Matrilin-2 and Lysine Enhanced Chitosan Scaffold within a Type 1 Collagen Conduit Promotes Peripheral Nerve Regeneration and Motor Function Return following a Segmental Nerve Defect in a Rat Model

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INTRODUCTION: Peripheral nerve injuries have the potential to be detrimental to a patient's independent function. Injuries with minimal nerve loss may be amenable to end-to-end repair. With larger defects, use of a conduit, processed allograft, or autograft are indicated. As conduits lack cross-sectional architecture, their use is best supported for small gaps, less than 1cm. For longer gaps, processed allografts or autografts are used. Processed allografts provide the native architecture of nerves, but detergent and processing removes the bioactivity of cellular and extracellular matrix (ECM) proteins conducive to nerve regeneration. Autograft delivers both the architecture as well as the cells, growth factors, and ECM needed for regeneration, but requires a loss of donor nerve function and possible neuroma formation. Given pros and cons of each of these modalities, this work sought to construct a readily available nerve graft with a biomimetic cross-sectional multi-channel architecture to reduce donor site morbidity and improve cellular chemotaxis and axonal regeneration. Matrilin-2 (MATN2), a protein from the Matrilin family of ECM proteins, has been shown to enhance axonal outgrowth and Schwann cell (SC) migration. Prior work in our lab found that MATN2 and chitosan, a biocompatible, biodegradable, polysaccharide derived from chitin, are able to form a multi-channel scaffold within a type I collagen conduit. In-vitro use of this scaffold found significantly increased SC adhesion and migration compared to an empty collagen conduit or chitosan alone. A dorsal root ganglion axonal outgrowth assay further found that lysine enhanced chitosan interaction with MATN2 provided greater axonal outgrowth compared to matrilin-2 and chitosan. The purpose of this study was to advance our matrilin-2 and lysine enhanced chitosan scaffold into a rat sciatic nerve model to determine whether this graft would offer an improved return of function, axonal outgrowth, and SC migration compared to an empty collagen conduit as well as to autograft.

METHODS: 30 Lewis rats were utilized for a 12-week study period. Rats were divided into three groups of 10: 1) untreated collagen conduit (UC), 2) collagen conduit treated with MATN2 and lysine enhanced chitosan (TC), and 3) reverse autograft (RA). Each rat underwent baseline motor testing via walking track assessment by recording ground reaction force over a pressure-sensing walkway. Rats then had a 10mm sciatic neurectomy of the left hindlimb (LHL) with implantation of a nerve graft. In the RA group, the resected sciatic nerve was flipped 180° and sutured. Walking track analysis was taken every 2 weeks. At 12-weeks, the normal right and experimental left sciatic nerves were exposed to measure the compound muscle action potential (CMAP). Sciatic nerves and associated grafts were then harvested along with the tibialis anterior (TA) and gastrocnemius (GSC) muscles from both right hindlimb (RHL) and LHL. For axon histomorphometry, 3mm of the distal graft and nerve was transversely sectioned into 1µm sections and stained with Toluidine Blue. The remaining nerve was then sectioned longitudinally into 10µm sections to visualize SC migration via immunofluorescence (IF) of S-100. One-way analysis of variance with Tukey's multiple comparisons test was utilized to assess for statistical differences between groups with $P \leq 0.05$ considered statistically significant.

RESULTS: Walking track analysis evaluated proportion of force placed on LHL compared to RHL. Baseline values averaged 95.1% ± 4.5% over all three groups, dropping to an average of 52.2% ± 3.9% following surgery. The UC group fell to 42.6% ± 8.2% by week 12 (Fig. 1A), while the TC and RA groups steadily rose to 78.5% ± 1.4% and 85.2% ± 5.6%, respectively (Fig. 1A). Analysis of week 12 force measurements found significant differences between UC vs TC groups (p=0.0035) and UC vs RA groups (p=0.001). CMAP evaluation of the UC group demonstrated a mean recovery of 37.3% ± 4.95% compared to the normal RHL, 62.9% ± 18.1% for the TC group, and 79.7% ± 15.9% for the RA group (Fig. 1B), with significant differences between UC vs TC (p=0.044) and UC vs RA groups (p=0.0013). TA and GSC weights of LHL normalized to RHL found significant differences in weights between the UC vs TC groups (p=0.0202), UC vs RA groups (P<0.0001), and TC vs RA groups (P=0.0030). Decreased axon density and myelination for the UC nerve (Fig. 2A) was seen when compared to an uninjured nerve (UN) (Fig. 2D). Greater density and number of myelinated axons was appreciated for the TC nerve (Fig. 2B) and RA nerve (Fig. 2C), similar to the UN (Fig. 2D). Quantitative analysis of axon number revealed a mean of 3,121 ± 908.4 axons for UC, 4,554 ± 1,740 axons for TC, 7,974 ± 2,283 axons for RA, and 9,710 ± 2,395 axons for UN (Fig. 2E) with significant differences between UC vs RA (P=0.0013) and TC vs RA (P=0.0313). S-100 IF, used to identify SC migration and distribution, demonstrated a narrow growth cone and reduced intensity of fluorescence of the UC nerve (Fig. 3A) when contrasted to the more robust migration and broader distribution seen in RA (Fig. 3C) and more so in UN (Fig. 3D). TC nerve (Fig. 3B) demonstrated a large growth cone with broader SC migration and fluorescent intensity compared to the UC nerve (Fig. 3A).

