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Strain and stress variations in the human amniotic membrane and fresh corpse autologous sciatic nerve anastomosis in a model of sciatic nerve injury☆

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Abstract

A 10-mm long sciatic nerve injury model was established in fresh normal Chinese patient cadavers. Amniotic membrane was harvested from healthy maternal placentas and was prepared into multi-layered, coiled, tubular specimens. Sciatic nerve injury models were respectively anastomosed using the autologous cadaveric sciatic nerve and human amniotic membrane. Tensile test results showed that maximal loading, maximal displacement, maximal stress, and maximal strain of sciatic nerve injury models anastomosed with human amniotic membrane were greater than those in the autologous nerve anastomosis group. The strain-stress curves of the human amniotic membrane and sciatic nerves indicated exponential change at the first phase, which became elastic deformation curves at the second and third phases, and displayed plastic deformation curves at the fourth phase, at which point the specimens lost their bearing capacity. Experimental findings suggested that human amniotic membranes and autologous sciatic nerves exhibit similar stress-strain curves, good elastic properties, and certain strain and stress capabilities in anastomosis of the injured sciatic nerve.

Key Words

sciatic nerve injury model; autologous nerve; amniotic membrane; anastomosis; tension; mechanical properties; neural regeneration

Research Highlights

The human amniotic membrane and autologous sciatic nerve exhibit similar stress-strain curves, good elastic properties, and certain strain and stress capabilities in anastomosis of injured sciatic nerves.

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INTRODUCTION

Growing evidence suggests that following anastomosis with nerves, the human amniotic membrane exhibits biological and immunological characteristics to inhibit fibrosis formation, prevent scar neovascularization, and promote inflammatory cell apoptosis, thereby providing a culture medium for axonal growth in the central nervous system[1-9]. These unique characteristics allow for increased applications of amniotic membranes in the medical field[10-11]. Previous studies focused on mechanical properties following sciatic nerve injury were primarily concentrated in experimental animals, such as rats[12-13]. However, previous studies suggest that mechanical properties of the sciatic nerve before and after injury are not representative of the human sciatic nerve, because of variations in tensile strain and stress mechanical

properties. Tensile strain and stress are important factors that influence anastomosis of the injured sciatic nerve under physiological loading.

Previous results have demonstrated tensile strength rate, elastic modulus, and stress-strain curves *via* tensile tests on monolayer human amniotic membranes, double-layer human amniotic membranes, and 3.5 cm × 1 cm cross-linked amniotic membranes[14]. According to Li *et al* [15], peripheral nerve mechanical properties were determined in un-dissected, male, adult cadavers fixed in formaldehyde solution for 3–10 years. Tensile strength and elongation rate of the sciatic nerve and other peripheral nerves were measured, as well as typical stress-strain curves of the spinal nerve, vagus nerve, and cranial nerve.

The present study aimed to determine the biomechanical properties of sciatic nerve injury models following anastomosis with human amniotic membrane and autologous nerve from normal Chinese fresh cadavers. Tensile strain and stress were determined at the anastomotic stoma under physiological loads and ultimate loads (stress after nerve suture mainly concentrated in anastomotic stoma), as well as the feasibility of amniotic membranes to repair nerve injuries at the biomechanical level.

RESULTS

Quantitative analysis of samples

A total of 31 human amniotic membrane tube specimens were included in the present study. Among these, 15 specimens were used to simulate sciatic nerve injury and anastomose with human amniotic membrane, 15 specimens served as the normal human amniotic membrane group, and 1 specimen served as the specimen for normal human amniotic membrane group. In total, 48 sciatic nerve specimens were harvested from normal Chinese fresh cadavers and were used to establish 10-mm long sciatic nerve injury models. The cadavers were randomly assigned to a normal sciatic nerve group, autologous nerve anastomosis group (autologous sciatic nerve anastomosis), and human amniotic membrane anastomosis group (human amnion anastomosis). In each group, 15 specimens were used for tensile test and 1 specimen was used for histological observation. In total, 79 specimens were used in the tensile test and in the result analysis, with no dropouts or losses.

Biomechanical properties of sciatic nerve specimens following anastomosis

Tensile mechanical properties of specimens were compared using the tensile test on the sciatic nerve in the normal human cadaver sciatic nerve group, the human amniotic membrane in the normal human amniotic membrane group, and the injured sciatic nerve following anastomosis with human amniotic membrane and autologous nerve, respectively. An electronic universal testing machine was used. Results showed significantly greater tensile maximum load, maximum stress, maximum displacement, maximum strain, elastic limit load, and elastic limit stress in the normal sciatic nerve group compared with the autologous nerve anastomosis group and normal human amniotic membrane group ($P < 0.05$). Indices from the normal human amniotic membrane group were greater than in the human amnion anastomosis group ($P < 0.05$; Table 1).

