This novel transgenic mouse model reliably distinguishes ENFs (GFP-CD26), embryonically-derived EPFs (GFP-CD26^+), and postnatally-derived EPFs (GFP^+). The scars and surrounding unwounded tissue were harvested upon complete wound healing (day 14), enzymatically-digested, and then sorted by fluorescence-activated cell sorting. Five sorted fibroblast populations (pEPFs from wounded skin; eEPFs from unwounded and wounded skin; and ENFs from unwounded and wounded skin) were then analyzed by bulk RNA-sequencing (experimental schematic in n = 2 biological replicates, 6 pooled mice each).

**Results:** Hierarchical clustering and principal components analysis of differentially expressed genes after wounding revealed that pEPFs clustered more closely with eEPFs than with ENFs. Both postnatally- and embryonically-derived EPFs showed increased expression of fibrosis-related genes in response to wounding, including Dpp4 (CD26). In contrast, ENFs showed increased expression of mechanotransduction signaling-related genes (Notch ligands Jag1, Dll1), suggesting that they are responsive to wound mechanical cues. Supporting these findings, gene set enrichment analysis of ranked whole genomes revealed that scar ENFs enriched for terms related to ECM adhesion and Notch signaling, while postnatal EPFs enriched for terms related to ECM production. Finally, we compared transcriptional activity of genes known to differentiate ENFs and eEPFs. Once again, pEPFs diverged from ENFs, exhibiting a gene expression profile more closely resembling that of eEPFs.

**Conclusion:** Postnatal En-1 activation in ENFs during wound healing is accompanied by the acquisition of a profibrotic transcriptional profile similar to that of embryonically-derived EPFs. These RNA-seq data also support our recent finding that ENFs activate En-1 through a canonical mechanotransduction mechanism involving YAP and Notch, before transitioning to an ECM-producing phenotype (pEPF). Thus, inhibition of mechanotransduction signaling may mitigate scarring by blocking this phenotypic switch. In future studies, we will compare the chromatin profiles of ENFs, eEPFs, and pEPFs to determine whether postnatal En-1 activation recapitulates the epigenomic shift that occurs during embryogenesis.

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**Purpose:** Wound healing is a complex process that involves the extensive coordination of different cell types, most significant of which is the fibroblast. Fibroblasts play a key role in wound healing by assisting in wound closure and tissue remodeling. Significant research efforts have gone into identifying and characterizing specific subsets of fibroblasts involved in wound healing. However, unanswered questions in wound healing remain including where these cells originate from and what their progenitor phenotypes are. Our lab has previously shown that scar fibrosis is dependent on focal adhesion kinase (FAK) signaling and mechanotransduction pathways. The aim of our research was to characterize fibroblast progenitor-type phenotypes in the setting of dermal injury and to understand the role of FAK-signaling in fibroblast proliferation during wound healing.

**Methods:** We used the Rainbow mouse model (Rosa26^{VT2/GK3}), which has a four-color reporter construct at the Rosa26 locus. Following the induction of Cre-recombinase, cells express one of four fluorescent proteins, and all daughter cells are labeled with the same color. We created wounds in the dorsal dermis of Rainbow mice using a stented wound-healing model which mimics human wound healing kinetics. We used local application of tamoxifen liposomes to induce Cre recombinase in tissue-resident cells at the time of injury. Confocal imaging was conducted on whole mount and sectioned wound specimens after tissue clearing. Using an unbiased FACS strategy, we isolated wound-healing fibroblasts based on their rainbow color and their position (outer edge vs. center of wound) for RNA-seq at various timepoints post-operatively (POD 2, 7 and 14). Topical FAK-inhibitor versus vehicle control was applied to Rainbow mouse dorsal dermal wounds and imaged with confocal microscopy.
**Results:** Confocal imaging shows distinct radial proliferation of tissue-resident, progenitor-type fibroblasts that are activated along the wound edge (area of greatest tension) and expand towards the center of the wound, using the Rainbow mouse model with an activated fibroblast (aSMACreERT2) driver. These linearly-expanding clones can be further appreciated in the dermis on wound cross section using a ubiquitous (Actin-CreERT2) driver with our Rainbow mouse model, compared with unwounded, control skin. Bulk RNA-seq of wound healing fibroblasts shows significant differences in gene expression patterns between fibroblasts isolated from the outer versus inner portions of the wound, and upregulation of FAK-pathway genes. Fibroblast heterogeneity is observed on single-cell RNA-seq of fibroblasts isolated based on their rainbow color. Application of an FAK-inhibitor shows disruption of fibroblast clonal proliferation compared with control wounds on confocal imaging.

