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The Cutting Edge: Surface Texture Analysis following Resection of Nerve Stumps Using Various Instruments

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Background: Preparation of nerve ends is an essential part of nerve repair surgery. Multiple instruments have been described for this purpose; however, no consensus exists regarding which is the least traumatic for tissue handling. We believe that various instruments used for nerve-end excision will lead to different surface roughness.
Methods: Median and ulnar nerves from fresh frozen cadavers were dissected, and 1–2 cm lengths were excised using a No. 11 blade, a razor blade, or a pair of scissors. Using electron microscopy, 3-dimensional surface analysis of roughness (Sa) for each specimen was performed using ZeeScan optical hardware and GetPhase software (PhaseView, Buisson, France). An ANOVA or Kruskal-Wallis test compared roughness measures among cutting techniques.
Results: Forty nerves were included. Of these, 13 (32.5%) were cut using scissors, 15 (37.5%) using a razor blade, and 12 (30%) using a No. 11 blade. An ANOVA test showed statistical differences in Sa among the cutting techniques ($P = 0.002$), with the lowest mean Sa noted in the scissors group (7.2 µM, 95% CI: 5.34–9.06), followed by No. 11 blade (7.29 µM, 95% CI: 5.22–9.35), and razor blade (11.03 µM, 95% CI: 9.43–12.62). Median Ra (surface profile roughness) was 4.58 (IQR: 2.62–5.46). A Kruskal-Wallis test demonstrated statistical difference in Ra among techniques ($P = 0.003$), with the lowest by No. 11 blade (3 µM, IQR: 1.87–4.38), followed by scissors (3.29 µM, IQR: 1.56–4.96), and razor (5.41 µM, IQR: 4.95–6.21).
Conclusion: This novel technique of 3-dimensional surface analysis found razor blade use demonstrated poor roughness, whereas a No. 11 blade or nerve-specific scissors led to equivocally smooth nerve ends. *(Plast Reconstr Surg Glob Open 2021;9:e3566; doi: 10.1097/GOX.0000000000003566; Published online 10 May 2021.)*

INTRODUCTION

Upper extremity peripheral nerve injury is a potentially devastating injury occurring in nearly 44 per 1 million people annually in the United States.[1] Although the incidence of nerve injury has decreased in recent years,[1] previous estimates denote nerve injuries to amount to $150 billion in annual US healthcare spending.[2] Following injury, peripheral nerve repair is often performed to achieve optimal guided reinnervation and facilitate functional recovery. Left untreated, many peripheral nerve injuries can lead to debilitating pain and disability whether from lack of function or neuroma formation.[3]

A neuroma occurs as a result of abnormal axonal outgrowth originating from improperly regenerating proximal nerve stumps. Any disorganized nerve regeneration can lead to either an end neuroma or a neuroma-in-continuity following aberrant end-to-end coaptation of proximal and distal stumps.[4] Patients with symptomatic neuromas may have significant pain in the distribution of the injured nerve, and although many treatment techniques have been described for neuroma management, there is no consensus on the standard method of symptomatic neuroma treatment.[5] However, it is widely agreed that prevention of neuroma formation is ideal, underscoring the need for proper technique in nerve injury repair.

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The costs of nerve injuries to the healthcare system are high and quantifiable. Yet, the cost to the patient, from loss of work, finances, and daily activities may not be.[6] Prompt surgical repair and improved outcomes are key to minimize the losses to the patients. Since Sunderland's 1945 study of intraneural topography mapping and repair using microsurgical techniques,[7] there have been few advances in nerve reconstruction. There are 2 basic techniques for nerve repair: end-to-end coaptation (primary repair) and nerve grafting. End-to-end coaptation is the preferred technique, for both acute and delayed repairs, as long as there is minimal tension at the suture line. When primary repair of nerve ends is not possible, debate continues regarding the use of autologous nerve donor grafts versus processed nerve allografts to restore continuity. Autologous nerve grafting has been the historical gold standard, with proponents recommending autologous nerve use in longer gaps (>3 cm), proximal injuries, and for critical peripheral nerves.[8] However, recent literature has challenged this and cites near-equal outcome with the use of processed nerve allograft.[9,10]

Regardless of repair technique employed, a core tenant of peripheral nerve repair is nerve end preparation by resection beyond the zone of injury to normal nerve tissue.[11–13] Preparation of the injured nerve ends is a must, as scar formation may provide an obstacle preventing further advancement of the axonal sprouts. Predictably, injured major peripheral nerves will demonstrate excessive fibrosis at the nerve ends beyond 3 weeks from injury date, requiring greater tissue resection from the nerve stumps.

