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Stress and strain analysis on the anastomosis site sutured with either epineurial or perineurial sutures after simulation of sciatic nerve injury[☆]

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Abstract

The magnitude of tensile stress and tensile strain at an anastomosis site under physiological stress is an important factor for the success of anastomosis following suturing in peripheral nerve injury treatment. Sciatic nerves from fresh adult cadavers were used to create models of sciatic nerve injury. The denervated specimens underwent epineurial and perineurial suturing. The elastic modulus (40.96 ± 2.59 MPa) and Poisson ratio (0.37 ± 0.02) of the normal sciatic nerve were measured by strain electrical measurement. A resistance strain gauge was pasted on the front, back, left, and right of the edge of the anastomosis site after suturing. Strain electrical measurement results showed that the stress and strain values of the sciatic nerve following perineurial suturing were lower than those following epineurial suturing. Scanning electron microscopy revealed that the sciatic nerve fibers were disordered following epineurial compared with perineurial suturing. These results indicate that the effect of perineurial suturing in sciatic nerve injury repair is better than that of epineurial suturing.

Key Words

sciatic nerve injury; epineurial suture; perineurial suture; strain electrical measurement; anastomosis site; tensile stress; tensile strain; elastic modulus; Poisson ratio; biomechanics; peripheral nerve injury; neural regeneration

Research Highlights

(1) The method used in this study is characterized by recording of the tensile strain and tensile stress values at each measurement point of the edge of the anastomosis site in sciatic nerve injury models following perineurial and epineurial suturing and recording of the elastic modulus and Poisson ratio of sciatic nerve specimens.

(2) Each specimen was independent; we sought to determine the differences in tensile strain and tensile stress at each measurement point of the edge of the anastomosis site following perineurial and epineurial suturing.

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INTRODUCTION

The tension of the anastomosis site using apposition sutures for peripheral nerve injury can affect the outcome of neural regeneration^[1-10]. Previous studies have

measured the tension of the anastomosis site using the uniaxial tension method and mini-force measure ruler following peripheral nerve injury, but the tension of the anastomosis site could not completely represent the tension of a normal anastomosis

site^[11-13]. The elastic modulus and Poisson ratio were previously measured using the uniaxial tension method, which results in a large error. To solve the above-mentioned problems, this study directly measured the tensile stress, tensile strain, elastic modulus, and Poisson ratio of anastomosis sites.

A previous study confirmed that the epineurium of the rat sciatic nerve contains collagen and can strongly resist tension^[11]. During suturing, the epineurium can bear a certain tension and cannot be avulsed^[11]. The anti-tension function of the nerve trunk is stronger than that of the epineurium during suturing^[11]. Hicks *et al*^[12] measured the tension of the ulnar nerve during elbow joint activity using the mini-force measure ruler and suggested that ulnar nerve decompression alone cannot relieve the tension of the ulnar nerve, but decompression combined with resection of the medial epicondyle of the humerus can lessen tension during elbow flexion. However, the error was large. Spinner *et al*^[13] evaluated the relationship of the number and type of brachial plexus root breakages with the traction size using a load sensor and displacement gauge. The above-described studies did not obtain the tensile stress and tensile strain values at the edge of the anastomosis site.

This study measured the tensile stress and tensile strain at each measurement point of the edge of the anastomosis site in fresh human cadaver sciatic nerve injury models using epineurial and perineurial suturing under the principle of strain electrical measurement.

RESULTS

Quantitative analysis of subjects

A total of 46 sciatic nerve specimens from fresh adult cadavers were randomly assigned to the perineurial suture group ($n = 15$), epineurial suture group ($n = 15$), or elastic modulus plus Poisson ratio test group ($n = 15$). One specimen served as the control (no treatment). A total of 46 specimens were included in the final analysis; and there were no drop-outs. One specimen from the epineurial suture group and one from the perineurial suture group were used for microstructure observation, and both were included in the final analysis.

Results of elastic modulus test

The elastic modulus was calculated according to Hooke's law. The formula is as follows:

$$E = \sigma / \varepsilon \quad [14] \quad (1)$$

Where E is the elastic modulus, σ refers to stress ($\sigma =$

P/A ; P : load; A : transverse section area), and ε represents strain. The strain value was substituted in formula (1), and the elastic modulus is listed in Table 1.