The tensile specimen data from each group was subjected to curve fitting on computers. The stress-strain curves of the normal sciatic nerve group and autologous nerve anastomosis group are shown in Figure 1. Results showed that the curve exponentially increased at the first phase, and the strain in both groups was 0–5%, respectively. At the second phase, the curve was directly proportional to elastic deformations; strain was 5.1–20.0% in the normal sciatic nerve group and 5.1–10.0% in the autologous nerve anastomosis group. At the third phase, the stress-strain curve, similar to the first phase, exhibited elastic deformations; strain increased to 20.1–28.0% in the normal sciatic nerve group and 10.1–17.8% in the autologous nerve anastomosis group.

Table 1 Mechanical indices of specimens in each group

Group	Maximum load (kN)	Maximum stress (MPa)	Maximum displacement (mm)	Maximum strain (%)	Elastic limit load (kN)	Elastic limit stress (MPa)
Normal sciatic nerve	0.57±0.09[ab]	11.63±1.80[ab]	5.92±0.47[ab]	30.28±3.19[ab]	0.33±0.05[ab]	6.71±0.93[ab]
Autologous nerve anastomosis	0.15±0.02	3.04±0.32	4.37±0.55	21.86±2.78	0.13±0.02	2.29±0.36
Normal human amniotic membrane	0.05±0.00	0.69±0.03	3.40±0.31	16.85±1.50	0.03±0.00	0.42±0.02
Human amnion anastomosis	0.01±0.00[ab]	0.23±0.03[ab]	2.30±0.27[ab]	11.50±1.37[ab]	0.008±0.00[ab]	0.17±0.12[ab]

[a]$P < 0.05$, *vs*. autologous nerve anastomosis group; [b]$P < 0.05$, *vs*. normal human amniotic membrane group. Measurement data are expressed as mean ± SD of 15 specimens in each group, and statistical analysis was performed using one-way analysis of variance and Scheffe's method.

However, specimens exhibited deformations and lost bearing capacities at the fourth phase; strain was

28.1–30.3% in the normal sciatic nerve group and 17.8–21.9% in the autologous nerve anastomosis group. Alterations in the stress-strain curve of the normal sciatic nerve group and autologous nerve anastomosis group indicated good elasticity in the sciatic nerve.

The stress-strain curves of the normal human amnion group and human amniotic membrane anastomosis group are shown in Figure 2. Results showed that the curve exponentially changed at the first phase in both groups, and strain was 0–2%, respectively. At the second phase, the curve was directly proportional to elastic deformations; strain was 2.1–8.0% in the normal human amnion group and 2.1–6.0% in the human amniotic membrane anastomosis group. At the third phase, the stress-strain curve, which was similar to the first phase, exhibited elastic deformations; strain increased to 8.1–14.0% in the normal human amnion group and 6.1–10.0% in the human amniotic membrane anastomosis group. However, specimens exhibited deformations and lost bearing capacities at the fourth phase; strain was 14.1–16.9% in the normal human amnion group and 10.1–11.5% in the human amniotic membrane anastomosis group. Changes in the stress- strain curves of the normal human amnion group and human amniotic membrane anastomosis group indicated good elasticity and plasticity in the human amnion.

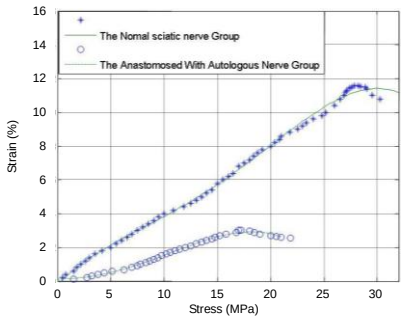

Figure 1 The tensile stress-strain curve of specimens in normal sciatic nerve group and autologous nerve anastomosis group.

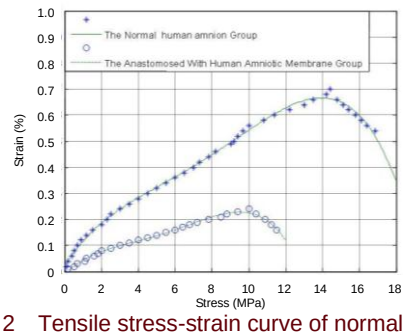

Figure 2 Tensile stress-strain curve of normal human amnion and amniotic membrane anastomosis on injured sciatic nerve (*Y* axis represents strain, *X* axis represents stress).

