High-Resolution Spatial Transcriptomics of Intra-Operative Muscle Biopsies Establishes a Platform for Biomarker Discovery in Nerve Repair

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Introduction: Muscle denervation following peripheral nerve injury triggers sensorimotor loss, driving progressive weakness, atrophy, and functional decline. While nerve repair or transfer can re-establish axonal contact, recovery of meaningful strength remains inconsistent across patients, reflecting biological factors that remain undefined. Prior work from our team has reported preserved motor-end-plate morphology in denervated muscle biopsies that may predict reinnervation success. Beyond morphology-based metrics, we apply high-resolution spatial transcriptomics of intra-operative human muscle biopsies to uncover reinnervation biomarkers that guide patient selection in nerve repair surgery.

Methods: With IRB approval, intra-operative muscle samples were obtained from consenting patients undergoing routine upper-extremity surgery. From a male patient, healthy innervated trapezius (control) and denervated deltoid biopsies, confirmed by pre-operative EMG, were flash-frozen using liquid nitrogen-cooled isopentane. 10× Genomics Visium HD's spatial matrix of 8-µm barcodes was utilized for high-density capture of mRNA molecules across the muscle samples. Quality control filtering was performed for low unique molecular identifiers (UMIs) and gene counts that deviated markedly from neighboring muscle architecture. Canonical marker genes were used to study spatial expression and annotate clusters into categories including fast/slow-twitch myofibers, fibro-adipogenic progenitors (FAPs), immune, endothelial, peripheral nerve, and epithelial populations.

Results: Visium HD detected 16,660 genes in 331,326 trapezius barcodes and 16,356 genes in 459,921 deltoid barcodes, with > 90% valid barcodes and > 98% on-target reads to the human probe set. At 8-µm resolution, healthy trapezius averaged 99.6 UMIs and 71.9 genes per barcode, while denervated deltoid averaged 173.2 UMIs and 125.8 genes per barcode, reflecting higher RNA density in denervation. Clustering of the healthy trapezius revealed multiple fast-fiber clusters (33.4%) and slow-fiber clusters (12.2%), along with a developmental-myosin cluster (9.7%). Other populations included fibro-adipogenic progenitors (11.2%), immune (7.9%), vascular (14.5%), and nerve-associated (1.3%) groups. In contrast, the denervated deltoid demonstrated a higher proportion of slow-fiber clusters (37.2%) and fewer fast-fiber clusters (16.6%), with a developmental-myosin cluster (5.7%). This shift reflects the well-recognized tendency for type II (fast) myofibers to undergo preferential atrophy with denervation, particularly in early stages of injury, leading to the relative predominance of slow-fiber profiles observed here. Other populations shifted towards an enrichment of fibro-adipogenic progenitors (24.5%), with immune (3.5%), endothelial (6.7 %), and epithelial (5.8%) populations. Notably, nerve-associated clusters were absent in the denervated deltoid.

Discussion: Each set of fast/slow-fiber clusters captured the layered architecture of individual myofibers, with clusters arising from the central contractile core versus those localized to the sarcolemma rim. This reflects the ability of 8-µm resolution to reveal layered organization within myofibers that would be obscured at lower resolution. Within each reported cluster category (immune, vascular, or FAPs), multiple genetically distinct clusters were identified, though they are reported here in aggregate for clarity. This underscores the capacity of high-resolution spatial transcriptomics to resolve heterogeneity within each cell population, yet still permit broad biological interpretations as seen above.

Significance: High-resolution spatial transcriptomics can be reliably applied to human muscle biopsies collected during routine orthopedic surgeries to generate transcriptomic maps from routine biopsies will allow us to create a foundation for tracking post-injury remodeling across multiple time points. By characterizing spatiogenomic shifts associated with denervation and reinnervation, this approach enables the identification of biomarkers that can refine patient selection and optimize the timing of nerve repair interventions.

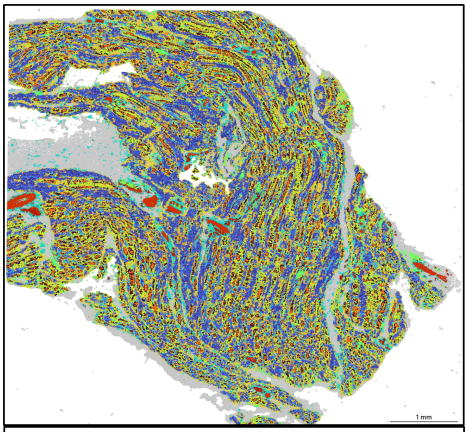

Figure 1: 8 by 8-µm Spatiogenomic Map of Intra-operative Denervated Deltoid Biopsy