Effect of the Number of Sutures on Nerve Repair: A Mechanical, Functional and Morphometric Study of Tibial Nerve Regeneration in Wistar Rats

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Abstract

Background: Nerve repair with microsurgical techniques is the gold standard and the most widely used surgical treatment for nerve reconstruction. However, the optimum number of microsutures for approximation of nerve ends has not been investigated thoroughly, as the focus of previous studies has been on in vitro examination of biomechanical strength of the repaired nerve.

Objectives: In this study, we investigated the effect of the number of suture on nerve repair, concerning mechanical, functional, and morphometric aspects.

Methods: Twenty-four male Wistar rats were subjected to tibial nerve transection and randomly divided into three groups: control with no repair; repair with 1 suture; and repair with 3 sutures. Tibial functional index (TFI) was determined weekly for two months to assess the functional recovery. At the end of the study, macroscopic and microscopic evaluations as well as adhesive strength quantification at the site of the injury were performed.

Results: There was no significant difference in the TFI measures between 1-suture and 3-suture groups during 8 weeks ($P > 0.05$). Adhesive strength was not significantly different between three groups ($P > 0.05$); however, it was higher in both suture groups compared with the control group. Scar index was 0.50 (SD = 0.06, $n = 2$) in control, 0.54 (SD = 0.08, $n = 4$) in 1-suture, and 0.58 (SD = 0.13, $n = 4$) in 3-suture groups. The comparison of the mean of differences in scar index and vascular index did not imply significant differences ($P > 0.05$).

Conclusions: Repair of rat’s tibial nerve with one or three microsutures gave comparable outcomes. This may suggest that for functional recovery of the fine nerves, simple approximation with minimum number of sutures is sufficient.

Keywords: Peripheral Nerve, Rats, Regeneration, Sutures, Tibial Nerve

1. Introduction

Peripheral nerve injuries are among the most prevalent and disabling clinical conditions (1). Peripheral nerve repair with epineural technique was first described in 1873 by Hueter (2). With advancement of microsurgical techniques, microsuturing was introduced as a gold standard and the most widely used surgical treatment for nerve reconstruction (3, 4). Nevertheless, conventional nerve suturing is technically demanding, time consuming, and may cause traumatic and inflammatory injuries to the nerve stumps, leading to unpredictable and suboptimal outcomes (5-8).

To achieve better concept of nerve repair with sutures and to optimize the design of future studies, it is necessary to investigate the interaction between microsuture and nervous tissue. To the best of our knowledge, the optimum number of microsutures for approximation of nerve ends has been previously studied in a limited extent and the main focus has been on in vitro examination of biomechanical strength of nerve repair (9-11).

The present study was therefore conducted to evaluate the effect of number of sutures on nerve repair. The evaluation involved mechanical, functional, and morphometric examination of tibial nerve regeneration in Wistar rats.

2. Material and Methods

2.1. Study Design

Twenty-four male Wistar rats (266 ± 44 g) were subjected to tibial nerve transection and randomly divided into three groups ($n = 8$ for each): control with no repair, repair with 1 suture, and repair with 3 sutures. Behavioral evaluation (Tibial functional index) was performed weekly for two months to compare the restored function of the
severed nerves. At the end of the study, macroscopic and microscopic evaluations were performed as well as adhesive strength quantification at the site of the injury. The care and use of laboratory animals were in accordance with the guidelines of the national institutes of health (12). All experiments were approved by the ethics committee of Tehran University of Medical Sciences (TUMS institutional review board).

### 2.2. Animal Surgery

Following general anesthesia with a mixture of ketamine (8 mg/100 g, intraperitoneal injection) and xylazine (0.4 mg/100 g, intraperitoneal injection), right sciatic nerve was exposed to aseptic conditions. Afterwards, tibial and common peroneal nerve branches were dissected and a complete transection was made in tibial nerve, 5 mm distal to the bifurcation of the sciatic nerve. Considering nerve retraction and in order to approximate nerve stumps to facilitate repair procedure, a 27-gauge needle was used to fix the distal stump of the nerve into the muscle adjacent to the proximal stump, similar to the method described by Kamath and Bhardwaj (13). In the control group, the needle was removed after 5 minutes without any attempt to repair the nerve injury. In other two groups, the nerve ends were alternatively repaired with one or three 10/0 nylon epi-perineural microsutures. After 5 minutes of approximation, the needle was removed and the wound was closed in layers. At the end, each rat was injected with 3 ml of normal saline intraperitoneally and 30 mg of cefazolin subcutaneously to prevent dehydration and infection. A daily application of picric acid (2,4,6-trinitrophenol, Sigma-Aldrich Co., St. Louis, MO) was performed over the operated limb to prevent autotomy (14).

### 2.3. Behavioral Assessments

During two months of follow-up, Tibial functional index (TFI) was determined weekly in all rats. For this purpose, we used a transparent corridor 100 cm in length and 15 cm in width, leading to a dark chamber at the end. A flat mirror was placed beneath the pathway with 45° angle to reflect the view of the plantar surface of the rat’s hind paw. Each rat had four non-hesitant passages. Mid-stance frames of the right foot were recorded with a digital high-speed camera (Sony, HDR-SR12 high definition, Japan). TFI was determined using a software program developed in our laboratory, based on a formula developed by Bain et al. (Figure 1) (15, 16).

