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ORIGINAL ARTICLE

Peripheral Nerve

Early Expression of MMP-9 Predicts Recovery of Tibialis Anterior after Sciatic Nerve Crush Injury

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Background: The purpose of this study was to assess the expression of molecular markers and epineural blood flow after differing degrees of nerve injury to identify potential tools to predict nerve recovery in a rat sciatic nerve model.
Methods: A total of 72 rats were divided into nine groups. Each group was subjected to one of three crush injuries, created by applying one of three vascular clamps for 30 seconds. Vascularity was assessed with laser Doppler flowmetry before and after crush, and at nonsurvival surgery. Nonsurvival surgeries were performed 6 hours, 2 weeks, or 6 weeks later with nerve conduction studies and muscle strength testing. Expression of matrix metalloproteinase 9 (MMP-9) and matrix metalloproteinase 2 (MMP-2) in each nerve was quantified using with enzyme linked immunosorbent analysis.
Results: Persistent hyperemia was noted in the zone of injury compared with baseline at 2 weeks and 6 weeks in the groups that displayed incomplete recovery. Expression of MMP-9 at 6 hours increased with increasing severity of crush and was inversely related to tibialis anterior muscle force recovery. The ratio of MMP-9:MMP-2 expression correlated well with recovery of compound nerve action potential amplitude at 6 weeks.
Conclusions: Resolution of nerve hyperemia may correlate with nerve recovery from trauma, but early measures of nerve blood flow after injury are not prognostic of recovery. Ratio of MMP-9:MMP-2 expression 6 hours after injury correlates with recovery of compound nerve action potential at 6 weeks, while MMP-9 expression alone predicts tibialis anterior recovery. These findings together suggest that increased MMP-9 expression is a potentially useful marker of more severe nerve injury. *(Plast Reconstr Surg Glob Open 2022;10:e4260; doi: 10.1097/GOX.0000000000004260; Published online 18 April 2022.)*

INTRODUCTION

Traumatic nerve injuries occur in approximately 2%–3%[1,2] of major limb traumas, causing significant pain, disability, and economic hardship. Distinguishing a critically injured nerve is key to appropriate treatment, as 97% of patients with Sunderland grade 1 injuries (neurapraxia)[3,4] regain normal function, while 83% of those with Sunderland grade 5 injuries (complete nerve transection) achieve little to no functional recovery.[2] Unfortunately, electrodiagnostic studies can produce unreliable results within the first 3–6 weeks after nerve injury.[5] Therefore, a critical need exists to identify physiologic or molecular markers corresponding to severity of nerve injury. Recent investigations have explored alterations in neural vascularity and expression of inflammatory markers after chronic compression,[6] but less is known about traumatic injury. Prior work at other institutions has demonstrated a local increase in matrix metalloproteinase 9 (MMP-9) 6 hours after peripheral nerve injury.[7] MMP-9 has been implicated in NGF-mediated neurite elongation[8] and is upregulated in models of murine neurotrauma.[9] In sharp contrast, matrix metalloproteinase 2 (MMP-2) is found within healthy nerves, and has different temporal expression than MMP-9 after injury. The release of pro-inflammatory cytokines such as MMP-9, IL-1, and TNF-alpha after peripheral nerve injury leads to degradation of the blood-nerve barrier

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and recruitment of macrophages to facilitate myelin clearance and nerve regeneration.[10]

New techniques in intraoperative imaging could be utilized to allow noninvasive assessment of such molecular markers with fluorophore probes and aid in surgical decision making.[11,12] The central hypothesis of this article is that a graduated crush injury to a peripheral nerve will result in a proportional change to epineural blood flow and changes in expression of MMP-9 and MMP-2.

Takeaways

Question: Can laser Doppler flowmetry and MMP expression predict potential for recovery after nerve crush?

Findings: Rat sciatic nerves were subjected to differing degrees of crush injury and assessed with laser Doppler flowmetry. Early measurements of laser Doppler flowmetry immediately after crush did not differentiate the degree of nerve crush. Early expression of MMP-9 reflected potential for late recovery of tibialis anterior function.

Meaning: Molecular markers expressed in nerves early after injury, such as MMP-9 and MMP-2, may be useful in predicting potential for clinical recovery at 6 weeks.

METHODS

To assess the effect of severity of injury on blood flow and MMP expression, a graduated crush model was developed using three vascular clamps. A total of 72 Lewis rats were divided into three separate groups of 24 each, with each group subjected to one of three degrees of unilateral sciatic nerve crush injury. All protocols were approved by our Institutional Animal Care and Use Committee. Baseline epineural blood flow was measured in the bilateral sciatic nerves using laser Doppler flowmetry and a crush injury then applied unilaterally with a vascular clamp. Eight rats in each crush group underwent nonsurvival procedures at 6 hours, 2 weeks, and 6 weeks to measure epineural blood flow and assess histology, nerve conduction, and muscle strength.

Validation of the Crush Injury

Three vascular clamps were obtained (Roboz Surgical Instrument Co., Gaithersburg, Md.), including a Johns Hopkins bulldog clamp, a DeBAKEY atraumatic bulldog clamp, and a Glover straight atraumatic bulldog clamp. Ultra Low Pressure Fuji film Prescale (FUJI CORP) was utilized to assess the pressure applied to each nerve. Each clamp was applied to the film for 30 seconds, and each trial performed seven times. Pressure maps were digitized and analyzed with ImageJ.[13] RGB pixel intensity of the exposed image was calculated and compared with a colorimetric scale.

Survival Surgery

Isoflurane anesthesia was utilized for sedation, and a transgluteal approach was made to the sciatic nerve. A reference point was marked with a 9-0 nylon 6 mm proximal to the sciatic trifurcation. Epineural red blood cell flux was measured with a laser Doppler flowmeter (Moor Instruments, Inc, Devon, UK) centered on the suture (Fig. 1). A region of interest was measured proximal and distal to the suture.

One of the clamps was applied to the nerve distal to the epineural suture for 30 seconds, released and applied again distal to the previous area for a crush width of 5 mm. Flowmetry measurements were taken again (Fig. 2). The contralateral sciatic nerve was exposed, suture placed, and flowmetry measured. Incisions were closed, and animals given oral Carprofen daily for 72 hours.

