

Original Article

Repair method of peripheral nerve defect in traumatic orthopedics

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Received December 14, 2017; Accepted February 2, 2018; Epub April 15, 2018; Published April 30, 2018

Abstract: Objective: To compare the effects of three different repair methods on the treatment of peripheral nerve injury in traumatic orthopedics. Methods: Twenty-four rats with similar body mass were randomly divided into four groups (A, B, C, and D). To establish a model of nerve injury, their right thighs were excised, and sciatic nerves were discontinued 15 mm. In group A (control group), autologous nerve transplantation was performed and the excised nerve was turned 180° and implanted into the defect part. Group B, C, and D served as the experimental group. Group B received allogeneic nerve graft and sciatic nerve segments excised from group C were suture-implanted to the two broken ends of group B. In group C, a nerve lengthening device was used to lengthen the broken ends of the sciatic nerve, and neural end-to-end suture was carried out. In group D, tissue engineering technology was used to implant poly(lactic-co-glycolic acid) nerve conduit stent at the broken ends. Four weeks after surgery, the control group and the experimental group were respectively subjected to nerve electrophysiological tests such as somatosensory evoked potential (SEP), muscle action potential (MAP) and motor nerve conduction velocity (NCV). Also, the general morphology of the experimental animals was observed. Eight weeks after operation, histological detection was performed for all the subjects and the number and area of myelinated nerve fibers was detected and observed, also, the triceps surae wet weight recovery rate was measured and calculated. Results: After 4 weeks, all the rats showed limb deformity, and had varying degrees of toe swelling. Twelve weeks after the operation, there was no adhesion in the nerve graft junction of group A and group B, and the peripheral neural vascular net of the nerve defect junction in group C and group D was clear. Compared with group A, the latency of SEP and MAP in group C and group D were significantly shortened with higher amplitude, and the NCV increased with statistically significant difference ($P < 0.05$). However, compared with group A, the latency of SEP, MAP in group B was increased with lower amplitude, and NCV decreased with significant difference ($P < 0.05$). Eight weeks after the operation, the number of myelinated nerves at the repair site of nerve injury broken ends in group C and D was more than that in group A; there was less in group B, with the order of $D > C > A > B$, and the difference was statistically significant ($F = 14.37$, $P < 0.05$). The area of myelinated nerve arranged in order: $D > C > A > B$, with significant difference ($F = 31.52$, $P < 0.05$). In addition, the rat triceps surae wet weight recovery rate was higher in group C and D than that in group A, and it was lower in group B than in group A and the difference was statistically significant ($P < 0.05$). Conclusion: In the repair methods of peripheral nerve in traumatic orthopedics, the new methods, nerve lengthening and tissue engineering technology, are expected to replace nerve graft.

Keywords: Traumatic orthopedics, peripheral nerve, repair method

Introduction

Traumatic orthopedic peripheral nerve injury is very common in the Department of Traumatology. About 3-8% people in the world have peripheral nerve injury [1]. Studies have shown that without prompt treatment or repair after injury, muscular atrophy can occur in surrounding muscles, leading to affected limb dysfunction or paralysis, seriously influencing the quality of life in patients [2]. Peripheral nerve injury

is divided into defects and no defects between broken ends. Direct suture can be performed for no defects, mainly relying on microsurgery technical epineurial or perineurial suture. Therapeutic methods such as nerve transplantation or tissue engineering are used for nerve injury with defects. Research and clinical practice have shown that autologous peripheral nerve graft is the gold standard for the treatment of traumatic peripheral nerve defects [3]. However, there are many influencing factors for the repair

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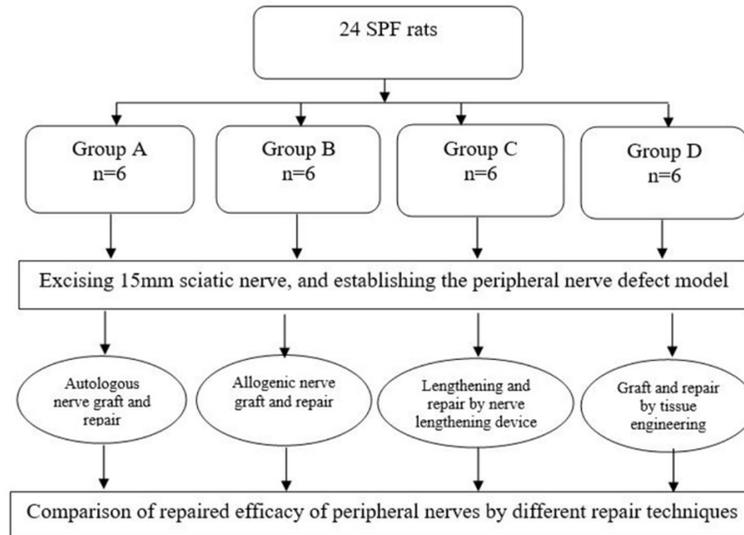


Figure 1. Experimental flow chart of nerve injury repair.

of peripheral nerve injury [4-6]. How to promote the regeneration of defective peripheral nerve and restore its function to the maximum extent have always been a hotspot and difficulty in medical research. In this paper, we focus on the reconstruction of defective peripheral nerves; animal models were established for research and comparison, hoping to provide a reference for the repair of peripheral nerve defects in clinic.