**Conclusions:** Dermal fibroblasts undergo clonal expansion in a distinct radial pattern in response to wounding, suggesting the presence of tissue-resident progenitor-type fibroblasts that are activated with injury. Differences in “outer” and “inner” fibroblasts are observed on RNA-seq, with significant heterogeneity amongst the fibroblasts isolated at various timepoints. The clonal proliferation of wound-healing fibroblasts is dependent on FAK-pathway signaling.

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**Electrophysiological And Histological Evaluation Of Composite Regenerative Peripheral Nerve Interfaces For Closed-loop Neuroprosthetic Control**

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**Purpose:** Current bionic limbs are capable of multi-degree-of-freedom, anthropomorphic motor function. However, insensate hardware without intuitive somatosensory feedback is visual/auditory cue dependent and burdensome. Both neuromuscular-like control and neurocutaneous-like feedback are important prosthetic qualities. The composite regenerative peripheral nerve interface (C-RPNI), constructed by implanting a transected mixed sensorimotor peripheral nerve between autologous free muscle and de-epithelialized skin graft components, is an innovation for bidirectional signal transduction. The aim of the current study was twofold: first, to determine electrophysiological signal transduction capabilities and second, to histologically characterize C-RPNI tissue viability, regeneration, and selective axon-to-target organ reinnervation. The overall goal is to develop a multifunctional C-RPNI that amplifies volitional effenter signals and simultaneously transduces sensory input.

**Methods:** Thirty rats had C-RPNIs surgically constructed by implanting the distal end of a transected common peroneal nerve between a contralaterally transferred extensor digitorum longus graft and a de-epithelialized glabrous skin graft harvested from an isogenic donor rat hindpaw. Animals were randomly assigned to one of three experimental endpoint groups (3, 6, or 9 months postoperatively) for ex-vivo electrophysiological testing. Electrodes were acutely placed. Three experimental models were evaluated: electrically stimulate 1) proximal nerve, 2) muscle, 3) skin while simultaneously recording a) muscle and skin, b) nerve and skin, c) nerve and muscle signals respectively. C-RPNI constructs were harvested and weighed at all endpoints. H&E stained cross-sections were evaluated for surgical construct health. Additional samples were immunolabeled and imaged using the three-dimensional iDISCO solvent cleared organ method to visually characterize reinnervation.

**Results:** Three month interval evaluation of C-RPNI electrophysiological parameters recorded CMAP amplitudes and conduction velocities of 8.7±1.6 mV and 10.0±1.2 m/s, and evoked peak-to-peak CSNAP amplitudes and conduction velocities of 140±35 µV and 9.1±1.4 m/s. Longer-term average recorded CMAP amplitudes and conduction velocities were 6.1±1.6 mV and 12.0±2.0 m/s at 6 months, and 10.2±2.1 mV and 9.5±0.6 m/s at 9 months. Evoked peak-to-peak CSNAP amplitude and conduction velocity averages were 278±163 µV and 11.1±1.3 m/s at 6 months, and 202±6.3 µV and 8.8±1.1 m/s at 9 months. All endpoint C-RPNI histology demonstrated healthy vascularized grafts maintaining 73±9% of original construct mass, and self-selective motor and sensory axon reinnervation of muscle and dermal components respectively.

**Conclusion:** The CRPNIs physiologically sort mixed sensorimotor nerve axons. Motor axons selectively reinnervate muscle and sensory axons selectively reinnervate skin target organs. Immunolabeled, three-dimensional imaging spatially mapped specific muscular and dermal components.