Nonsurvival surgery

Nonsurvival surgeries were performed at 6 hours, 2 weeks, and 6 weeks. Anesthesia was induced, the incisions were re-opened, and laser Doppler flowmetry measurements were obtained of the same region of interest for both control and experimental sides, followed by nerve conduction studies and muscle force measurements. The injured nerve was harvested, samples from the nerve proximal to and within the zone of injury were preserved for with enzyme linked immunosorbent analysis assays of MMP-9 and MMP-2, histomorphologic measurements, and immunohistochemistry.

Measurement of MMP-2 and MMP-9 Expression

Quantification of MMP-9 and MMP-2 protein expression was accomplished with enzyme linked immunosorbent analysis using commercially prepared kits for MMP-9 (Aviva Systems Biology, San Diego, Calif.) and MMP-2 (ScienCell Research Laboratories, Carlsbad, Calif.). A 2-mm section was taken from the injured and uninjured portion of the nerve, protein was extracted using N-PER Neuronal Protein Extraction Reagent (Thermo Scientific #87792), and total protein concentrations were measured using Pierce Coomassie Plus (Bradford) Assay kit (Thermo Scientific #23236). Protein samples were diluted to equal concentrations for all samples, 10 µg/mL for MMP-9 assay and 50 ug/mL for MMP-2 assay; all enzyme linked immunosorbent analysis assays were performed in 96-well, optical-bottom plates. Similar samples were harvested from the control nerve and analyzed.

Electrodiagnostic Studies

A functional assessment system (Red Rock Laboratories, St. Louis, Mo.) was utilized to asses compound nerve action potential latency and amplitude bilaterally. The stimulus probe was placed on the sciatic nerve just proximal to the crush, and the recording probe placed on the common peroneal nerve at least 1 cm apart. For nerve conduction study recordings, the stimulus was 1000 µA with a pulse duration of 50 µs. Once two satisfactory waveforms were captured, the average amplitude and latency were recorded.

Maximal tetanic force of bilateral tibialis anterior and gastrocnemius muscles was measured. Tendons were transected and sutured to metal hooks connected to the transducer using 5-0 nylon suture after immobilizing the knee

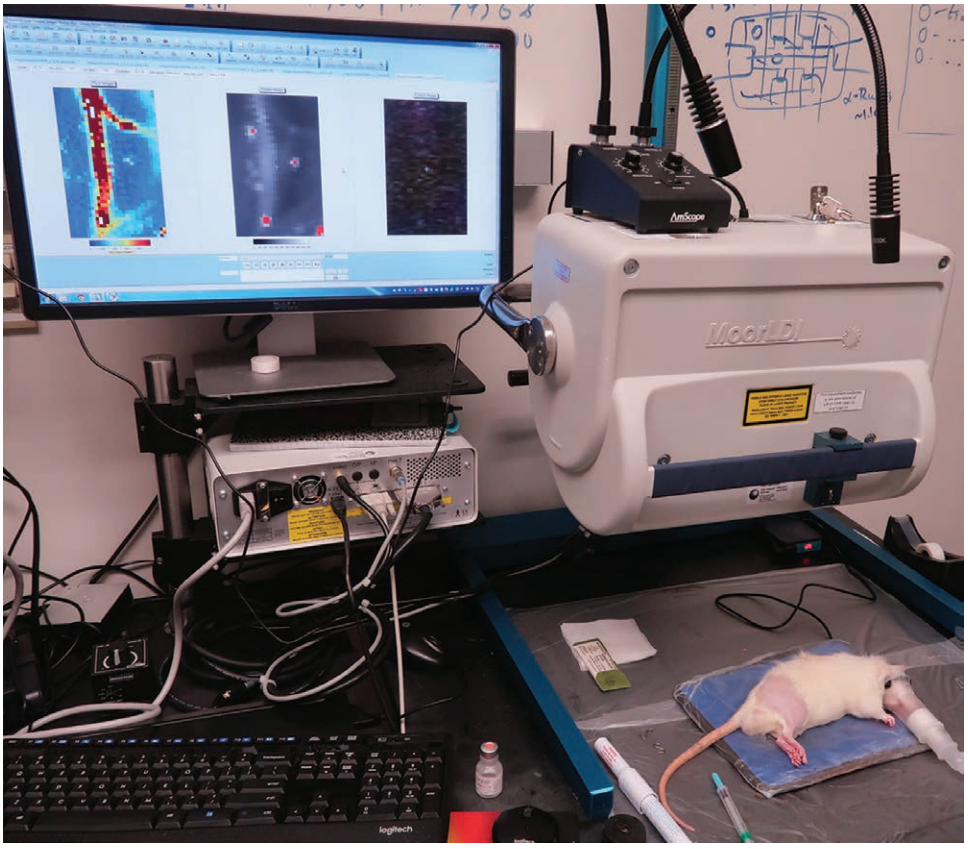

Fig. 1. Experimental setup of laser Doppler flowmetry before exposure, flowmetry measurement, and crush injury to nerve.

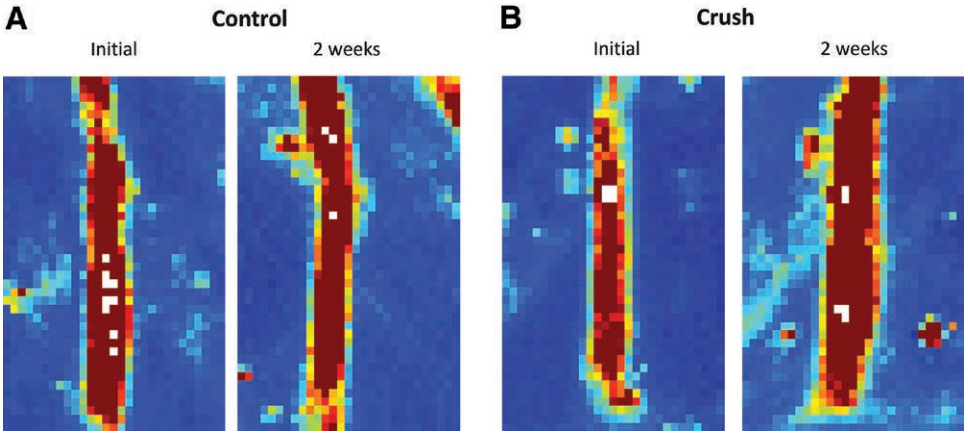

Fig. 2. Laser Doppler flowmetry was utilized to assess blood flow in control and injured nerves before and after injury. Flowmetry image as measured by laser Doppler demonstrating hyperemia in crushed nerve (B) compared with baseline. Control nerve (A) demonstrates no significant change at 2 weeks.

with a rigid clamp. The sciatic nerve was stimulated proximal to the injury with a stimulus signal of 1000 μA, burst width of 0.3 s, pulse duration 200 μs, and frequency 80 Hz. Tendon length/tension was adjusted to achieve maximal isometric tetanic force with a consistent waveform.

