**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 (aSMA-CreERT2) 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 effferent 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.
Bimodal topography allows independent EMG recording and sensory stimulation. CRPNI components electrophysiologically demonstrated appropriate efferent CMAP and afferent CSNAP signaling. Constructs were stable over the 9 month period without neuroma formation or disabling scar tissue. The results support C-RPNI potential for closed-loop neuroprosthetic control.

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Silk Fibroin Based Conduits Filled With Native Spider Silk Fibers Successfully Promoted Nerve Regeneration In A 10 Mm Sciatic Nerve Defect In Rats

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Purpose: The surgical repair of nerve transection injuries remains a challenging task and often results in unsatisfactory functional recovery. If a direct coaptation is not possible, the current gold-standard is the use of an autograft. However, the availability of autologous nerve tissue is limited and the harvest of a donor nerve entails functional loss and possible donor site morbidity. In the search for alternatives, different synthetic and biological materials are currently tested to bridge nerve gaps. Recent studies supported silk as promising material for tissue engineering and the development of artificial nerve conduits. In addition, nerve conduits that contain an internal framework as guiding structures could enhance a directed axonal re-growth. Spider silk possess excellent mechanical properties such as an adequate tensile strength, long-term degradability and a non-immunogenic nature, which support their use as promising conduit filling material. In this study, we investigated the performance of a silk fibroin-based conduit filled with spider silk fibers to bridge a 10 mm sciatic nerve defect in rats.

Methods: In 18 male Sprague-Dawley rats, a 10mm piece of the sciatic nerve was resected and immediately bridged with 1) autografts (control group, n=6), 2) empty silk conduits (experimental group one, n=6), and 3) silk conduits filled with spider silk fibers (experimental group two, n=6). Walking track analysis was performed for each animal prior to surgical intervention and every 14 days over a course of 14 weeks. Functional recovery was evaluated by calculating the sciatic functional index (SFI) according Bain et al. At the endpoint, animals were sacrificed and the nerves were harvested to assess axon re-growth and myelination by histomorphometric as well as immunofluorescence analyses on paraffin sections.

Results: The walking track results showed that there was no statistical difference in the mean SFI of animals treated with the autograft or the silk fiber containing silk conduits. Moreover, the immunofluorescence stainings of nerve sections illustrated a similar pattern of regenerated nerve tissue in sections of autografts and filled silk conduits, while a less advanced nerve regrowth was seen in the samples containing empty silk conduits. The histomorphometric parameters are currently evaluated.

Conclusion: Taken together, our study demonstrated that the functional recovery of a 10 mm sciatic nerve defect bridged with silk conduits containing spidersilk fibers as internal guiding structure was comparable to autologous nerve grafts.

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Multichannel Carbon Fiber Electrode Arrays For Selective Stimulation And Recording Of Sensory-motor Signals In Peripheral Nerve Interfaces

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Purpose: Advanced motorized prosthetic limbs are capable of fine multiple joint manipulations and possess the potential to emulate the intricate functions of the native extremity. Equipped with force or pressure sensors, these devices are also able to provide tactile information to the user. However, increasing a prosthetic’s manipulative degrees of freedom requires additional controlled inputs from the amputee. The development of a reliable interface between amputee and prosthetic device is therefore crucial for closing the