Formula of stress-strain functional relations

The dependency relationship between variables and measurement errors were measured to establish a formula for a stress-strain functional relationship, as follows:

Normal sciatic nerve group:

$\sigma(\varepsilon) = 0.024e^5 - 0.0411e^4 + 0.6113e^3 - 0.1614e^2$

Autologous nerve anastomosis group:

$\sigma(\varepsilon) = 0.0002e^5 + 0.0148e^4 + 0.0068e^3 + 0.1022e^2$

Normal human amniotic membrane group:

$\sigma(\varepsilon) = 0.0016e^5 - 0.0158e^4 + 0.1060e^3 + 0.0250e^2$

Human amniotic membrane anastomosis group:

$\sigma(\varepsilon) = 0.0024e^5 - 0.0171e^4 + 0.0690e^3 - 0.0104e^2$

Histological changes in cross-sections of normal sciatic nerve and sciatic nerve specimens following anastomosis

Scanning electron microscopy showed that normal sciatic nerve fibers were orderly arranged, axons were surrounded by a myelin sheath, and the endoneurium-encased myelin sheath and axons were clear and visible on the nerve fiber surface (Figure 3A). Following anastomosis with autologous nerve anastomosis, the injured sciatic nerve fibers were disorderly arranged, and the surface connective tissue, endoneurium, myelin sheath, and axons morphology changed and even ruptured, thus blocking the basilar membrane cavity (Figure 3B).

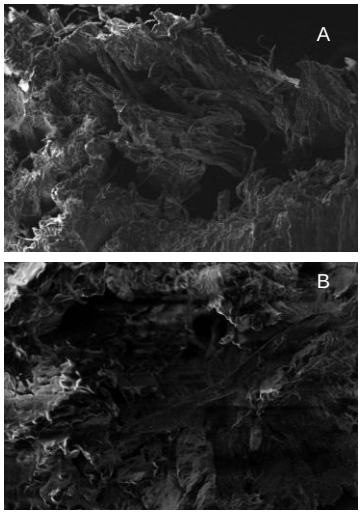

Figure 3 Morphology of sciatic nerve cross-sections in normal sciatic nerve group (A) and autologous nerve anastomosis group (B) in tensile test (scanning electron microscopy, × 2 000).

In the normal human amniotic membrane group, longitudinal fibers were neatly arranged (Figure 4A). In the human amniotic membrane anastomosis group, the longitudinal cross-section fibers were disorderly arranged at the ends after the tensile test (Figure 4B).

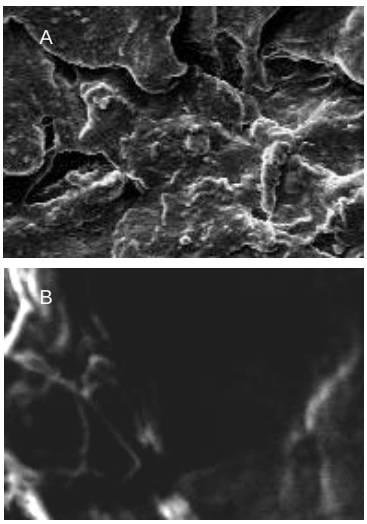

Figure 4 Morphology of human amnion cross-sections in normal human amnion group (A) and amniotic membrane anastomosis group (B) in tensile test (scanning electron microscopy, × 2 000).

DISCUSSION

Scanning electronic microscopy results from the present study revealed an ordered arrangement of normal sciatic nerve fibers and clear axons. Following injury to the sciatic nerve, the nerve fibers were disorderly arranged, and the connective tissue, endoneurium, myelin sheath, and axons on the nerve fiber surface were altered. Normal human amniotic membrane fibers were arranged in rows, while longitudinal cross-section fibers of human amniotic membrane were disorderly arranged and structural changes appeared following anastomosis. Under loading conditions, the specimen microstructures were damaged if the load was greater than the bearing capacity.