Experimental and control sciatic nerves were harvested and prepared for immunohistochemistry and histormorphologic analysis to include total axon counts and G-ratio. Samples were incubated with primary antibodies to axonal neurofilaments, MMP-9, MMP-2, and CD31, followed by a wash, blocking and fluorescent secondary antibodies.

Statistical Analysis

One-way ANOVA was used to compare FUJI film pixel intensity after crush validation for each clamp. Mixed effects analysis was used to compare flowmetry data, and histomorphometry data, including G-ratio and normalized total

number of axons in the zone of injury for each of the clamps at each time point. All flux values were reported as a ratio of the value at each time point compared with its pre-injury flux. One-way ANOVA tests were also used to compare CNAP amplitude, CNAP latency, and gastrocnemius and tibialis anterior muscle forces, between the different crush groups at each time interval. MMP-9 and MMP-2 expression were also calculated as a ratio of the expression of each protein compared with the uninjured zone proximal to the crush injury and compared amongst injury groups at each time interval using one-way ANOVA. A Dunnett's test was used to adjust for the effect of multiple comparisons at each time point for one-way ANOVA, and Tukey's multiple comparisons test was utilized for the mixed effects model.

Sample Size Justification

A-priori power analysis found that MMP-9 expression was expected to increase in the zone of injury in the severe crush group, based on a literature review of work in a chronic compression model.[6] A sample size of eight per group at each time point (total 72 rats) achieves 80% power to detect a 68% increase in expression of MMP-9 ratio between the crush levels assuming a null hypothesis of no difference between the crush severity and a significance level of 0.05 using an analysis of variance F-test.

RESULTS

Force Validation

Results of the pressure film measurements demonstrated that the Hopkins bulldog clamp exerted a mean pressure of 0.393 MPa, the Debakey clamp exerted a pressure of 0.455 MPa, and the Glover bulldog clamp resulted in 0.683 MPa. One-way ANOVA showed a significant difference in pixel intensity on FUJI film between the Hopkins bulldog and Glover bulldog crush groups ($P = 0.01$), and between the Debakey and the Glover bulldog clamps ($P = 0.03$). No significant difference was found between the Hopkins bulldog clamp and the Debakey clamp ($P = 0.71$). To simplify nomenclature, each of these clamps was designated as mild A (Hopkins), mild B (Debakey), and severe (Glover), respectively (Fig. 3).

Flowmetry Data

All but two of the rats survived throughout the study; these two (both from mild B group) were removed and subsequently replaced to ensure adequate numbers. All nerves undergoing surgery demonstrated increased hyperemia at 6 hours after the index surgery, but no significant difference was found between groups. The mild B crush and severe crush maintained persistently elevated red blood cell flux levels (hyperemia) compared with

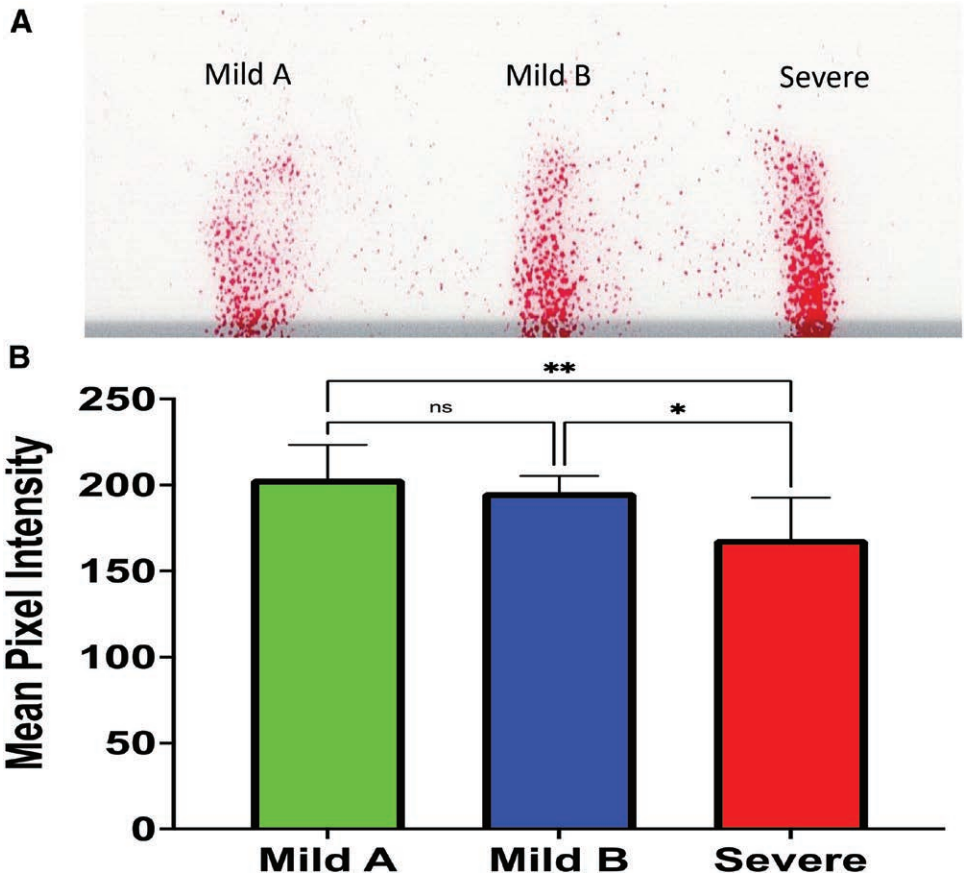

Fig. 3. Crush injuries were validated using Fuji film. Prescale ultra low pressure film demonstrating pressure maps from mild A, mild B and severe crush injuries (A) with calculated average pixel intensity as measured in ImageJ. (B).

controls at 2 weeks (with a mean difference of −0.61 flux units; 95%CI [−0.87, −0.37] and −0.65 flux units; 95%CI [−0.90, −0.40]) (Fig. 4). This difference in flux persisted even at 6 weeks in the mild B and severe crush groups compared with controls, but the magnitude of difference did not correlate with severity of injury (−0.47 flux units; 95%CI [−0.64, −0.29] for mild B and −0.32 flux units; 95%CI [−0.59, −0.06] for severe).

