices, mitigating a major burden of disease.

### Disruption Of Mechanotransduction Signaling Preserves Fibroblast Heterogeneity And Promotes Tissue Regeneration In Healing Wounds

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**Purpose:** The ability of cells to respond to injury through either a fibrotic or regenerative phenotype plays an important role in wound healing processes throughout the body. Currently, clinically effective treatment strategies for scars and fibrotic conditions are very limited. In the past decade, we have demonstrated that mechanical stress is a critical component of hypertrophic scar formation, acting via mechanisms that promote fibrotic cellular activities.

**Methods:** We created large deep partial-thickness wounds on the dorsum of red duroc pigs. Immediately upon injury, wounds were treated with either focal adhesion kinase inhibitor (FAKI) or placebo-releasing hydrogels or with standard bandages as controls. Wound healing and scar quality was assessed over time by serial quantification of scar area in photographic images as well as cutometer measurements of the scars over time. We then isolated and cultured dermal fibroblasts from both porcine skin as well as human skin collected from various surgical procedures, including a mastectomy, abdominoplasty, and thighplasty. Fibroblasts were incorporated into 3D collagen lattices. The cell-populated collagen lattices were either stretched, stretched and treated with FAKI, or left unstretched for 2 days before being processed for droplet-based microfluidic single cell RNA sequencing (scRNAseq) using the 10X Genomics platform. Data were log-normalized and partitioned using UMAP based density mappings.

**Results:** Disruption of mechanotransduction using FAKI resulted in significantly accelerated wound closure day 15 vs. day 24, p<0.0001, lower Visual Analog Scale (VAS) score (VAS 59 vs. 100, p<0.0001), decreased firmness and increased elasticity (p<0.05) in the cutometer measurements, and regrowth of hair follicles as well as other dermal appendages (p<0.05). FAKI treated wounds also demonstrated significantly lower expression of alpha smooth muscle actin (αSMA). ScRNAseq analysis indicated that mechanical stretch in the 3D collagen lattice system shifted fibroblast gene expression profiles to distinctly different subpopulations in UMAP based density mappings which showed a higher expression of ACTA1/ACTA2 (coding for αSMA), as well as classical pro-fibrotic markers (PDGFR, SCA1, COL1A1). Treatment of stretched fibroblasts with FAKI then shifted fibroblast heterogeneity into a subpopulation that had decreased expression of pro-fibrotic markers. These findings were observed in porcine fibroblasts as well as fibroblasts originating from various human patients and procedures, demonstrating the robustness of our findings.
Conclusion: Small molecule disruption of FAK-mediated mechanotransduction promotes wound healing and improves the quality of resultant scar likely due to an enhanced regeneration of dermal appendages. Moreover, our data indicate that FAKI treatment is able to mitigate the transcriptional pro-fibrotic signatures which we induced in our 3D collagen lattice system by shifting fibroblast heterogeneity from a pro-fibrotic into a more regenerative subpopulation.

Improved Wound Healing With A Novel Chemical Compound That Modulates Neuronal Pas Domain 2 (Npas2) Gene: In Vitro And In Vivo Studies

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Purpose: Attempts to modulate the wound healing process to minimize scarring remain among the most basic yet difficult challenges facing plastic surgeons. In this study, we focused on a new medical agent that has the potential to modulate the wound healing process to facilitate rapid healing of an incisional dermal wound with minimal scarring. This agent modulates the neuronal PAS domain 2 (Npas2) gene, which is integral to circadian rhythm function. In a previous study, we reported that Npas2 was associated to some degree in the modulation of wound healing. In this study, we performed high-throughput screening (HTS) to identify the compounds that suppress Npas2 and accelerate fibroblast migration to the wound site and have analyzed the effect of Npas2 on fibroblast behavior using one of Npas2 suppressor molecules on in vitro and in vivo wound healing.

Methods: Primary fibroblasts were isolated from WT and Npas2 heterozygous knockouts with reporter gene (Npas2+-/-) mice. Npas2+-/- fibroblasts were seeded on 384 plates, treated with 1,120 FDA-approved compounds and we then observed reporter gene expression. We also tested cell migration under the 1,120 compounds using Oris Cell Migration Assay. Then, WT fibroblast behaviors were characterized using scratch tests and floating collagen gel culture under one of hit compounds, named Dwn1. The effects of Dwn1 on fibroblast migration in vitro and in the full-thickness dorsal skin punched-out wound healing model in vivo were tested.

Results: Several hit compounds were discovered to simultaneously suppress Npas2 and thus increase fibroblast migration. Fibroblasts treated with Dwn1 showed increased cell migration and contraction capabilities relative to untreated FB. Furthermore, gross imaging of Dwn1-treated dermal wounds show statistically significant accelerated wound healing vs non-treated wounds.

Conclusions: This study demonstrates that in vitro cultures of primary FBs treated with Dwn1 show greater migration and contractility. In addition, dorsal punch wounds treated with Dwn1 show similarly enhanced wound healing versus non-treated models. Our observations suggest that the down-regulation of the Npas2 gene might contribute to more efficient dermal wound healing. The clinical ramifications of these in vitro findings warrant further investigation.

Amniotic Fluid Stem Cells Ameliorate Wound Healing In A Diabetic Murine Model

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Purpose: Adult multipotent stromal cells are being widely investigated for treatment of diabetic chronic ulcers that pose a $96 billion annual healthcare burden. One less explored source of these cells is human amniotic fluid with