The goal of nerve end preparation is to create flat matching surfaces that allow near perfect coaptation and alignment of the fascicles, with no overlap of fascicles that pass beyond the epineurial ends. A well-described principle to assess nerve viability involves continuing resection until pouting fascicles are visualized, owing to increased endoneurial fluid pressure in healthy nerve tissue. Additionally, healthy nerve tissue will display pinpoint bleeding from the cut ends of the severed vaso nervorum.[14] The nerve ends must be handled delicately by the epineurium only, with care to prevent proximal retraction of the epineurium and subsequent exposure of the fascicular bundles within.

There are various instruments described for nerve end preparation, including a razor blade, No. 11 blade, No. 15 blade with tongue depressor, various fine scissors, and specialized nerve-cutting guide with straight blade, to name a few. Yet there is a paucity of data in regard to the technique of resection that would lead to perfectly cut, well-matched surfaces with no overlap. In this study, we aimed to quantitatively compare cut geometry of various nerve-cutting instruments, using a novel surface texture analysis, thereby filling the gap in the existing literature.

MATERIALS AND METHODS

Study Design

We elected to use fresh frozen human cadaver upper extremities to mimic the in vivo environment as close as possible, which were received following standard protocols in accordance with institutional guidelines for ethical use of cadaver specimens. A total of 10 upper extremities distal to mid-humerus level were acquired and dissected. Mixed motor median and ulnar nerves were identified and chosen at the proximal forearm level. Upon exposure, these nerves were followed distally until the wrist level. Any branches of the nerves in the forearm were dissected out, and the segment including the branch was excised and not used for evaluation. The nerves were dissected circumferentially from the surrounding soft tissue and 1–2 cm lengths of segments of nerves were excised, labeled, and placed into individual containers. Excision took place under tension on a tongue depressor to mimic a surgical environment. Each nerve was excised using a No. 11 blade, razor blade, or straight iris scissors (Miltex, Integra LifeSciences, Plainsboro Township, N.J.), which made up the 3 different arms of our study. Each portion of the excised nerve in the blade arms used a new No. 11 blade or razor blade. After excision, the nerve segments were prepared for electron microscopy by immediate placement into a 10% formalin solution for 1 week then washed in ethanol. Before imaging, specimens were washed with several solutions of 70% ethanol to remove the formalin, then dehydrated by a graded series of ethanols up to 100%, and promptly critical point dried. The cut nerve surface was then placed in plane to the optical axis of the optical topometry system. Example electron microscopy images can be found in Figure 1.

Three-dimensional surface analysis of roughness (Sa) for each specimen was performed using ZeeScan optical hardware assembly and GetPhase software (PhaseView, Buisson, France). The ZeeScan was mounted on a Zeiss Axio Imager M1m (Carl Zeiss Microscopy, LLC, Thornwood, N.Y.), using a Zeiss Epiplan 5× microscope objective lens (NA = 0.13), the system providing 1.412 µm/pixel lateral and 18.5 µm axial resolution over a 1.9 × 1.425 mm field of view. GetPhase software was configured for extended depth of field imaging, with the x-y cutoff set to achieve a profile roughness (Ra) of 3.096 µm on the Standard Reference Material 2073a Sinusoidal Roughness Specimen standard (National Institute of Standards & Technology, Gaithersburg, N.Y.). Once the roughness profile (Ra)—an arithmetical mean height of a line between the peaks and troughs of the cross-sectional area—was measured, calculation of Sa—the extension of Ra to a surface (3D)—could be performed. GetPhase provided Z-depth measurement and 3-dimensional reconstruction of surfaces for quantifying Sa and for providing simulated 2-dimensional images of the surface for qualitative analysis.

Statistical Methods

Stata v13.1 (StataCorp LP, College Station, Tex.) was used for all statistical analyses. We used proportions to summarize categorical variables and means or medians, as appropriate according to data distribution, to summarize continuous variables. We then used ANOVA or Kruskal-Wallis test, as appropriate according to data distribution, to compare roughness measures among cutting techniques. We defined $P < 0.05$ as statistically significant.