Nerve Conduction Studies

Nerve conduction studies demonstrated no change in latency at 6 hours but a uniformly significant increase in latency among all groups when compared with controls at 2 weeks (Fig. 5A). However, this increase was transient, with a return to normal latency by 6 weeks in all groups ($P = 0.46$). A significant drop in amplitude was seen in all crushed nerves at 6 hours postinjury, and diminished amplitude persisted in all groups at both 2 weeks and 6 weeks compared with controls ($P < 0.0001$ for both) (Fig. 5B). Intergroup comparison at 6 weeks between varying degrees of crush demonstrated persistent amplitude difference between the mild A and severe injury (mean difference of 1.47 mV; 95%CI [0.33, 2.60]) but no significant difference in amplitude between the mild A and mild B groups (mean difference 0.54 mV; 95%CI [−0.60, 1.67]), or the mild B and severe groups (mean difference 0.93 mV; 95%CI [−0.21, 2.07]).

Muscle Force Testing

At the 6 hour and 2 week time points, all injured legs demonstrated a significant decrease in both gastrocnemius and tibialis anterior force compared with controls ($P < 0.0001$). By 6 weeks, no significant difference was observed in gastrocnemius muscle strength between injured and control groups (Fig. 6A). Tibialis anterior muscle force proved to have a slower recovery in comparison to the gastrocnemius muscle. In contrast, all crush groups had significantly diminished tibialis anterior muscle strength compared with control ($P < 0.0001$) at 2 weeks and showed significant differences in recovery between the crush groups. At 6 weeks, the mild A crush group had regained strength equivalent to the controls, whereas mild B had slightly less strength compared with controls. The severe crush demonstrated significant persistent weakness, compared with control 4.02 N; 95%CI [3.28,4.75]) and the other crush groups (Fig. 6B).

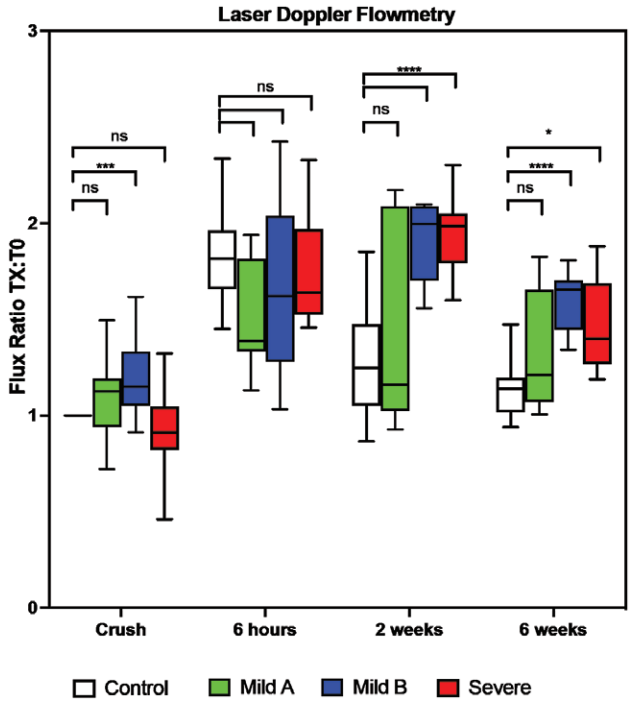

Fig. 4. Flux ratios compared with pre-crush baseline measurement. Persistent increased flux was noted in the more severely injured groups at 2 weeks and 6 weeks.

Histomorphometry

The total number of axons at 6 hours did not vary between groups, but was significantly different amongst all groups at 2 weeks. A significant difference was also seen in the severe crush at 6 weeks compared with the two milder injuries (Fig. 7A). G-ratio was also significantly different amongst all groups at 2 weeks, and this difference persisted in the severe crush injury compared with the mild crush injuries, even at 6 weeks (Fig. 7B).

MMP-2 and MMP-9 Expression

Severe crush demonstrated a significant increase in MMP-9 expression compared with controls ($P < 0.0001$) 6 hours after injury, but this was not found in mild A ($P = 0.9923$) or mild B ($P = 0.075$) crush injuries (Fig. 8A). However, severe crush did demonstrate an increase in MMP-9 expression compared with the mild A and mild B crushes ($P < 0.0001$). No significant difference was found in MMP-9 expression between any group at 2 or 6 weeks. MMP-2 was uniformly decreased in the crushed area compared with control with all degrees of crush at 6 hours ($P < 0.0001$), but significantly elevated compared with controls in all groups at 2 weeks. By 6 weeks, MMP-2 expression demonstrated no significant difference between crush and control groups. The ratio of MMP-9 to MMP-2 at 6 hours postinjury in the severe crush group demonstrated an increase three times greater than that seen in the mild A crush (mean difference −4.59; 95%CI[−5.96, 3.21] and −1.43; 95%CI[−2.81, −0.06] respectively) (Fig. 8B). Immunohistochemistry stains confirmed the presence of MMP-9 within 6 hours of injury (Fig. 9).

DISCUSSION

The results of this study suggest that laser Doppler flowmetry and MMP-9 expression correlate with degree of nerve injury, but at differing timepoints. Early changes in laser Doppler flowmetry did not predict long-term recovery, but persistent nerve hyperemia at 6 weeks was indicative of incomplete tibialis anterior recovery and likely reflects persistent disruption in the blood nerve barrier (BNB) associated with incomplete axonal regeneration. This finding is in agreement with prior studies showing increased signal on T2-weighted MRI images for up to 2