DISCUSSION: This study found that a scaffold composed of MATN2 and lysine enhanced chitosan within a collagen conduit, used to repair a 10mm rat sciatic nerve defect, performed better than an empty collagen conduit, but not as well as autograft. However, the TC group demonstrated no significant differences from the RA group when evaluating return of motor function with walking force or between axonal function with CMAPs. Muscle weight, axon counts, and SC migration and distribution were better for RA compared to the TC group. When compared to the UC group, the TC group demonstrated significantly better differences in return of walking force, CMAP, muscle weight, axon histomorphometry, and SC distribution and migration, a paramount feature needed to generate the necessary environment for axonal regeneration. Taken together, the TC group demonstrated better outcomes compared to the UC group and was comparable to the RA group in terms of functional outcome measures involving walking force and CMAP.

SIGNIFICANCE/CLINICAL RELEVANCE: This study demonstrates that a novel scaffold built upon the biomimetic activity of MATN2 linked with lysine enhanced chitosan to form a multichannel microarchitecture is able to provide a microenvironment that supports SC migration and axonal regeneration compared to an empty collagen conduit. Thus, this work provides a basis for the use of MATN2 as an integral protein for the development of novel nerve repair strategies along with the versatility of chitosan to form a readily available scaffold that avoids the morbidity associated with autograft and delivers bioactivity that is poorly present in processed allograft.

ACKNOWLEDGEMENTS: Funded by American Foundation for Surgery of the Hand Basic Science Grant #2837

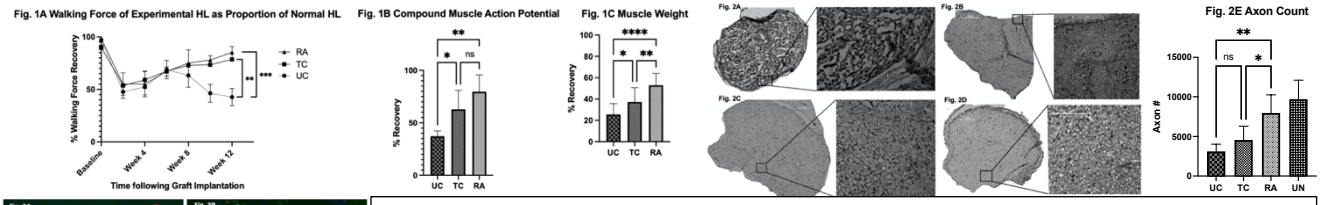

Figure 1. (A) Walking track analysis from 0 to 12 weeks expressed as % force of normal right HL. (B) Compound Muscle Action Potential of experimental left HL at 12 weeks expressed as % recovery to normal right HL. (C) Muscle weights of tibialis anterior and gastrocnemius from experimental left HL normalized to % of right HL.

Figure 2. (A) 10x and 20x cross-section of nerve distal to UC graft. (B) 10x and 20x cross-section of nerve distal to TC graft. (C) 10x and 20x cross-section of nerve distal to RA. (D) 10x and 20x cross-section of distal UN. (E) mean number myelinated axon counts from all 4 groups. Scale bar at 100µm.

Figure 3. Immunofluorescence staining of S-100 (Green) and DAPI (Blue). (A) 4x longitudinal section of distal UC graft. (B) 4x longitudinal section of distal TC graft. (C) 4x longitudinal section of distal RA. (D) 4x longitudinal section of distal UN. Scale bar at 500µm *P≤ 0.05, ** P ≤ 0.01, *** P≤ 0.001, **** P≤ 0.0001 detailing statistically significant differences between groups. UC: Untreated Conduit, TC: Treated Conduit, RA: Reverse Autograft, UN: Uninjured Nerve, HL: Hindlimb