Tensile test results showed that 6.71 MPa stress was the limit for sciatic nerve physiological stress in the normal sciatic nerve group, as well as the critical point of sciatic nerve injury. When stress reached 11.63 MPa, the sciatic nerve is ruptured. In the autologous nerve anastomosis group, injured sciatic nerve stress was 2.29 MPa, which was also the critical point of sciatic nerve injury; when stress reached 3.04 MPa, the sciatic nerve was damaged. The limit value of normal human amniotic membrane physiological stress was 0.42 MPa, when the stress reached 0.69 MPa, and human amnion specimens appeared deformed, the cross-sections tapered, and bearing capacity was not available, although the specimens exhibited sufficient plasticity and elasticity. In the human amniotic membrane anastomosis group, 0.17 MPa stress was the critical point of sciatic nerve injury, and the specimens were damaged at 0.23 MPa stress. These experimental findings indicated that sciatic nerve injury models anastomosed with human amniotic membrane exhibited specific strain and stress characteristics. Results showed that physiological stress limits of the normal human amnion and stress leading to sciatic nerve injury were greater than in the rabbit median nerve, as previously described[12]. This suggested different mechanical properties of nerves among different species. According to a previous study[15], sciatic nerve tensile strength of an undissected male, adult cadaver, which was fixed for 3–10 years, was greater than in the normal human fresh cadaver. These results suggested that a formalin fixation for more than 3 years altered the mechanical properties of the human cadaveric sciatic nerve. Therefore, this is not an ideal specimen for sciatic nerve injury *in vitro*. Fresh cadaver sciatic nerves and peripheral nerve specimens exhibit greater potential for biomechanical studies.

In the present study, the sciatic nerve stress-strain curve initially underwent exponential change in the normal sciatic nerve group and autologous nerve anastomosis group, and this alteration was also visible in the normal human amniotic membrane group and human amniotic membrane anastomosis group.

One-dimensional tensile test is an important method for evaluating anastomosis and comparing mechanical properties of a normal sciatic nerve with a normal human amniotic membrane, a sciatic nerve injury model with autologous nerve anastomosis, and a sciatic nerve injury model with human amniotic membrane anastomosis. However, this method has some limitations, such as race, gender, age, health status, and occupation, even among the same species. The present study utilized fresh sciatic nerve specimens from normal, young, healthy, male Chinese, and human amniotic membrane specimens were collected from healthy female placentas following caesarean sections. The placenta and sciatic nerves were identically preserved and were tested within 24 hours after removal. Prior to the experiment, each specimen underwent repeated loading and unloading 10 times to ensure reliable experimental results.

Results from the present study quantitatively analyzed the mechanical properties of normal sciatic nerves and normal human amniotic membranes, as well as the biochemical properties of a sciatic nerve injury model following anastomosis with human amniotic membrane and autologous nerve. Results showed significant differences in maximum stress and strain between the sciatic nerve and human amniotic membrane. Although tensile mechanical properties were apparently different in the sciatic nerve and amniotic membrane, the human amniotic membrane exhibited good biological characteristics and

biocompatibility[16-17], and human amniotic membrane transplantation has been shown to be an ideal method for repairing sciatic nerve injury[18-19]. Following allograft amniotic membrane anastomosis, the injured sciatic nerve exhibited good rheological properties, which was conducive to injured nerve anastomosis and reconstruction, suggesting that the human amniotic membrane was an ideal nerve graft. Considering the biological characteristics, biocompatibility, stress relaxation, and creep rheological characteristics of the human amniotic membrane, results from the present study suggest that the crosslinking method was an effective method for improving mechanical properties of human amniotic membranes[14]. In addition, a modified human amniotic membrane could be used as a peripheral nerve graft. Because human cadaveric specimens are difficult to harvest and sciatic nerve specimens are limited, only 10-mm sciatic nerve injury specimens were created. Due to these limitations, further studies are required to conclusively analyze the multiple defect models and variety of stress types on amniotic membranes following transplantation. In conclusion, human amniotic membranes and sciatic nerve specimens exhibit similar tendencies in stress-strain alterations, good elastic properties, and certain strain and stress characteristics.

MATERIALS AND METHODS

Design

A comparative, observational, biomechanical experiment.

Time and setting

Experiments were performed from December 2009 to August 2011 in Mechanics Experimental Center of Jilin University in China.

Materials

Fresh cadavers of eight, male, adult, normal Chinese, who died due to acute head trauma, with an average age of 25–30 years, as well as 16 bilateral sciatic nerve gluteus maximus muscle specimens, were provided by the Department of Anatomy, Jilin University School of Medicine, China. Human amniotic membranes were derived from 10 healthy female placentas from patients, who underwent cesarean sections at Jilin University China-Japan Friendship Hospital. According to the *Administrative Regulations on Medical Institution*, issued by the State Council of the People's Republic of China[20], all cases were informed of the human amniotic experimental program and provided written informed consent. Human amniotic membranes were preserved in 4°C saline immediately upon removal.