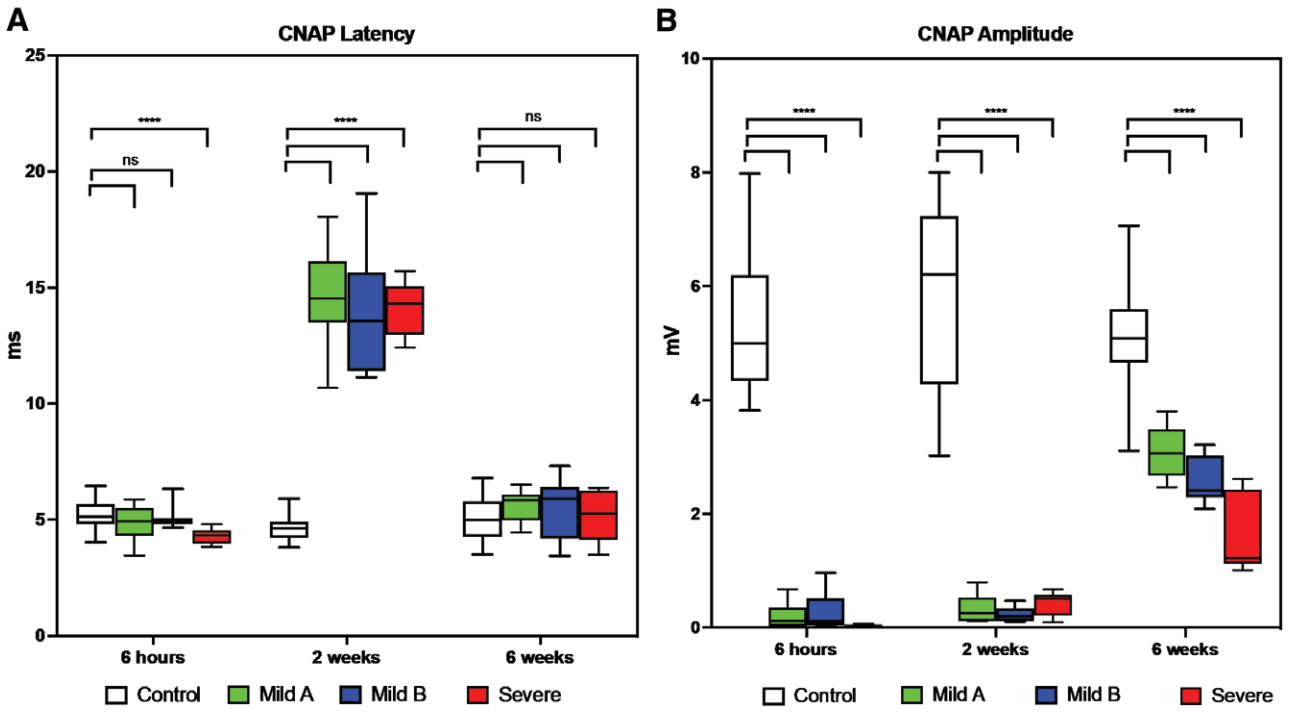

Fig. 5. CNAP latency and amplitude were calculated in injured and control nerves during non-survival surgery. A, CNAP latency measurements at different time intervals. Note significant elevation in latency 2 weeks after injury, which returned to normal by 6 weeks. B, CNAP amplitude decreased immediately and did not fully recover after 6 weeks in any group, consist with axonotmetic injury.

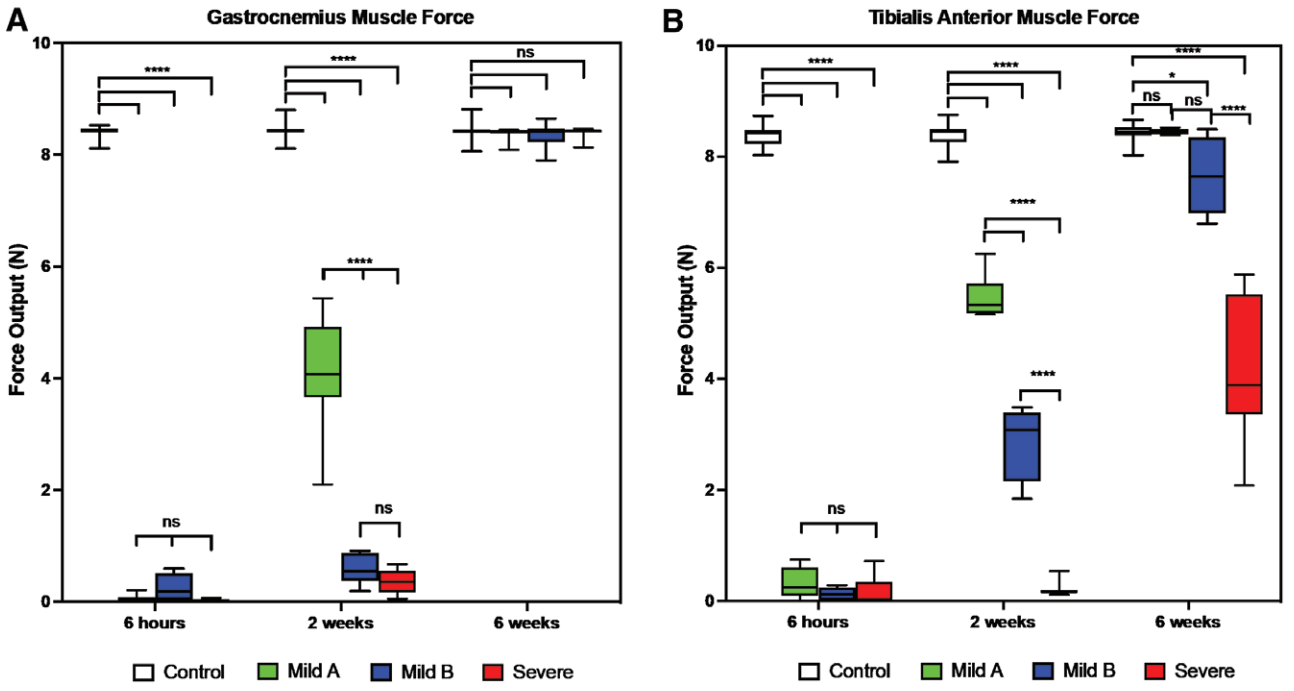

Fig. 6. Maximal isometric tetanic force was assessed in each hindlimb during non-survival surgery A, Gastrocnemius muscle force correlated to degree of injury at 2 weeks, with complete recovery across all groups at 6 weeks. B, Tibialis anterior muscle force correlated with degree of injury and time from injury at both 2 weeks and 6 weeks, with persistent dysfunction in the most severe injuries.

weeks[14] after a rat sciatic nerve crush injury. A separate rodent model of axonotmetic nerve injury demonstrated that return of normal CMAP occurred within 2 weeks of resolution of T2-weighted signal enhancement,[15] while others have found persistent nerve enhancement on MRI 3 weeks after crush.[16] Although we did not use MRI, the persistent hyperemia seen at 2 weeks in two of the three groups likely corresponds with signal enhancement seen