### 2.4. Macroscopic and Biomechanical Assessments

At the end of the study, all rats were euthanized and tibial nerves were re-exposed. Four animals from each group were subjected to biomechanical assessment. Adhesive strength was measured using a universal testing machine employing a hooklike accessory applied proximally to the repair site. The device had one gram precision and a stretching velocity of 5 mm per minute (17).

### 2.5. Microscopic Assessments

Microscopic assessment was performed on four animals from each group. A 4 mm segment of the tibial nerve, including the lesion region, was removed. The tissue samples were fixed in 4% paraformaldehyde solution, dehydrated in gradually increasing solutions of ethanol and embedded in paraffin. Transverse sectioning was performed and 2 µm sections were stained with hematoxylin and eosin (H and E) and Masson’s trichrome. In order to assess perineural scar formation, the scar index was calculated by dividing the cross-sectional area of the scar tissue by the whole area of the nerve, using Image J software (18, 19). To this end, digitalized images were taken from the slides stained by Masson’s Trichrome under 100 × magnification (Figure 2A).

To assess acute inflammation, presence of polymorphonuclear (PMN) cells was examined in H and E-stained sections by a pathologist blinded to group assignments (20). For chronic inflammation, vascular index was calculated as ratio of the total area of vessels to the whole nerve area, suggesting the congestion (Figure 2B) (21-23).

### 2.6. Statistical Analysis

SPSS software (version 19.0; IBM Corp., Armonk, NY) was used for statistical analysis. We used two-way ANOVA with LSD post hoc test for comparison of TFI measures, and one-way ANOVA for analysis of adhesive strength, scar index, and vascular index. Chi-square test was used for occurrence of dehiscence. P values < 0.05 were considered to be statistically significant.

### 3. Results

Two rats (both from the control group) were excluded due to post-operative death. None of the animals showed automutilation or infection.

#### 3.1. Behavioral Assessments

There was no significant difference in TFI measures between 1-suture and 3-suture groups during 8 weeks (Two-way ANOVA, P > 0.05). However, both groups had a significantly higher TFI score compared with the control group in the 5th (P = 0.04), 6th (P = 0.02), and 8th (P = 0.004) week post-injury (Figure 3).
First three different measures were obtained for each experimental (E) and normal (N) legs: 1, print length (PL), distance from the heel to the tip of the third toe; 2, toe spread (TS), distance from the first to the fifth toe; 3, intermediary toe spread (ITS), distance from the second to the fourth toe. The TFI was calculated using the formula developed originally by Bain et al. (15). TFI = (-37.2 × PLF) + (104.4 × TSF) + (45.6 × ITSF) - 8.8

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\text{print length factor (PLF)} = \frac{EPL - NPL}{NPL}, \quad \text{toe spread factor (TSF)} = \frac{ETS - NTS}{NTS}, \quad \text{intermediary toe spread factor (ITSF)} = \frac{EITS - NITS}{NITS}.
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3.2. Macroscopic and Biomechanical Assessments

In the control group, the nerve gap was present in three out of six surviving rats. In other three rats, the nerve continuity was restored without any intervention. No gap was observed at the repair site in either of the suture groups. This finding showed a statistically significant difference ($\chi^2 (2) = 9.26, P = 0.01$). Adhesive strength was not significantly different in the three groups (One-way ANOVA, $P > 0.05$); however, it was higher in both suture groups compared with the control group. The mean adhesive strength was 296 g (Range = 83-283 g, $SD = 100, n = 4$) in 1-suture group and 270 g (Range = 170 - 374 g, $SD = 89, n = 4$) in 3-suture group. The adhesive strength in the control group was only measured in one rat (95 g), due to the dehiscence in the others.

3.3. Microscopic Assessments

No acute inflammatory reaction was detected by assessment of PMN cells in samples. Nonetheless, foreign body giant cells around the suture material were detected in a few specimens of microsuture groups (Figure 2C).

Scar index was 0.50 ($SD = 0.06, n = 2$) in the control group, 0.54 ($SD = 0.08, n = 4$) in 1-suture, and 0.58 ($SD = 0.13, n = 4$) in 3-suture groups. The mean of differences in scar indices was not significantly different (One-way ANOVA, $P > 0.05$), neither were the differences of the vascular indices among the groups (One-way ANOVA, $P > 0.05$). The mean vascular surface was 0.028 ($SD = 0.01, n = 2$) in control,
Figure 2. Cross Sections of Tibial Nerve Taken at the Repair Site

A, the scar index was measured by dividing the cross-sectional area of the scar tissue by the whole area of the nerve and surrounding scar tissue. Dotted line shows the scar area and solid line shows the nerve area. (Masson’s trichrome stain, original magnification × 100). B, vascular index was calculated as the ratio of the total area of vessels (solid lines) to the whole nerve area (dotted line), using ImageJ software (H and E stain, original magnification × 100). C, foreign body giant cells around the suture material (Masson’s trichrome stain, original magnification × 1000).