Fig. 1. SEM of nerve ends cut with each instrument: A, Razor blade; B, scissors; C, No. 11 blade.

Table 1. Surface Area (Sa) Calculated for Each Study Arm

Sa (Surface Area Roughness, in µm)			
Technique	**Mean**	**95% CI**	***P****
Scissors	7.2	5.34–9.06	0.002
Razor blade	11.03	9.43–12.62	
11 blade	7.29	5.22–9.35	

*Mean value from the ANOVA test.

Table 2. Roughness Area (Ra) Calculated for Each Study Arm

Ra (Surface Profile Roughness, in µm)			
Technique	**Median**	**IQR**	***P****
Scissors	3.29	1.56–4.96	0.003
Razor blade	5.41	4.95–6.21	
11 blade	3	1.87–4.38	

*Mean value from the Kruskal-Wallis test.

RESULTS

Forty cut sections of nerves taken from the median and ulnar nerves were included in our study. Of these, 13 (32.5%) were cut using iris scissors, 15 (37.5%) using a razor blade, and 12 (30%) using a No. 11 blade. Mean Sa was 8.96 µm (SD: 3.25). An ANOVA test demonstrated a statistical difference in Sa among the cutting techniques ($P = 0.002$), where the lowest mean Sa was seen in surfaces cut with iris scissors (7.2 µm, 95% CI: 5.34 to 9.06), followed by the No. 11 blade (7.29 µm, 95% CI: 5.22 to 9.35), and razor blade (11.03 µm, 95% CI: 9.43 to 12.62). Table 1 shows a summary of these findings. A post hoc *t*-test comparing Sa between surfaces cut with scissors and those cut with No. 11 blades did not show any statistically significant difference ($P = 0.944$). Ra was not normally distributed and we therefore used non-parametric tests (Kruskal-Wallis) for analysis. Median Ra was 4.58 (IQR: 2.62–5.46). The Kruskal-Wallis test also showed strong evidence for a difference in Ra among cutting techniques ($P = 0.003$), where the lowest median Ra was observed among nerves cut with No. 11 blades (3 µm, IQR: 1.87–4.38), followed by scissors (3.29 µm, IQR: 1.56–4.96), and razor blade (5.41 µm, IQR: 4.95–6.21). Table 2 shows a summary of these findings. A post hoc test using Wilcoxon rank-sum test to compare surfaces cut with a pair of scissors with those cut with No. 11 blades also did not show a significant difference ($P = 0.757$).

DISCUSSION

Our results confirm that the lower the surface area roughness (low Sa value), the less inconsistencies of the exposed fascicles beyond the epineurial ends. Uniformity of the two nerve ends undergoing coaptation theoretically increases the likelihood of nerve growth through the repair site. In the strive toward the ultimate goal of improving nerve healing after injury, we developed a novel technique to quantitatively assess the surface roughness of different cutting instruments. In evaluating the surface area roughness after various instruments were used, we found the smoothest nerve ends from those excised with straight iris scissors and No. 11 blades. A straight razor blade performed the poorest in regard to surface area roughness. This was statistically significant. A post hoc analysis between the scissors and eleven blades showed no statistical difference. Therefore, both scissors and No. 11 blades performed better than the razor blade.

These results are unsurprising when considering the mechanism of how these instruments cut. A No. 11 blade is used to "saw" through the nerve by applying minimal force and using only the cutting edge to make a precise cut without distorting the tubular shape of the nerve being held under tension. A No. 15 blade can be used in a similar manner; however, care must be taken not to use excess force to compress the nerve with the belly of the blade. An iris scissor works similarly, with 2 sharp cutting edges one on the inside of each scissor leaf that each slice the nerve from both ends without forceful compression of the nerve. However, a razor blade, when used for nerve resection, will unintentionally cause forceful compression of the nerve as the blade belly is pressed down against it, flattening the nerve against the firm background of the tongue depressor. This leads to inconsistencies of the cut end, as flattening of the previously tubular structure will herniate the internal fascicles beyond the epineurium during the cutting motion, leaving a fish-mouth flap of epineurium and heterogeneity of the fascicular bundles at the new cut end. Likely the key to nerve preparation is not the instrument used to cut the nerve end, but maintaining the anatomic tubular structure of the nerve during the cutting motion.