Table 1 Results of elastic modulus and Poisson's ratio of sciatic nerve specimens ($n = 15$)

No.	Longitudinal strain (%)	Transverse strain (%)	Elastic modulus (MPa)	Poisson's ratio
1	0.396	0.143	39.47	0.36
2	0.366	0.139	42.63	0.38
3	0.414	0.145	37.64	0.35
4	0.353	0.131	44.26	0.37
5	0.403	0.137	38.71	0.34
6	0.379	0.148	41.22	0.39
7	0.391	0.149	39.96	0.38
8	0.366	0.132	42.68	0.36
9	0.427	0.149	36.54	0.35
10	0.339	0.125	46.12	0.37
11	0.378	0.147	41.32	0.39
12	0.398	0.147	39.25	0.37
13	0.392	0.133	39.84	0.34
14	0.358	0.129	43.61	0.36
15	0.379	0.140	41.22	0.37
Mean \pm SD	0.38 \pm 0.24	0.14 \pm 0.01	40.96 \pm 2.59	0.37 \pm 0.02

Results of Poisson coefficient test

The formula for calculating the Poisson's ratio is as follows:

$$\mu = | \varepsilon^* / \varepsilon | \quad [14] \quad (2)$$

Where ε is the Poisson's ratio, ε^* is the transverse strain, and ε is the longitudinal strain. The elastic modulus was 40.96 ± 2.59 MPa, and the Poisson ratio was 0.37 ± 0.02 (Table 1).

Changes in stress and strain at each measurement point of the edge of the anastomosis site in the epineurial and perineurial suture groups

In accordance with Hooke's law, the results of stress and strain on the edge of the anastomosis site were calculated in the perineurial and epineurial suture groups (Table 2).

Table 2 Results of stress and strain in models of sciatic nerve injury in the perineurial and epineurial suture groups

No.	Strain (%)		Stress (MPa)	
	Perineurial suture group	Epineurial suture group	Perineurial suture group	Epineurial suture group
1	0.347 \pm 0.015	0.384 \pm 0.012 ^a	0.142 \pm 0.006	0.157 \pm 0.005 ^a
2	0.365 \pm 0.012	0.393 \pm 0.013 ^a	0.150 \pm 0.005	0.162 \pm 0.005 ^a
3	0.370 \pm 0.015	0.399 \pm 0.012 ^a	0.151 \pm 0.006	0.164 \pm 0.005 ^a
4	0.355 \pm 0.012	0.386 \pm 0.010 ^a	0.146 \pm 0.005	0.158 \pm 0.004 ^a
5	0.381 \pm 0.010	0.409 \pm 0.012 ^a	0.156 \pm 0.004	0.168 \pm 0.005 ^a
6	0.385 \pm 0.010	0.408 \pm 0.018 ^a	0.158 \pm 0.004	0.167 \pm 0.007 ^a
7	0.355 \pm 0.012	0.384 \pm 0.012 ^a	0.146 \pm 0.005	0.157 \pm 0.005 ^a
8	0.381 \pm 0.012	0.408 \pm 0.014 ^a	0.156 \pm 0.005	0.167 \pm 0.005 ^a

^a $P < 0.05$, vs. perineurial suture group. Measurement data are expressed as mean \pm SD. Paired t -test was used. There were 15 specimens in each group

Stress and strain were lower in the perineurial suture group than in the epineurial suture group ($P < 0.05$).

Results of histological analysis of sciatic nerve specimens in each group

Scanning electron microscopy revealed that normal sciatic nerve fibers were regularly arranged. Axons were wrapped by a myelin sheath. Endoneurium formed by connective tissues was present on the surface of nerve fibers wrapped in myelin sheaths and on axons (Figure 1A). In the perineurial suture group, sciatic nerve fibers were disordered, and the morphology of connective tissues on the surface of nerve fibers, endoneurium, myelin sheaths, and axons was changed (Figure 1B). In the epineurial suture group, sciatic nerve fibers were obviously scattered (Figure 1C).

