Ultrasonographic Evaluation Of Nerve Regeneration After An Artificial Nerve Graft For Complete Transection Of The Sciatic Nerve In Rats.

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Disclosures: Koshiro shimbo (6-Nipro Inc.), Atsushi Yokota (N), Keitaro Fujino(N), Yukiko Yoshimura(N), Shuhei Otsuki(N) (In this study, we used Renerve® provided by Nipro.)

INTRODUCTION: Recently, reconstruction surgeries using a bioabsorbable nerve conduits have become a common treatment for peripheral nerve injuries. While imaging modalities, such as MRI have limitations in evaluating postoperative regeneration of the repaired nerves, ultrasound scanning has emerged as a promising tool for this purpose. However, the correlation between ultrasonographic findings and functional or histological alterations remains unestablished, making the clinical utility of ultrasound unclear. This study aimed to assess whether the nerve regeneration process can be monitored with ultrasound by comparing ultrasound findings with histological evaluations and measurements of the ankle joint range of motion (ROM).

METHODS: All experiments were performed with Institutional Animal Care and Use Committee (IACUC) approval. Rat sciatic nerve transection surgery: Nine 8-week-old female Sprague-Dawley rats were used. Under general anesthesia (medetomidine, midazolam, and butorphanol), a 5-mm nerve defect was created in the sciatic nerve by sharp transection. A 7-mm bioabsorbable collagen conduit (Renerve®, Nipro, Osaka, Japan) was used to bridge the defect. The nerve ends were inserted 1 mm into each end of the conduit and secured with an 8-0 nylon horizontal mattress suture. Three rats were euthanized at 8 weeks post-surgery, and the remaining six at 12 weeks. The conduits were harvested at the time of euthanasia. Ultrasound Scanning: Examinations were performed weekly under general anesthesia, with an ultrasound system "Aplio me" (Canon Medical Systems, Tochigi, Japan) using a 22MHz hockey transducer. The transplanted conduit was evaluated in both longitudinal and transverse sections. Range of Motion (ROM) Measurement: To measure ankle joint ROM, a weekly swim test was performed by filming the rats from the side as they swam in a 90-cm glass basin [1]. Immunohistochemistry: Harvested samples were immediately fixed in 4% paraformaldehyde, embedded in optimal-cutting-temperature (OCT) compound, and sectioned (6 µm). Immunofluorescence staining was performed to examine NF200 expression.

RESULTS SECTION: Ultrasound examination revealed hypoechoic areas within the conduits around week 7. The cross-sectional area (CSA) of these hypoechoic areas progressively increased over time in the transverse view (Fig.1). Correspondingly, the maximum ankle joint angle, measured by the swim test, also showed a gradual increase starting from week 7. A larger CSA on ultrasound corresponded to a greater maximum ankle angle (Fig. 2). Immunofluorescence staining at 8 weeks confirmed the presence of nerve fibers within the remaining conduit. By week 12, the conduit was almost absorbed and the nerve bundles had thickened (Fig. 3).

DISCUSSION: Our findings suggest that the hypoechoic area identified by ultrasound represents a regenerating nerve bundle, given that its CSA was consistent with that of the nerve fibers confirmed by immunofluorescence staining. This is further supported by the correlation between the progressive increase in the CSA of this area and the increase of the maximum ankle joint angle over time. These results indicate that ultrasound scanning may be a viable method for evaluating the extent of recovery after peripheral nerve injury. However, this study has several limitations, including a small sample size that may affect the generalizability of our findings, and the lack of assessment for intra- and inter-examiner variability, which may limit the reproducibility of our measurements.

SIGNIFICANCE/CLINICAL RELEVANCE: The results of this study suggest that ultrasound scanning is a useful method for evaluating the nerve regeneration process following treatment with a nerve guidance conduit in a simple and non-invasive way.

REFERENCES: [1] S. Targosinski, et al. A swim test for functional assessment of rodent peripheral nerve regeneration. J. Neurosci. Methods. 379 (2022) 109663

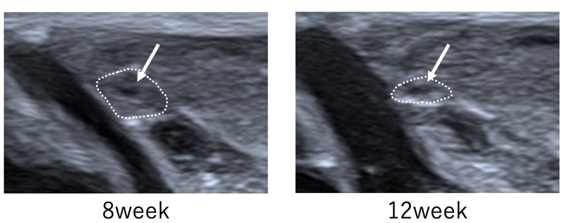

Figure 1. Ultrasound scanning (transverse view).
The dotted line indicates the conduit, and the white arrow indicates the hypoechoic area. Compared to the image at 8 weeks (Left), the conduit is absorbed, and the hypoechoic area has become larger at 12 weeks (Right).

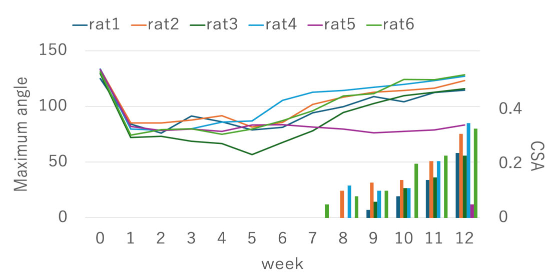

Figure 2. The maximum angle of the ankle joint and the CSA of the hypoechoic area.
The maximum ankle joint angle gradually increased from around week 7. Hypoechoic areas began to appear around week 7, and their cross-sectional area (CSA) progressively enlarged. The individuals with a smaller ankle angle also had a smaller CSA.

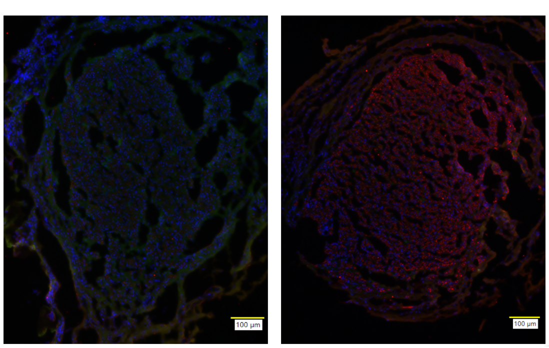

Figure 3. Immunohistochemistry (transverse section).
NF200 is shown in red and DAPI in blue.
(Left) At 8week, nerve fibers with faint NF200 staining are sporadic within the remaining conduit.
(Right) At 12week, as the conduit's absorption progresses, the NF200 staining becomes more intense and the nerve fiber bundle becomes thicker.