Materials and methods

Animal grouping and model building

Twenty-four male Sprague-Dawley rats (specific pathogen-free (SPF) experimental animals, same type) weighing 250 ± 20 g were randomly divided into 4 groups, with 6 rats in each group, and the models were established with right sciatic nerve cut off. Group A received autologous nerve transplantation and group B received allogeneic nerve graft. A nerve lengthening device was used to lengthen the nerve in group C and end-to-end suture was performed. Group D was dealt with tissue engineering technology. **Surgical methods:** Rats were anesthetized with 1% pentobarbital sodium (40 mg/kg) by intraperitoneal injection. Revealing the right sciatic nerve after anesthesia, the sciatic nerve was cut off at the midpoint. In group A, the excised nerve was turned 180° and implanted into the defect part as a control group. In group B, the sciatic nerve segment resected from group C was sutured to the broken ends of nerve defect.

In group C, suture was performed after the broken ends of sciatic nerve were lengthened by the nerve lengthening device. In group D, tissue engineering technology was used to implant polylactic-co-glycolic acid nerve conduit stent at the broken ends. Experimental flow chart is shown in **Figure 1**.

Detection index

General morphology: Four weeks after surgery, the general morphology of rats was observed. After 12 weeks, the sciatic nerve was revealed to observe the gross appearance of the suture.

Electrophysiological examination: Four weeks after the operation, the electrodes were placed at 5 cm away from both suture sides or stent sides of the nerve defect. The somatosensory evoked potentials (SEP) and muscle action potentials (MAP) were measured by electromyography, and the nerve conduction velocity (NCV) was also measured [7].

Histological detection: Eight weeks after operation, 0.5 cm proximal and distal nerve tissues at graft location of nerve defect were excised and stained with osmic acid for observation under light microscope. The myelinated nerve fibers were then counted in the unit field of vision [8]. The mean area of myelinated fibers was calculated as the average of all regular shaped areas of myelinated fibers in the field of view.

Triceps wet weight recovery rate: After the nerve electrophysiological examination, bilateral triceps were removed from the starting to the ending points of rat triceps surae and the fat and fascia on the muscle surface were peeled off for weighing by an analytical balance, and the triceps wet weight recovery rate was calculated [9].

Statistical analysis

SPSS19.0 software was used for statistical analysis, and the data were expressed as mean \pm standard deviation. Multiple group comparisons were conducted using an F test. The difference was significant when $P < 0.05$.

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Figure 2. General morphology of each group of rats 4 weeks after surgery.

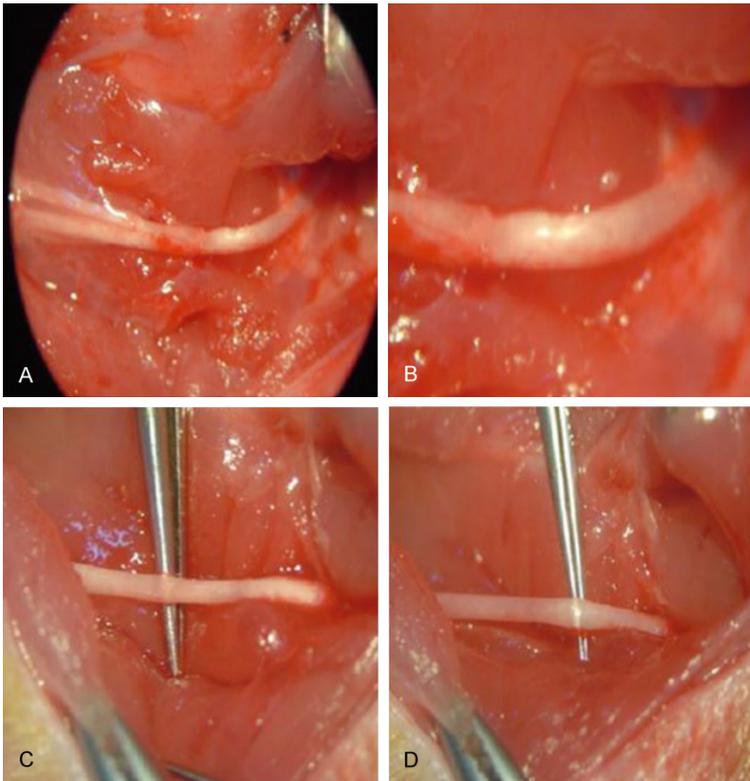


Figure 3. General morphology of sciatic neural graft location 12 weeks after surgery.