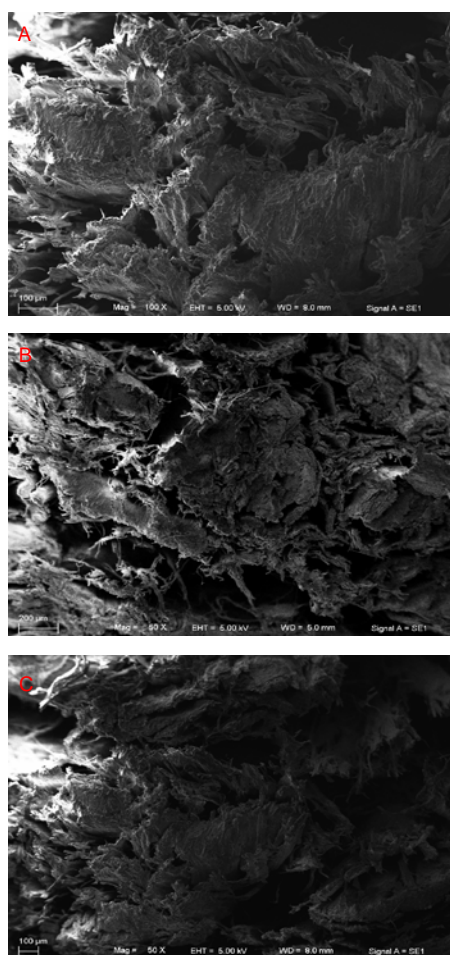


Figure 1 Ultrastructure of sciatic nerve cross section in the perineurial suture group (scanning electron microscope, $\times 2\,000$).

Sciatic nerve fibers were arranged regularly in the control group (A) and disorderly in the perineurial suture group (B) and epineurial suture group (C).

DISCUSSION

The method used in this study is characterized by the following: (1) The tensile strain and tensile stress was recorded at each measurement point on the edge of the anastomosis site in the sciatic nerve injury models following perineurial and epineurial suturing. (2) The elastic modulus and Poisson ratio of the sciatic nerve specimens were recorded. (3) Specimen grouping was random (experimental design, grouping analysis). (4) Each specimen was independent; we sought to determine the difference in tensile strain and tensile stress at each measurement point on the edge of the anastomosis site following perineurial and epineurial suturing. (5) Analysis of variance was used. The obtained standard deviation could be applied to quantitatively compare tensile stress and tensile strain in each group.

The strain electrical measurement results suggested that the elastic modulus was 40.96 ± 2.59 MPa and the Poisson ratio was 0.37 ± 0.016 . The elastic modulus is an important parameter with which to evaluate the function of a biomaterial. For example, different temperatures, load velocities, and sampling directions would affect measurement results.

In the present study, specimens were stored in the same conditions. The elastic modulus was tested at the same temperature under the same load velocity. The influences of anisotropy and non-homogeneity were excluded. Before loading, the specimens were pre-adjusted to provide enough relaxation time for the sciatic nerve fibers, and the data had good reproducibility.

Previous studies have confirmed that the ultimate tensile strain is 0.49 ± 0.09 , the ultimate stress is 7.14 ± 1.50 MPa, and the elastic modulus is 19.33 ± 2.00 MPa^[15-16]. Strain electrical measurement results suggested that strain and stress in the epineurial suture group were larger than those in the perineurial suture group at measurement points No. 1–8. The strain and stress were lower in this study than in previously published studies^[15-16]. The elastic modulus of the sciatic nerve of humans was larger in the present study than that of normal rats in previous studies^[16-17]. These results indicate that the anti-elastic deformation ability differs between human and rat sciatic nerves. The elastic modulus obtained in previous studies^[15-16] was calculated by stress-strain data in the elastic segment of the stress-strain curve, and the error was large. Nevertheless, in this study, the resistance strain gauge was directly pasted, and the strain value was measured by a resistance strain

gauge. The elastic modulus of specimens was calculated according to Hooke's law, with high precision and small error.

Connective tissue in the peripheral nerve trunk comprises endoneurium, perineurium, and epineurium. Endoneurium wraps each nerve fiber, creating bundles of many nerve fibers. The endoneurium displays disposed bundles of collagen fibers. Epineurium-wrapped nerve trunks are formed by many nerve bundles containing three types of extracellular fibers that mainly comprise longitudinally disposed collagen fibers followed by longitudinally disposed lamellated elastic fibers^[17]. Collagen fibers are the main component of connective tissue of peripheral nerves, and the distribution and number of collagen fibers determine the biomechanical properties of peripheral nerves^[18]. Collagen fibers and elastic fibers under loads have various characteristics. Collagen fiber is a ductile material, and elastic fiber is a brittle material^[19]. The epineurium mainly contains longitudinally disposed lamellated elastic fibers, which are brittle material; thus, the tensile resistance of epineurium is poorer than that of perineurium.