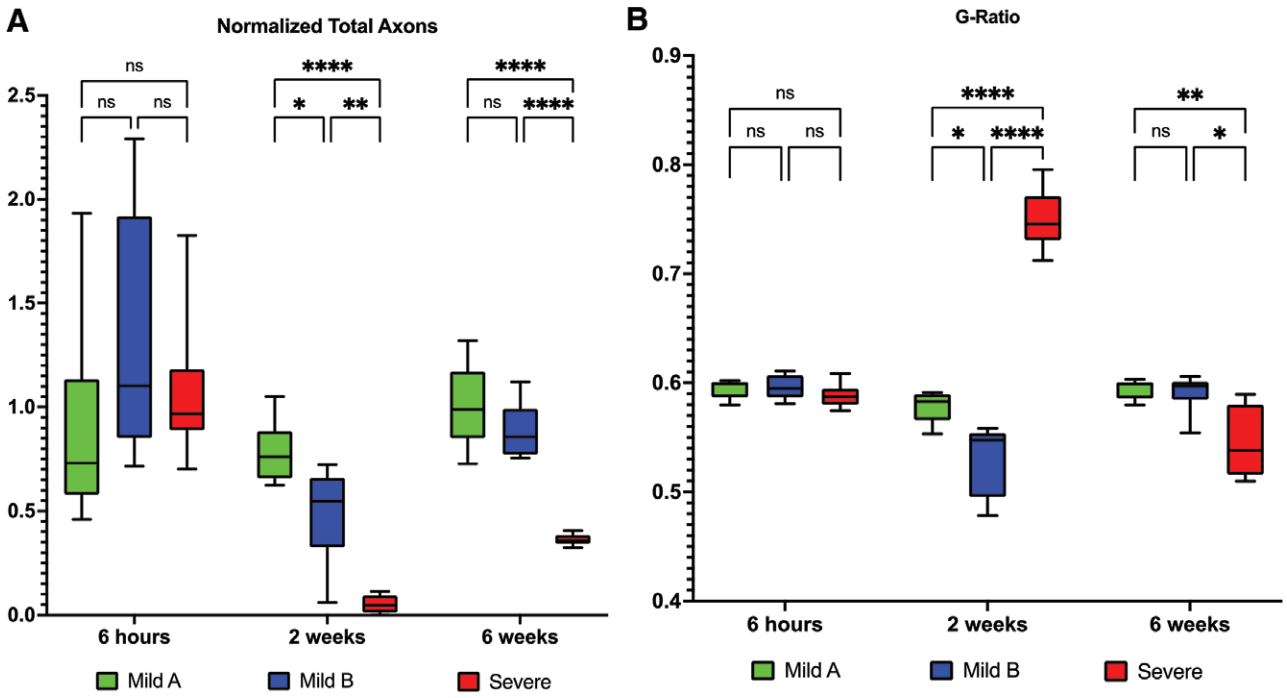

Fig. 7. Histomorphometric measurements of injured nerves were obtained and normalized against the contralateral control nerve. A, Total number of axons correlated with time from injury and degree of crush. B, G-ratio within the zone of injury at different time points varied by degree of crush.

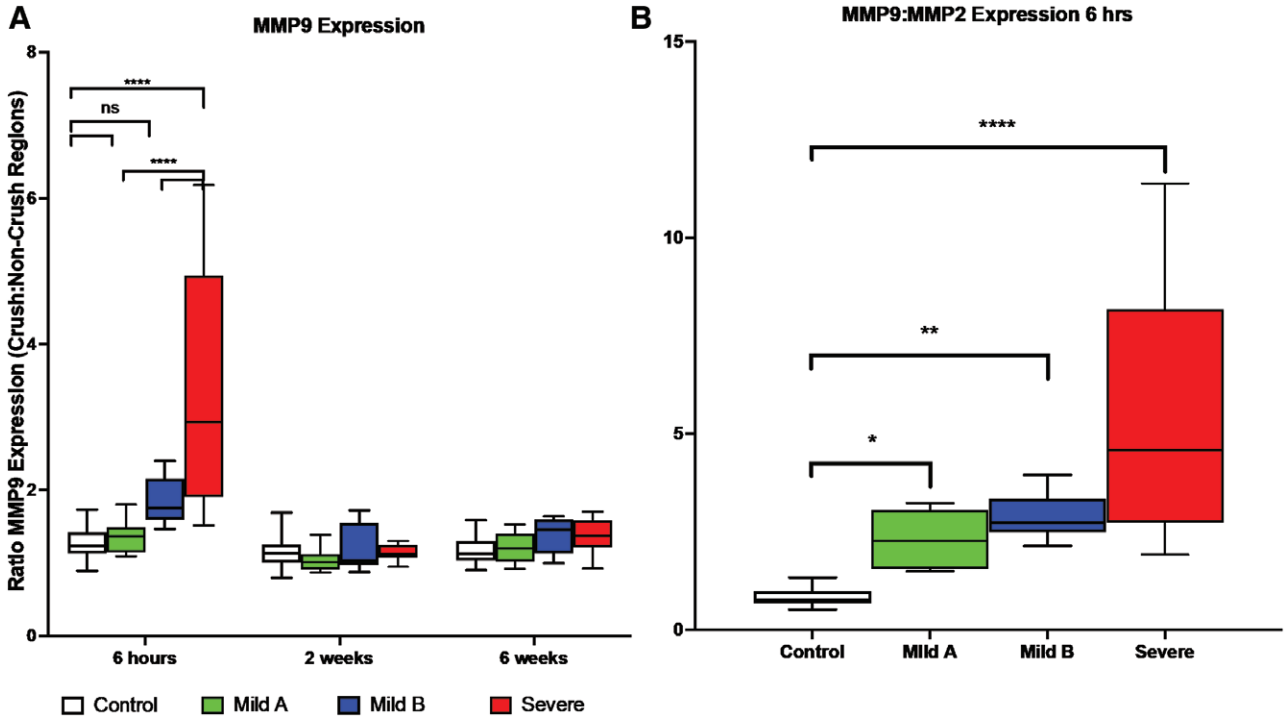

Fig. 8. ELISA assay was used to calculate MMP-9 and MMP-2 expression in injured and control nerves. A, Expression of MMP-9 in the crushed portion of nerve as a ratio of the expression in the more proximal uninjured portion of nerve at differing time points. B, Expression of MMP-9:MMP-2 in the crushed portion of nerve compared with the uninjured portion at 6 hours corresponded to recovery of CNAP amplitude at 6 weeks.

on T2 images due to disruption of the BNB. Bouldin et al[17] found that the presence of regenerating axons correlated with restoration of impermeability of the BNB, as did Omura, who noted that the restoration of the BNB occurs in a proximal to distal direction and lags behind nerve regeneration by 1 week.[18]