Figure 3. Tibial Function Index (TFI) during eight weeks (± SE)

Both 1-suture and 3-suture groups had a significantly higher TFI score compared with the control group in the 5th, 6th and 8th week post-injury.

0.024 (SD = 0.01, n = 4) in 1-suture, and 0.021 (SD = 0.004, n = 4) in 3-suture groups.

4. Discussion

The present study showed that 1 suture and 3 sutures have similar effects on mechanical, functional, and morphometric outcomes of primary repair in rat’s tibial nerve.

Previously, Goldberg and colleagues examined the effect of suture number, suture gauge, and suture purchase length on repair strength in digital nerves of fresh-frozen cadaver (10). They found that suture number was the only significant, effective variable, and four epineural sutures being mechanically stronger than two sutures (10). In another cadaveric study, Giddins and colleagues investigated the optimum gauge of epineural suture for median nerve repair (9). The number of sutures was not assessed as an individual factor, because 9 to 13 sutures were used for each median nerve to neatly restore continuity of epineurium. The number increased slightly when finer material was used. However, the best gauge to repair the gap was the one with the highest number of sutures (9). Temple and colleagues studied different nerve repair techniques in a rabbit sciatic nerve model (11). The sciatic nerves of rabbits were dissected and studied in vitro. The final strength was tested biomechanically and techniques with higher number of epineurial grasping strands had the best biomechanical outcome (11).

All three mentioned studies were in vitro while biomechanical results may be different in a viable nerve (10). Thus, the effects of edema, adhesions, and normal tissue healing that occur in a live tissue were not investigated. Moreover, nerve function during normal activity is an important issue that could not be analyzed in these studies (10).

Furthermore, such studies have evaluated the mechanical aspects of repair in an acute setting. It is evident that the initial repair site strength does not correlate with the strength in a few next days (17). Nishimura and colleagues showed this trend by comparing fibrin tissue adhesive to conventional nerve repair in a rat sciatic model.
Although the repair resistant was significantly lower for the glue, it became equivalent for the two types of repair at 14 days post-surgery (17). The importance of this finding is that usually the repair should be protected in the first two weeks after surgery and only limited motion can be started afterwards (17). Therefore, in vivo studies with time dependent manner could be of value on this subject.

We found that the number of sutures in fine nerves (e.g. tibial nerve of rat) does not have a direct effect on nerve repair, as may be expected. One suture was not significantly different from three sutures in outcomes, suggesting that for functional recovery of fine nerves, simple approximation is sufficient (i.e. it is not necessary to have a precise end-to-end approximation). Nevertheless, we could not extend this finding to the nerves with large diameters such as human median nerve.

On the other hand, higher number of sutures in fine nerve could be associated with worse outcome. Martins and colleagues reported that the electrophysiological outcome of six-suture repair was inferior to three-suture repair in sciatic nerve of rat (24). The six-suture repair was related to a significant increase in collagen of epineurium, compared with the three-suture repair.

The granulomatous tissue formation around the suture material has also been a concerning issue (6). In histological assessments, we did not find excessive scar formation or inflammatory reaction; however, foreign body giant cells around the suture material were detected in a few specimens.

For nerve approximation, a simple nerve approximator was used as described by Kamath and Bhardwaj with slight modifications (13). A fine needle was used and distal stump was fixed. Similar to their findings, we observed that the passage of a fine needle through rat’s tibial nerve did not disturb its function. The results of the present study also confirm the utility of this technique as an inexpensive, simple method that could reduce operation time.

This study had some limitations. The adhesive strength in the control group was only measured in one rat, because out of four animals anticipated for this assessment, three had nerve dehiscence that made measurement impossible. We did not measure electrophysiological data in the current experiment. Future studies may therefore expand toward these observations by electrophysiological evidence.

In conclusion, the repair of rat’s tibial nerve with one or three microsutures had comparable outcomes. This may suggest that for functional recovery of the fine nerves, simple approximation with minimum number of sutures is sufficient.

Footnote

Authors’ Contribution: Study concept and design, Reza Shahryar Kamrani, Mohammad Hossein Nabian, Shayan Abdollah Zadegan; acquisition of data, Reza Erfanian, Shayan Abdollah Zadegan; analysis and interpretation of data, Shayan Abdollah Zadegan, Mohammad Hossein Nabian; drafting of the manuscript, Shayan Abdollah Zadegan, Mohammad Hossein Nabian, Reza Erfanian; critical revision of the manuscript for important intellectual content, Masoumeh Firoouzi, Leila Orlady Zanjani, Reza Shahryar Kamrani; statistical analysis, Shayan Abdollah Zadegan, Mohammad Hossein Nabian; administrative, technical, and material support, Mohammad Hossein Nabian, Shayan Abdollah Zadegan, Leila Orlady Zanjani, Reza Shahryar Kamrani, Reza Erfanian; study supervision, Reza Shahryar Kamrani, Masoumeh Firoouzi, Mohammad Hossein Nabian.

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