Zhu *et al*^[20] verified that perineurial suturing of the anastomotic location of nerve tracts reduces their adhesion to one another and promotes neural regeneration. Sunderiand^[21] stated that peripheral nerve perineurium has the greatest tensile resistance. The results of this study suggest that the tensile stress and tensile strain at each measurement point on the edge of the anastomosis site differed between perineurial and epineurial suturing in the sciatic nerve injury models. This study concludes that perineurial suturing is more effective in sciatic nerve injury treatment because it results in low tensile stress and strain at the anastomosis site under physical stress and contributes to neural regeneration and functional reconstruction.

This study only measured the tensile strain at the anastomosis site of sciatic nerve injury models following end-to-end neurorrhaphy. The measurement of tensile strain at the anastomosis site using other anastomotic methods, such as neural transplantation, deserves further investigation. Although experimental data are dispersed because of individual differences in cadaver height, weight, and health status, such data have some referential clinical value.

MATERIALS AND METHODS

Design

This was a biomechanical comparative analysis study.

Time and setting

Experiments were performed at the Mechanics Experimental Center, Jilin University, China from March to August 2011.

Materials

Sciatic nerves were collected from the bilateral gluteus maximus of eight male adult cadavers who died of acute head injury at 25 to 30 years of age. The cadavers were supplied by the Department of Anatomy, School of Medicine, Jilin University, China. The specimens were used after obtaining the informed consent of family members.

Methods

Preparation of sciatic nerve specimens

A total of 16 sciatic nerves from the bilateral gluteus maximus were collected within 20 hours following death. These specimens were stored at -20°C for 1 day, and then thawed at room temperature. A total of 45 specimens (30 mm in length, 8.8–9.2 mm in diameter) were obtained using an operating knife. Sciatic nerve injury models were created by cutting the sciatic nerves by the same clinical physician using an S-5 sterile operating knife with a plastic handle (Xuyi County Huaian United Yikang Medical Supplies, China) in the perineurial suture and epineurial suture groups in accordance with a previous method^[13]. A regular stump represented successful models. The specimens were sutured by tensionless perineurial or epineurial sutures with No. 10-0 nylon thread (Qingdao Naike Co., Ltd., Qingdao, Shandong Province, China) under a microscope. Qualified suture patterns comprised precise alignment at the stump following suturing. Those with unqualified suture patterns were excluded.

Strain of sciatic nerve anastomosis site and electrical measurement of elastic modulus and Poisson ratio of normal sciatic nerves

According to the requirements of gluing technology, a professional senior experimentalist pasted the resistance strain gauge on the edge of the anastomosis site of the sciatic nerve specimens following suturing using 502 flash-dry glue (Beijing Chemical Plant, Beijing, China). Two 1 mm × 1 mm impregnated base foil strain gauges (521 Factory, China) were separately transversally and longitudinally attached to the central part of the specimens to measure the elastic modulus and Poisson ratio. Specimens that did not meet the gluing technology requirements were excluded. The distribution of the resistance strain gauge for the elastic modulus plus Poisson ratio test group is displayed in Figure 2.

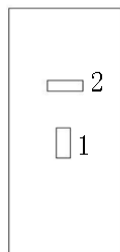


Figure 2 Distribution of resistance strain gauge in the elastic modulus plus Poisson's ratio test group.
1: Longitudinally, 2: transversally.

The distribution of the resistance strain gauge at each measurement point in the perineurial and epineurial suture groups is listed in Figure 3.

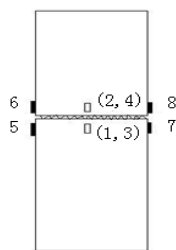


Figure 3 Distribution of resistance strain gauge in the perineurial and epineurial suture groups.
1: Edge of anastomosis site in front of (main vision) and below the specimen; 3: edge of anastomosis site posterior to (back vision) and below the specimen.
5: Edge of anastomosis site to the left of and below the specimen; 6: edge of anastomosis site to the left of and above the specimen.
2: Edge of anastomosis site in front of (main vision) and above the specimen; 4: edge of anastomosis site posterior to (back vision) and above the specimen.
7: Edge of anastomosis site to the right of and below the specimen; 8: edge of anastomosis site to the right of and above the specimen.