Results

Observation of general morphology

Four weeks after operation, all the four groups of animals showed limb deformity as well as red and swollen toes, and it was lighter in group A

than in the other three groups. See **Figure 2**. Eight weeks after operation, red and swollen was improved in all the 4 groups, and the improvement degrees in group C and D were better than that of group B when compared with group A. After 12 weeks, the nerve transplant in group A and group B showed no significant adhesions with the surrounding tissues, and the blood vessels at the junction of the two broken ends were basically clear. The vascular net at the suture end of lengthened neural segment in group C was clearly visible. In group D, the vascular network of the surrounding surface of the stent-graft at the nerve defect was clear. See **Figure 3**.

Neural electrophysiological change

Four weeks after surgery, compared with group A, the latency of SEP and MAP in group C and D were significantly shortened with higher amplitude, and the NCV increased with a statistically significant difference ($P < 0.05$). However, compared with group A, the latency of SEP and MAP in group B increased with lower amplitude, and NCV decreased with significant difference ($P < 0.05$). See **Table 1**. The latency and amplitude of MAP in each group is shown in **Figure 4**.

The number and average area of myelinated fibers

Eight weeks after surgery, the number and average area of medullary fibers in each group was shown in **Table 2**. The order of the number of myelinated fibers in each group was $D > C > A > B$. Compared with group A, the differences in group B, C and D were statistically significant ($F = 14.37$, $P < 0.05$). The order of the aver-

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Table 1. Comparison of latency and amplitude of SEP and MAP as well as changes of NCV in each group 4 weeks after surgery ($\bar{x}\pm\text{sd}$)

Group	Group A	Group B	Group C	Group D	P
Case	6	6	6	6	
SEP Latency (ms)	22.12±1.56	26.35±2.16*	17.22±5.49*	15.63±4.26*	<0.05
SEP Amplitude (μV)	9.36±3.44	4.32±2.56*	22.32±8.12*	27.32±9.56*	<0.05
MAP Latency (ms)	14.24±3.67	16.17±4.72*	9.85±3.47*	8.43±2.38*	<0.05
MAP Amplitude (μV)	34.55±4.91	25.68±3.22*	44.04±7.35*	48.32±9.63*	<0.05
NCV (m/s)	18.03±3.26	16.53±2.15*	21.31±2.76*	26.12±2.58*	<0.05

Note: Compared with group A, in group B, C and D, *P<0.05. SEP, somatosensory evoked potential; MAP, muscle action potential; NCV, motor nerve conduction velocity.

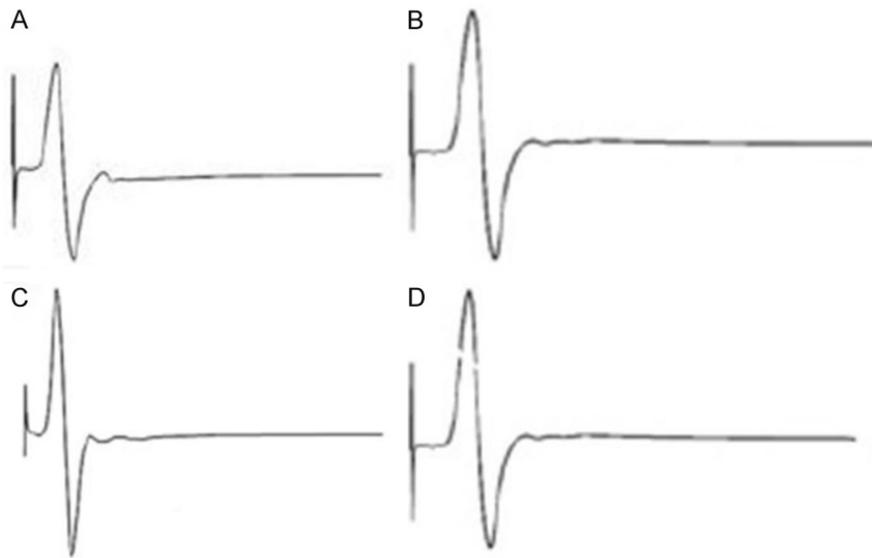


Figure 4. Latency and amplitude of muscle action potential in each group four weeks after surgery.

age area of myelinated fibers was D>C>A>B. Compared with group A, the differences in group B, C, and D were statistically significant (F=31.52, P<0.05).

Triceps surae wet weight recovery rate

The triceps surae wet weight recovery rate in group C and group D was higher than that in group A (P<0.05), while that of group B was lower than that of group A (P<0.05). See **Table 3**.