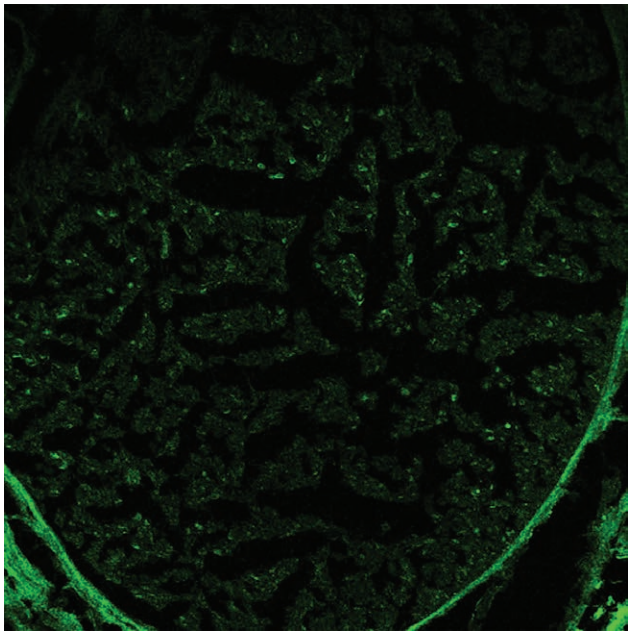

Fig. 9. Confocal microscopy with immunohistochemical stains of injured nerve demonstrating presence of MMP-9 within the zone of injury 6 hours after severe crush.

Total axon count and muscle force are thought to be sensitive indicators of nerve regeneration in crush injuries.[19] Our findings of persistent tibialis anterior weakness and reduced total axon count at 6 weeks validate a clear distinction between the milder and more severe crush injuries to the peroneal division of the sciatic nerve. Previously published animal models of mild nerve crush injuries mimic the clinical scenario of a grade 2 crush, with subsequent Wallerian degeneration, and recovery by 2–3 weeks.[20,21] In contrast, we induced a severe crush resulting in a persistent difference in tibialis anterior force but not gastrocnemius force at 6 weeks. Similar findings of earlier recovery of plantarflexion at 6 weeks, with incomplete dorsiflexion recovery have been reported in a rat sciatic nerve model.[22]

Curiously, our G-ratio did not mirror functional recovery; however, a relatively poor correlation between G-ratio and nerve conduction velocity has been described and likely reflects a mix of small and large diameter fibers with varying degrees of myelin.[23] This is consistent with changes in G-ratio between groups at different time points in our data, which appeared to be related to wide variations in fiber size in the more severe crush group (Fig. 10).

Finally, we found a proportional early increase in MMP-9 expression with increasing degree of crush severity, likely reflective of more severe injury to Schwann cells with a resulting increased inflammatory cascade.[24,25] Furthermore, significant increases in MMP-9 expression at 6 hours postinjury were able to predict recovery of tibialis anterior muscle force and total number of axons at 6 weeks. The ratio of MMP-9:MMP-2 at 6 hours inversely corresponded to the CNAP amplitude at 6 weeks but did not predict muscle recovery. This ratio likely reflects the severity of intraneural fibrosis conferred by each of the nerve injuries. Indeed, intraoperative ultrasound evaluation of traumatic nerve injuries has demonstrated decreased CNAP amplitudes in more severe injuries with significant intraneural scarring.[26] MMP-9 expression alone showed a sharp rise at 6 hours, but this was not sustained at 2 weeks or 6 weeks in any of the crush mechanisms, as previously reported.[7] Although MMP-9 is scarce in normal Schwann cells, it is upregulated in axonal injury.[27] Our results showed an increase in latency at 2 weeks, but not at 6 hours despite an early drop in CNAP amplitude. Stecker et al found similar results in an acute nerve compression model, where CMAP amplitude (but not latency) significantly declined immediately after injury.[28] Late changes in latency in our data likely represent the sequelae of Wallerian degeneration via myelin breakdown and the subsequent transdifferentiation of Schwann cells into a pro-regenerative phenotype to aid in nerve recovery.[29] MMP-2 is expressed in normal nerves but increases in expression 5 days after injury, peaks around 10 days, and remains elevated for up to 9 weeks.[30] Given the above, MMP-9 may prove to be a sensitive prognostic marker of functional nerve recovery, while the ratio of MMP-9:MMP-2 may better predict overall axonal injury.

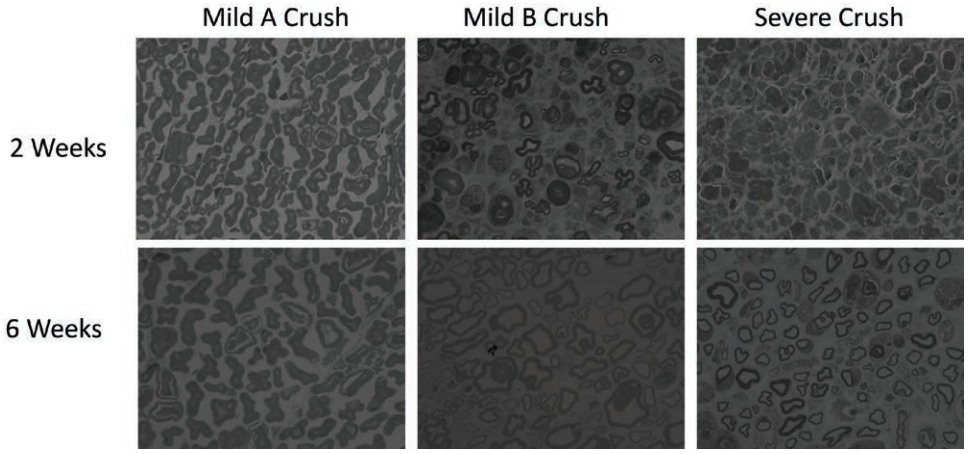

Fig. 10. Photomicrographs of representative zones of nerve injury taken at 2 weeks and 6 weeks. More severe injury resulted in disorganized repair with lower percent nerve.

This study is not without limitations. The rat sciatic nerve model may prove to be more resilient to nerve injury than human models, potentially leading to imperfect correlation of these findings to the clinical setting. More investigation is needed before this can be applied clinically, as MMP-9 did not predict gastrocnemius muscle strength at 6 weeks, suggesting that it may only be useful to identify the most severe nerve injuries. Despite this, this study raises interesting questions about the exploitation of the inflammatory cascade to quantify nerve injury. Work is currently underway in our laboratory to better understand the inflammatory response of nerve injury in a nerve transection model and to create molecular markers specific for MMP-9.

