Quantitative Evaluation of Rat Sciatic Nerve Degeneration/Regeneration Using High-frequency Ultrasound

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INTRODUCTION: Peripheral nerve injury is a common condition caused by traumatic injuries such as blast injuries or vehicular accidents. Functional recovery is often poor, in part due to limited diagnostic tools for assessing the severity of injury or the progress of regeneration after repair. In this study, we evaluated the utility of high-frequency ultrasound to non-invasively track degeneration and regeneration in a rat model of peripheral nerve injury.

METHOD: All procedures were approved by the institutional IACUC. A 10mm segment of sciatic nerve was excised from 12-week-old Lewis rats under anesthesia. A chronic nerve degenerative model (i.e., no repair) was created by suturing Polydimethylsiloxane (PDMS) block between the proximal and distal stumps (Figure 1A). An acute regeneration model was created by immediately repairing the transected nerve with an autograft; the excised segment was reversed and sutured into the gap with 8-0 monofilament sutures. A VevoMD system (VisualSonics) with a 48MHz frequency probe was used for high frequency ultrasound assessment. This study explored spatial and temporal changes in quantitative backscatter coefficient (BSC) spectrum-based outcomes and B-mode textural outcomes, using gray-level co-occurrence matrices (GLCMs), during the progressive transition from acute to chronic injury. Immunolabeling of axons (beta3-tubulin) and laminin as well as Masson’s trichrome stain were used to suggest a structural basis for ultrasound outcomes.

RESULTS: In the chronic injury model, both mean BSC spectrum-based and mean GLCM-based measures exhibited significant spatial and temporal differences, with distal stumps enclosed in proximity to the injury site being particularly affected. Both integrated BSC (iBSC) and GLCM contrast sensitively detected peripheral nerve degeneration at 1-month and 2-month post-injury time points (Figure 1B-C). In the acute repair model, iBSC values were intermediate to those of unrepaired and control values at 1-month post-injury (Figure 1D vs. Figure 1B). Outcomes also indicated that BSC spectrum-based and GLCM-based parameters moderately correlate with each other (R > 0.6 for most of the parameters), suggesting the possibility of a shared structural basis for these parameters (Figure 1E). IHC and trichrome stain confirmed structural changes of degenerated sciatic nerve shown in Figure 1F-I.

DISCUSSION: This study investigated the efficacy of BSC spectrum-based and GLCM-based quantitative high frequency ultrasound parameters in distinguishing between degenerating and regenerating nerves in vivo. Both strategies have the potential to be useful tools for diagnosing nerve injury. The moderate positive correlation between the two sets of parameters may suggest there is an intrinsic biological effect on both sets of parameters, but also potentially different structural influence on each outcome. In conclusion, this work has important implications for understanding the application of quantitative HFUS approaches to the evaluation of peripheral nerve injury and repairs.

SIGNIFICANCE/CLINICAL RELEVANCE: In clinical application, BSC spectrum-based and GLCM-based analyses could be utilized to effectively diagnose peripheral nerve degeneration and regeneration.

Figure 1: Quantitative evaluation of peripheral nerve degeneration/regeneration. A) Longitudinal B-mode image showing the sciatic nerve (orange dashed lines) and PDMS implant (gray dashed box) for chronic degeneration model. B-C) Imaging outcomes at different time points and at different sites for degeneration model (no repair). The data was divided into four individual sites and one-way ANOVA across different time points was performed for mean B) iBSC, C) GLCM contrast (* p < 0.05, ** p < 0.01, *** p < 0.001 vs pre-surgical time point). D) iBSC outcome 1 month post-acute repair. E) Scatter plot
between GLCM contrast and iBSC; $R = 0.628$ was shown. F) Immunohistochemistry of contralateral healthy sciatic nerve G) Near distal (close to the PDMS implant) H-I: Trichrome labeling: H) healthy contralateral sciatic nerve vs. I) loss of epineurial integrity in near distal stump.