After attaching the resistance strain gauge in each group, a wiring terminal was attached to the proximal end of the strain gauge. The outlet of the electrical strain gauge was welded to the wiring terminal, and the guide wire was then welded.

Sciatic nerve specimens attached to the resistance strain gauge in each group were separately packed in the clamp of an electronic universal machine. Load was transmitted by a load sensor, and the ultimate range of the sensor was 200 N. Pre-adjustment was conducted according to previous methods^[16-17]. The guide wires at each measurement point on specimens were connected to the subcabinet of the static electric resistance strain gauge using the half-bridge external-compensation technique. The specimens

received a 10-N tensile load at 2 mm/min in the elastic modulus plus a Poisson ratio test in the perineurial and epineurial suture groups. Strain ($\mu \varepsilon, \times 10^{-6}$) at each measurement point was read using the static electric resistance strain gauge. Photographs in the elastic modulus plus Poisson ratio test group are shown in Figure 4A. Photographs of electrical strain measurements at the anastomosis site in the epineurial suture group are listed in Figure 4B. Photographs of electrical strain measurements at the anastomosis site in the perineurial suture group are listed in Figure 4C.

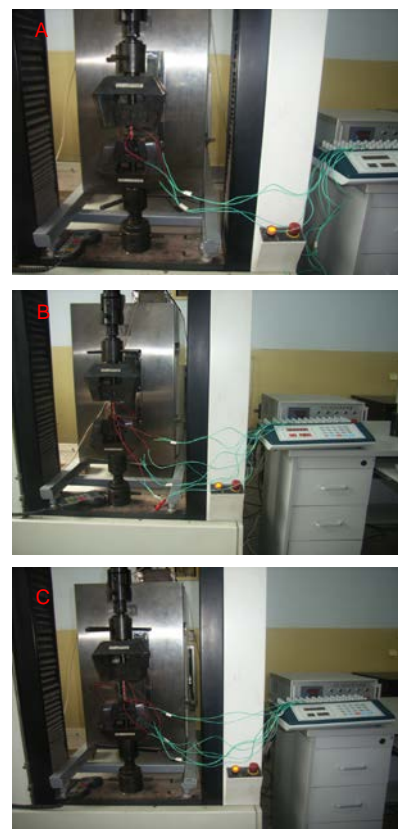


Figure 4 Electrical strain measurement at the anastomosis site in each group.

Sciatic nerve specimens were separately packed in the clamp of an electronic universal machine and received a 10-N tensile load after guide wires were connected to the resistance strain gauge in the elastic modulus plus Poisson ratio test group (A), epineurial suture group (B), and perineurial suture group (C). Only slight deformation appeared.

Histological ultrastructural observation of sciatic nerves

One sciatic nerve was randomly selected from the control group. One sciatic nerve was separately obtained from the perineurial and epineurial suture groups

following electrical strain measurement. Specimens were collected from the same region of the edge of the anastomosis site, trimmed into 5 mm segments, prefixed in 4% glutaraldehyde, postfixed in 1% osmic acid, dehydrated in acetone, dried by the critical point method, vacuum-coated, and metal-sprayed. A Supra-55 sapphire Field Emission Scanning Electron Microscope (Carl Zeiss, Jena, Germany) was used to observe nerve cross sections and understand the structural changes in neural cells, myelin sheaths, axons, and basal membranes.

Statistical analysis

Measurement data are expressed as mean \pm SD and analyzed using SPSS 11.0 software (SPSS, Chicago, IL, USA). Stress and strain data were compared using paired *t*-tests in the perineurial and epineurial suture groups. A value of *P* < 0.05 was considered statistically significant.

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Author contributions: Guangyao Liu participated in the study concept and design. Guangyao Liu, Qiao Zhang, and Yan Jin were in charge of data integration, analysis, and management. Guangyao Liu wrote the manuscript. Qiao Zhang participated in statistical analysis. Guangyao Liu and Yan Jin were in charge of manuscript authorization. Zhongli Gao obtained the funding.

Conflicts of interest: None declared.

Ethical approval: This study was approved by the Ethics Committee, China-Japan Union Hospital, Jilin University, China.

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