PURPOSE: Regenerative Peripheral Nerve Interfaces (RPNIs) show promise in controlling neuroprosthetic devices. We have implanted and recorded from RPNIs in 3 human subjects. Here, we present the results from our longest implanted subject with a distal transradial amputation.

METHODS: An RPNI consists of a muscle graft that is neurotized by the distal end of a transected peripheral nerve. Once revascularized and reinnervated, the RPNI muscle graft serves as a stable bioelectric amplifier for efferent nerve action potentials and produces recordable electromyography (EMG) signals. The subject was implanted with RPNIs on the residual median, ulnar, and dorsal radial nerves. Using ultrasound, RPNIs were located, and percutaneous fine-wire bipolar electrodes were inserted for acute EMG recordings. Temporal features of the EMG waveforms (100-500Hz) were used for decoding algorithms.

RESULTS: Eight months post-surgery, we recorded 300–400µV EMG signals from the median RPNI with signal-to-noise ratio (SNR) of 24.2 and 100–120µV EMG signal from the ulnar RPNI with SNR of 5.84. Additionally, EMG from residual muscles was obtained including the flexor digitorum superficialis with 100–120µV signals, SNR of 6.30, and flexor pollicis longus with ~1mV signals, SNR of 47.8. With these signals, the subject controlled a virtual robotic hand in real time with 96% accuracy, choosing 1 of 4 movements within 212 trials. Importantly, the subject controlled a physical Touch Bionics iLimb neuroprosthetic hand with 100% accuracy, choosing 1 of 3 movements within 100 trials.

CONCLUSION: RPNIs harness neural signals from transected peripheral nerves with sufficient amplitude and fidelity to control an advanced neuroprosthetic limb.

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Discovery Of Unique Immune Cell Subsets During The Development Of Capsular Fibrosis

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PURPOSE: Capsular fibrosis is the most common long-term complication after breast implant-based augmentation. The cellular alterations that underlie capsular fibrosis are still barely understood. The identification of cell types in the implant area can provide clues in determining the pathophysiology of the fibrotic changes to find a medical cure. Here, using an in vivo mouse model with silicone implants we analyze the cell types present in the capsule over time. Next, we use single cell analysis to characterize these cells to determine their involvement in fibrotic capsule development.

METHODS: Capsular fibrosis was induced by inserting a customized silicone implant (same shape and filling as a human silicone breast implant) in C57/BL6 mice. A paravertebral incision was performed on the dorsum of the mice and the implant was placed in a subcutaneous pocket. Explantation was done on day 15, day 30 and day 90 by resecting the implant with the surrounding capsule en-block for SEM and TEM. The capsule itself was digested and cells were immunostained with CD45, EpCam, CD31, CD11b, F4/80 and Col I. Cells were subjected to further analysis by fluorescence-activated cell scanning (FACS). Single cells were sorted and subjected to single cell transcriptional analysis. H&E, Trichrome and Picosirius Red as well as immuno-staining were performed on the capsules.

RESULTS: FACS analysis revealed that the major cell types in the fibrotic capsule across all time-points were immune cells, not fibroblasts or endothelial cells. The immune cells could be classified as: (i) CD45+/CD11b+/F4/80+ (macrophages), (ii) CD45+/CD11b+/F480- (myeloid cells, including dendritic cells) and (iii) CD45+/CD11b-/F4/80- cells. On day 15, the maximum number of cells was CD45+/CD11b+/F4/80+ (mean:76.61%, SEM:5.13%) and there were no cells that were CD45+/CD11b-/F4/80-. On day 90 there were no cells that were CD45+/CD11b-/F4/80-. On day 30 the CD45+/CD11b+/F4/80+ cells were reduced and the CD45+/CD11b+/F4/80+ cells were the statistically significant population (mean:57.35%, SEM:8.78%). The most interesting finding was that CD45+/CD11b-/F4/80- cells that were not found at earlier time-points were seen on day 90 (mean: 27.2%, SEM:3.67%) and there was a further reduction in the macrophage population. The macrophages were found to deposit Coll both by FACS and immunostaining of sections. Scanning electron microscopy and Transition electron microscopy were used to confirm the presence of these immune cells and collagen deposition. We are currently using single cell analysis to understand the
CONCLUSIONS: For the first time, our results reveal that the most prominent cells at every time-point in the capsule are immune cells. Macrophages comprise the largest subset of immune cells at day 15 but the macrophage numbers decrease subsequently. At day 30 and day 90 CD45+/CD11b+/F4/80- myeloid cells, most likely dendritic cells, comprise the largest immune subset. Importantly on day 90, CD45+/CD11b-/F4/80-, most likely T cells arise in the capsule. We are currently conducting single cell and functional studies on these immune subsets to understand their identity and role in capsule formation over the course of time. Our findings have promising therapeutic implications for the treatment of capsular fibrosis.

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Identifying The Role of and Treatment Targeting Bone Progenitor Cell VEGF Secretion On The Niche Supporting Traumatic Heterotopic Ossification

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PURPOSE: Patients who sustain mechanical trauma, spinal cord injury, burns, or extremity surgeries are at risk for developing heterotopic ossification (HO), the pathologic formation of extraskeletal bone. HO is formed through a process of endochondral ossification initiated by acute inflammation. Vascular endothelial growth factor (VEGF) has been shown to be critical for both normal bone development and for fracture repair. We hypothesized that VEGF plays a central role during ossification of the cartilaginous matrix present during pathologic HO formation and that therapeutic targeting of the vascular niche is sufficient to prophylax against traumatic HO.

METHODS: Male C57BL/6J mice underwent Achilles’ tendon transection and 30% of total body surface area (TBSA) dorsal burn injury to induce HO. Mice underwent Microfil CT and near infra-red imaging with intravascular injection of Angiosense to survey local vascularity. CDH5 endogenous reporter lines were imaged with confocal microscope. Hindlimb sections from injured mice were immunostained and injury site was harvested for flow cytometry and PCR. To further validate these findings and define the source of VEGF, co-staining with PDGFRa and VEGF was performed. Mice with genetic loss of Vegf in cells of mesenchymal lineage (Vegf cKO: Prx-cre/Vegffl/fl) and their littermate controls underwent burn/tenotomy (n=4–7). Human histological sections from analogous early HO sites were also examined via immunohistochemistry to confirm the translational value of these findings. Finally, a subset of mice after burn/tenotomy were treated with bevacizumab (biweekly injections of drug 10mg/kg) or vehicle control and were analyzed by histology (3 weeks) and MicroCT (9 weeks, n=5–6) for therapeutic testing.

RESULTS: In wild-type mice, vascular density was intimately associated with the HO anlagen as demonstrated by near infrared imaging with Angiosense, Microfil imaging 5 weeks after burn/tenotomy, and endogenous CDH5 signal. Immunostaining of early human HO specimens with VEGFa and PDGFRa confirmed co-localization, corroborated by observations in mouse histology. Mice treated with bevacizumab, a potent anti-VEGF antibody, formed significantly less HO when compared at 9 weeks with vehicle control (2.64 v. 6.85 mm³, p=.0013). Genetic targeting produced parallel findings, with Vegf cKO mice forming significantly less HO near the distal tibia when compared with littermate controls (2.52 v. 4.53 mm³/mm, normalized to tibial cortical thickness compared to littermate control).

CONCLUSIONS: Leveraging our knowledge of normal bone development, these findings suggest that VEGF from the mesenchymal niche plays a critical role in the formation of HO and can be successfully targeted to attenuate this process via bevacizumab, an FDA-approved pharmacologic agent used for its anti-VEGF properties. Given the