Previous studies have compared different methods of nerve transection in relation to post-injury neuroma formation. Fischer et al[15] made a direct comparison of neuroma formation in opposite limbs of Sprague-Dawley

rats using a scalpel or CO_2 laser to transect each sciatic nerve. After 30 days following nerve transection with each method, the sciatic nerves were harvested and specimens underwent histologic axonal composition studies and scanning electron microscopy (SEM) evaluation. The CO_2 laser group specimens were found to have less scar tissue formation and perineural cell proliferation, but a unique foreign body reaction with multi-nucleated giant cells surrounding carbonaceous debris. However, both groups demonstrated clinical neuroma formation at 30 days without differences in gross appearance and percentage composition of axons compared with control nerves.

Rummings et al[16] evaluated the formation of sciatic neuromas in Sprague-Dawley rats following nerve transection and repair using a No. 15 blade and tongue depressor, micro-serrated scissors, nerve-cutting guide forceps and straight razor, or bipolar cauterization. Six weeks following this procedure, each of the 15 rats was euthanized and the sciatic nerve previously repaired was harvested to assess clinical neuroma formation and neuroma morphology under SEM. All rats independent of cutting instrument demonstrated increased cross-sectional area corresponding to neuroma formation in the transected sciatic nerve when compared with the unaffected contralateral limb sciatic nerve, and all sciatic specimens displayed abnormal neural architecture under SEM. However, those nerve ends transected with micro-serrated scissors or the nerve-cutting guide forceps and straight razor had smooth surface edges with uniform axonal distribution, whereas those cut with the No. 15 blade and tongue depressor were found to have much greater disorganization of the neural micro-architecture. This difference was attributed to the crushing force created by performing the cutting motion with the No. 15 blade against the firm, rigid surface of the tongue depressor. Although qualitatively assessing SEM images may be prone to inter-observer reliability differences, they concluded that microscopic differences between the cut ends are attributable to the technique used, albeit without any clinical difference in neuroma formation.

We are not the first to stress the importance of anatomic micro-architecture preservation during nerve end preparation. There has been a great deal of research into the effects of the Saint-Venant's principle of tensile force distribution within elastic bodies, relating to tension causing deformational forces at cut nerve ends.[17] By applying this principle, de Medinaceli postulated first that when manipulating cut nerve ends, all mechanical traction placed on a nerve should be a distance of at least 1.5× the diameter of the nerve away from the cut end. Additionally, de Medinaceli expounded that any deformational force would lead to a loss of symmetry between 2 cut ends, as internal displacement of the fascicles from flattening leads to asymmetric alignment of the neurites within the endoneurium. Without proper alignment of neurites, the skeletal framework of the nerve, de Medinaceli believed proper nerve healing would be impossible. Therefore, his second postulate regarded using a perfectly cylindrical force to the nerve during transection, to yield 2 symmetrically cut nerve ends, with the internal nerve fibers still in equal configuration.[18]

To better maintain nerve architecture during end trimming, de Medinaceli developed an experimental technique involving temporary freezing of nerve ends using a specialized irrigation fluid. The frozen nerve would further resist deformation by decreasing elasticity, and allow for a much smoother transection with minimal crush injury. By careful selection of fluid composition and close monitoring during the freezing process, causes of freeze/thaw injury to nerve cells via increased extra-cellular salt concentration or formation of intra-cellular icicles are minimized.[19] This new technique of temporary freezing (dubbed "cell surgery") remains experimental but did demonstrate good preliminary results in small follow-up study.[20]

There are several limitations to the study presented here. Although fresh frozen cadavers are the standard for cadaver-based studies, it is difficult to extrapolate this to in vivo, intraoperative conditions. Secondly, one of this study's biggest strengths is also a weakness. This is a novel technique for evaluating the nerve roughness after excision, yet there is not a gold standard to validate our technique. Lastly, the surface roughness is quantifiable with our novel approach, yet there is not a clinical correlation with nerve outcomes and technique. Further studies using calculated surface roughness are necessary to fully elucidate the impact of the topographical architecture on nerve healing.