Discussion

The peripheral nerve is a nervous system other than the central nervous. Peripheral nerve injury is one of the frequently-occurring diseases in traumatic orthopedics, which is mainly caused by the damage of nerve structure or function

due to traction, squeezing or incision of peripheral nerves, leading to disorder and deletion of limb sensation and function [10]. Due to the relatively slow rate of repair and reconstruction of traumatic peripheral nerve and the feature of incomplete repair, the repair and reconstruction of traumatic orthopedic peripheral nerve injury has been the focus and difficulty of clinical research [11, 12]. Currently, autologous nerve transplantation is considered the gold standard for the treatment of peripheral nerve injury. However, in autologous nerve repair, nerve sources are subject to greater restrictions, and it is

easy to cause new damage to patients [13, 14]. Researchers have done some studies to determine how to effectively promote repair of nerve injury [15-17]. More effective repair methods have been tried for treating patients with nerve injury [18-20]. Compared with the previous studies, this paper mainly focuses on the comparison of different repair methods of peripheral nerve in traumatic orthopedics, and more intuitively analyzes its effective repair methods.

The results of this experiment showed that the gross morphological observation 4 weeks after operation revealed lighter red and swollen in group A than in the other three groups, indicating that the autologous nerve graft method has high biocompatibility. The phenomena of other three groups indicate that there is a certain level of exclusion in body to the allogeneic

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Table 2. Distal medullary fiber counts and mean area of medullary fibers in each group ($\bar{x} \pm sd$)

Group	Case (n)	Number of myelinated fiber (piece)	Average area of myelinated fibers (μm^2)
Group A	6	218.224 \pm 13.415	868.476 \pm 13.443
Group B	6	146.347 \pm 12.364*	765.347 \pm 11.089*
Group C	6	221.403 \pm 12.676*	853.145 \pm 12.226*
Group D	6	226.136 \pm 10.378*	876.347 \pm 10.532*
F		14.37	31.52
P		<0.05	<0.05

Note: Compared with group A, in group B, C and D, *P<0.05.

Table 3. Triceps surae wet weight recovery rate in each group ($\bar{x} \pm sd$)

Group	Case (n)	Triceps surae wet weight recovery rate (%)
Group A	6	78.341 \pm 1.455
Group B	6	76.482 \pm 2.236*
Group C	6	78.513 \pm 2.647*
Group D	6	78.916 \pm 1.486*

Note: Compared with group A, *P<0.05.

implantation of the nerve or tissue. However, after 12 weeks, there was no adhesions in both group A and B, which indicated that autograft or allograft could repair the nerve fracture after a certain period of time. The peripheral neural blood vessels at the broken ends were clear in group C and D, indicating that the repair effect was favorable. In this study, electromyography was used to detect the results of rat SEP and MAP treated with a different repair method, and to measure the NCV for direct reflection of nerve conduction. The experimental results showed that in the SEP and MAP test, the NCV in group C and D was significantly better than that in group A and B. The latency and amplitude of SEP and MAP in group C and D were significantly better than that in group A, however, the SEP, MAP and NCV in group B were weaker than those in group A with statistically significant difference (P<0.05). This result indicates that the nerve conduction in group C and D is better than that in group A and B. Compared with autologous nerve transplantation, nerve conduction in the allogenic nerve transplantation is weak with certain level of exclusion. Eight weeks after the operation, observation by optical microscopy found that group C and D had a larger number and increased area of myelinated nerve fibers, sug-

gesting that repair methods of nerve lengthening device and tissue engineering technology forming new nerve fibers had achieved a better nerve repair effect. In addition, the triceps surae wet weight recovery rate of the experimental rats in group C and D were 78.513 \pm 2.647 and 78.916 \pm 1.486, respectively, slightly higher than that of group A and B, indicating that it is ideal to perform end-to-end suture after nerve lengthening by the device and to use tissue engineering stent for

the repair of peripheral nerve defects. By the above two ways, preferable nerve regeneration can be achieved. In this study, there was no significant difference in the experimental data between group C and D, whereas both of them have their advantages in clinical realization and operability. However, nerve lengthening device method has no need for nerve graft and external stent implantation, but it needs secondary surgery (anastomosis after lengthening), so the indications of this technique need to be explored. At present, tissue engineering technology is not limited by donor sources and does not require a second surgery. However, its clinical application is based on the comprehensive consideration of scaffold, seed cells and growth factors, and the limitations still need to be studied clinically.

In summary, with the rapid development of related disciplines, such as tissue engineering and materials science, emerging orthopedic traumatic peripheral nerve repair techniques will achieve even greater effects and can be widely used to replace autologous nerve graft for clinical treatment. Due to restrictions of time and quantity of experimental models, the experimental data in this paper have some limitations and will be supplemented in later experimental research.

Disclosure of conflict of interest

None.

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