To our knowledge, clinical data are lacking regarding the timing and degree of MMP-9 expression in human peripheral nerve injury. Further translational research may pave the way for applications of novel molecular tracers sensitive to MMP-9 that might be used clinically in the operating room to assess nerve injury. Optical measurements of fluorescence could then negate the need for nerve biopsies to assess MMP-9 activity. Given the lack of sufficient tools for intraoperative evaluation of nerve injuries, there is an inherent need for new technologies to assist with identification of degree of nerve injury to allow for prognostication of recovery, but more investigation is needed to optimize this technology.

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REFERENCES

1. Taylor CA, Braza D, Rice JB, et al. The incidence of peripheral nerve injury in extremity trauma. *Am J Phys Med Rehabil.* 2008;87:381–385.
2. Noble J, Munro CA, Prasad VS, et al. Analysis of upper and lower extremity peripheral nerve injuries in a population of patients with multiple injuries. *J Trauma.* 1998;45:116–122.
3. Sunderland S. A classification of peripheral nerve injuries producing loss of function. *Brain.* 1951;74:491–516.
4. Seddon H. A classification of nerve injuries. *Br Med J.* 1942;2:237–239.
5. Levenson D, Rosenbluth J. Electrophysiologic changes accompanying Wallerian degeneration in frog sciatic nerve. *Brain Res.* 1990;523:230–236.
6. Jung J, Hahn P, Choi B, et al. Early surgical decompression restores neurovascular blood flow and ischemic parameters in an in vivo animal model of nerve compression injury. *J Bone Joint Surg Am.* 2014;96:897–906.
7. Shubayev VI, Myers RR. Upregulation and interaction of TNFalpha and gelatinases A and B in painful peripheral nerve injury. *Brain Res.* 2000;855:83–89.
8. Shubayev VI, Myers RR. Matrix metalloproteinase-9 promotes nerve growth factor-induced neurite elongation but not new sprout formation in vitro. *J Neurosci Res.* 2004;77:229–239.
9. Vecil GG, Larsen PH, Corley SM, et al. Interleukin-1 is a key regulator of matrix metalloproteinase-9 expression in human neurons in culture and following mouse brain trauma in vivo. *J Neurosci Res.* 2000;61:212–224.
10. Fregnan F, Muratori L, Simões AR, et al. Role of inflammatory cytokines in peripheral nerve injury. *Neural Regen Res.* 2012;7:2259–2266.
11. Akers WJ, Xu B, Lee H, et al. Detection of MMP-2 and MMP-9 activity in vivo with a triple-helical peptide optical probe. *Bioconjug Chem.* 2012;23:656–663.
12. Liu Y, Zhao YM, Akers W, et al. First in-human intraoperative imaging of HCC using the fluorescence goggle system and transarterial delivery of near-infrared fluorescent imaging agent: A pilot study. *Transl Res.* 2013;162:324–331.
13. Schneider CA, Rasband WS, Eliceiri KW. NIH image to ImageJ: 25 years of image analysis. *Nat Methods.* 2012;9:671–675.
14. Cudlip SA, Howe FA, Griffiths JR, et al. Magnetic resonance neurography of peripheral nerve following experimental crush injury, and correlation with functional deficit. *J Neurosurg.* 2002;96:755–759.
15. Bendszus M, Wessig C, Solymosi L, et al. MRI of peripheral nerve degeneration and regeneration: correlation with electrophysiology and histology. *Exp Neurol.* 2004;188:171–177.
16. Hill B, Padgett K, Kalra V, et al. Gadolinium DTPA enhancement characteristics of the rat sciatic nerve after crush injury at 4.7 T. *Am J Neuroradiol.* 2018;39:177–183.
17. Bouldin TW, Earnhardt TS, Goines ND. Restoration of blood-nerve barrier in neuropathy is associated with axonal regeneration and remyelination. *J Neuropathol Exp Neurol.* 1991;50:719–728.
18. Omura K, Ohbayashi M, Sano M, et al. The recovery of blood-nerve barrier in crush nerve injury—a quantitative analysis utilizing immunohistochemistry. *Brain Res.* 2004;1001:13–21.
19. Wood MD, Kemp SW, Weber C, et al. Outcome measures of peripheral nerve regeneration. *Ann Anat.* 2011;193:321–333.
20. Bridge PM, Ball DJ, Mackinnon SE, et al. Nerve crush injuries–a model for axonotmesis. *Exp Neurol.* 1994;127:284–290.
21. De Koning P, Brakkee JH, Gispen WH. Methods for producing a reproducible crush in the sciatic and tibial nerve of the rat and rapid and precise testing of return of sensory function. Beneficial effects of melanocortins. *J Neurol Sci.* 1986;74:237–246.
22. Varejão AS, Cabrita AM, Meek MF, et al. Functional and morphological assessment of a standardized rat sciatic nerve crush injury with a non-serrated clamp. *J Neurotrauma.* 2004;21:1652–1670.
23. Ikeda M, Oka Y. The relationship between nerve conduction velocity and fiber morphology during peripheral nerve regeneration. *Brain Behav.* 2012;2:382–390.
24. Ydens E, Lornet G, Smits V, et al. The neuroinflammatory role of Schwann cells in disease. *Neurobiol Dis.* 2013;55:95–103.
25. Martini R, Fischer S, López-Vales R, et al. Interactions between Schwann cells and macrophages in injury and inherited demyelinating disease. *Glia.* 2008;56:1566–1577.
26. Koenig RW, Schmidt TE, Heinen CP, et al. Intraoperative high-resolution ultrasound: a new technique in the management of peripheral nerve disorders. *J Neurosurg.* 2011;114:514–521.
27. Kim Y, Remacle AG, Chernov AV, et al. The MMP-9/TIMP-1 axis controls the status of differentiation and function of myelin-forming Schwann cells in nerve regeneration. *PLoS One.* 2012;7:e33664.
28. Stecker MM, Baylor K, Chan YM. Acute nerve compression and the compound muscle action potential. *J Brachial Plex Peripher Nerve Inj.* 2008;3:1.
29. Arthur-Farraj PJ, Latouche M, Wilton DK, et al. c-Jun reprograms Schwann cells of injured nerves to generate a repair cell essential for regeneration. *Neuron.* 2012;75:633–647.
30. Demestre M, Wells GM, Miller KM, et al. Characterisation of matrix metalloproteinases and the effects of a broad-spectrum inhibitor (BB-1101) in peripheral nerve regeneration. *Neuroscience.* 2004;124:767–779.