CONCLUSIONS

Excision of nerve endings with razor blades, No. 11 blades, and straight iris scissors did produce different surface roughness in cadaveric mixed nerves. In regard to both surface roughness and surface profile roughness, the No. 11 blade and scissors did statistically better than a razor blade. The post hoc analysis showed the scissors and the No. 11 blade were not statistically different. Our novel approach brings forth a method to evaluate the homogeneity within the topography of a cut surface of a nerve. Although the system is not validated, secondary to a lack of gold standard, we believe there is limited patient risk in recommending No. 11 blades or iris scissors for excision of injured nerve endings. Furthermore, No. 11 blades are cheap and easily accessible in comparison with nerve-specific cutting scissors. This study can be a platform for further studies comparing other accepted methods of nerve cutting and for other research in nerve repairs and nerve recovery, utilizing the methodology described.

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REFERENCES

1. Karsy M, Watkins R, Jensen MR, et al. Trends and cost analysis of upper extremity nerve injury using the National (Nationwide) Inpatient Sample. *World Neurosurg.* 2019;123:e488–e500.
2. 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.
3. Grinsell D, Keating CP. Peripheral nerve reconstruction after injury: A review of clinical and experimental therapies. *Biomed Res Int.* 2014;2014:698256.
4. Sunderland S. Nerves and nerve injuries. *J Neurol Neurosurg Psychiatry.* 1968;32:165.
5. Vernadakis AJ, Koch H, Mackinnon SE. Management of neuromas. *Clin Plast Surg.* 2003;30:247–68, vii.
6. Rosberg HE, Carlsson KS, Höjgård S, et al. Injury to the human median and ulnar nerves in the forearm–analysis of costs for treatment and rehabilitation of 69 patients in southern Sweden. *J Hand Surg Br.* 2005;30:35–39.
7. Sunderland S. The intraneural topography of the radial, median and ulnar nerves. *Brain.* 1945;68:243–299.
8. Pfister BJ, Gordon T, Loverde JR, et al. Biomedical engineering strategies for peripheral nerve repair: Surgical applications, state of the art, and future challenges. *Crit Rev Biomed Eng.* 2011;39:81–124.
9. Cho MS, Rinker BD, Weber RV, et al. Functional outcome following nerve repair in the upper extremity using processed nerve allograft. *J Hand Surg Am.* 2012;37:2340–2349.
10. Safa B, Buncke G. Autograft substitutes: Conduits and processed nerve allografts. *Hand Clin.* 2016;32:127–140.
11. Means KR Jr, Rinker BD, Higgins JP, et al. A multicenter, prospective, randomized, pilot study of outcomes for digital nerve repair in the hand using hollow conduit compared with processed allograft nerve. *Hand (N Y).* 2016;11:144–151.
12. Saltzman EB, Villa JC, Doty SB, et al. A comparison between two collagen nerve conduits and nerve autograft: A rat model of motor nerve regeneration. *J Hand Surg Am.* 2019;44:700.e1–700.e9.
13. Woo A, Bakri K, Moran SL. Management of ulnar nerve injuries. *J Hand Surg Am.* 2015;40:173–181.
14. Panse N, Bindu A. Basic techniques of peripheral nerve repair. *J Peripher Nerv Surg.* 2018;2:41–8.
15. Fischer DW, Beggs JL, Shetter AG, et al. Comparative study of neuroma formation in the rat sciatic nerve after CO_2 laser and scalpel neurectomy. *Neurosurgery.* 1983;13:287–294.
16. Rummings WA, Honeycutt PB, Jernigan EW, et al. Effect of nerve-cutting technique on nerve microstructure and neuroma formation. *J Hand Microsurg.* 2019;11:28–34.
17. De Saint-Venant A. Mémoire sur la torsion des prismes. *Mem Acad Sci.* 1856;14: 233–560.
18. De Medinaceli L. How to correctly match 175,000 neurites: Two postulates for a quick solution. *Biosystems.* 1987;20:307–315.
19. de Medinaceli L, Merle M. How exact should nerve stump coaptation be? A new answer given by "cell surgery". *J Hand Surg Br.* 1991;16:495–498.
20. de Medinaceli L, Merle M. Applying "cell surgery" to nerve repair: A preliminary report on the first ten human cases. *J Hand Surg Br.* 1991;16:499–504.