Methods

Amnion specimen preparation

Amniotic epithelial cells were removed using the ammonia digestion method[21]. The human amniotic membranes were then processed into 15 multilayer coiled specimens with an 8.8–11.4 mm diameter and 10 mm length, according to previously described methods[22], and served as the tensile specimens for the human amnion anastomosis group. An additional 15 specimens 25 mm in length and 9.8–10.2 mm in diameter were used as test specimens for the normal human amniotic membrane. Two multilayer, coiled, amniotic specimens, 8.8–11.4 mm in diameter and 10 mm in length, were used for observation of normal human amniotic specimen cross-sections.

Sciatic nerve specimen preparation

Sixteen bilateral sciatic nerve gluteus maximus muscle specimens, which were harvested within 20 hours after death, were preserved at –20°C, then thawed at room temperature, and finally cut into specimens 25 mm in length and 8.6–11.4 mm in diameter. Sciatic nerve specimens from the autologous nerve anastomosis group and human amniotic membrane anastomosis group were intermediately cut using a S-5 sterile plastic-handle surgical knife (Huaian Uniecom Medical Supplies, Xuyi, Jiangsu Province, China), thereby establishing 10-mm sciatic nerve injury models. The mutilated models were sutured using 7-0 nylon line (Qingdao Nike Medical Material, Qingdao, China). Following anastomosis, a sciatic nerve injury model was successfully established according to accurate contraposition. Specimens < 8.6 mm in diameter were removed. All models were established by a clinical physician, who used 8 stitches for each sample.

Tensile test

The automatic control electronic universal testing machine was provided by Changchun Institute of Testing Machine, China. Specimen length and diameter were measured using a reading microscope (Changchun Third Optical Instrument Factory, Changchun, China). Specimens from the normal sciatic nerve group, autologous nerve anastomosis groups, and human amnion anastomosis group were 25 mm long and 8.6–11.4 mm in diameter. Specimens from the normal human amniotic membrane group were 25 mm long and 9.8–10.2 mm in diameter. Each specimen was preset by 10 repeated loading and unloading. The experimental temperature was close to normal human body temperature (36.5 ± 1.0°C). Four groups of specimens were placed in the

testing machine, with a loading speed of 5 mm/min. To maintain humidity in the specimens, a liquid spray was continuously administered. Upon experimental completion, the following indices were automatically generated from the automatic control electronic universal testing machine (Changchun Institute of Testing Machine, China): maximum load, maximum displacement, maximum stress, maximum strain, elastic limit load, elastic limit stress, and stress-strain curve.

Scanning electron microscopic observations of sciatic nerve and human amnion cross-sectional ultrastructure

One normal sciatic nerve and one injured sciatic nerve were randomly selected and trimmed into 5-mm segments, pre-fixed with 4% glutaraldehyde, post-fixed with 1% osmium tetroxide, dehydrated with acetone gradient, dried at the critical point, undyed, and treated with a vacuum-spray coating. Nerve cross-sections were observed under a scanning electron microscope (Zeiss, Carle, Germany) to detect changes of nerve cells, myelin sheath, axons, and nerve basement membrane structure.

Following anastomosis, one normal human mniotic membrane and one injured human amniotic membrane were randomly selected and trimmed into 5-mm segments, pre-fixed with 4% glutaraldehyde, post-fixed with 1% osmium tetroxide, dehydrated with acetone gradient, dried at the critical point, undyed, and treated with a vacuum-spray coating. Nerve cross-sections were observed under a scanning electron microscope (Carl Zeiss, Jena, Germany) to detect changes in nerve cells, myelin sheath, axons, and nerve basement membrane structure.

Statistical analysis

Data were statistically analyzed using SPSS 16.0 software (SPSS, Chicago, IL, USA). Data differences between groups were compared using one-way analysis of variance and Scheffe's method. $P < 0.05$ was considered significantly different. The stress-strain functional relationship formula was established using the normalized analysis method.

Author contributions: Chuangang Peng and Qingsan Zhu had full access to the study concept and design. Chuangang Peng, Qiao Zhang, and Qi Yang integrated data. Chuangang Peng was responsible for statistical processing and statistical analysis, as well as drafting of the manuscript. Qingsan Zhu was responsible for manuscript validation.

Conflicts of interest: None declared.

Ethical approval: This pilot was approved by Ethics Committee, China-Japan Friendship Hospital, Jilin University, China.

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