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*Original Article*

# Rat sciatic nerve regeneration through type I collagen-derivedartificial tubular implants

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**Abstract:** Objective: To investigate the efficacy of a type I collagen-derived artificial tubular implant on sciatic nerve regeneration. Materials and Methods: Sprague-Dawley rat sciatic nerve injury model was built by cutting a 5.0 mm defect on the sciatic nerve of the right thigh. Rats with sciatic nerve injury were treated with type I collagen-derived artificial tubular implants (Group A), autologous common carotid artery implants (Group B), or autologous sciatic nerve implants (Group C). Two control groups were also included: Group D (mock injury group: same surgery was performed on the rats without generation of nerve injury) and Group E (normal rats without surgery). After treatment, all rats were monitored for change in claw reflection, nerve conduction velocity, evoked potential, latency and histological assessment on week 2, 4, 8 and 12. Results: Comparing to Group B, Group A showed faster claw reflection recovery as well as higher nerve conduction velocity and volatility and shorter latency time. Also, histological assessment also revealed that Group A demonstrated less gastrocnemius wet weight loss than Group B. However, Group C was better than Group A in all the above assessments. Conclusion: Type I collagen-derived artificial tubular implantation is a feasible way for sciatic nerve regeneration, but its efficacy is still not as good as autologous nerve implantation.

**Keywords:** Sciatic nerve regeneration, artificial tubular implant, type I collagen

## Introduction

Due to trauma or deliberate surgical resection, peripheral nerve injury, a common clinical complication, represents a major cause of morbidity and disability worldwide. In general, about 2.8% of the trauma patients would suffer from peripheral nerve injuries and many of them would have life-long disability [1]. Usually, nerve graft implantation is required to bridge the nerve defect. Autologous nerve graft has been considered as the "gold standard" technique for treatment of nerve defect, which requires nerve autograft harvested from another site of the same patient [2]. Although the autologous nerve graft implantation often provides good outcome, there are some serious limitations for this method, including donor site morbidity, secondary deformities, tissue availability and possible differences in tissue structure and size [2-4]. Besides autologous grafts, allografts have also been tried in the nerve defect treatment. However, due to immunosuppression, the results have been not satisficing [5-7]. Therefore, novel techniques for treatment on peripheral nerve injuries are still needed.

Beside the conventional autologous and allogeneic nerve implantation techniques, artificial nerve implants have offered an alternative option with promising results [8, 9]. Artificial nerve grafts, usually in the form of nerve tubes or conduits, can be made of many types of materials, including type I collagen, chitosan and polyglycolic acid etc [10-13]. These types of artificial implants are made of bioresorbable materials that can be biodegraded in the body after its temporary function as nerve scaffolds [13]. Moreover, their unique structure could allow sufficient nutrition infiltration into the inner cavity of the implants [11, 14]. Their characteristics in biocompatibility and biodegradation render these artificial nerve implants very strong candidates for peripheral nerve regeneration.

**Table 1.** Claw reflection recovery time after surgery (n=12)

| Group | Recovery initiation time (Day) | Total recovery time (Day) | Average recovery time (Day) |
|---|---|---|---|
| A | 45 | 52 | 48.50±3.67[a] |
| B | 55 | 63 | 58.08±3.78 |
| C | 37 | 45 | 42.00±3.49[b] |
| D | NA | NA | NA |
| E | NA | NA | NA |

[a], $P < 0.05$ (vs. Group B); [b], $P < 0.05$ (vs. Group A and B); NA: Not applicable.

In this study, using Sprague-Dawley (SD) rats as a model, we have investigated the feasibility of a type I collagen-derived artificial implant for sciatic nerve regeneration.

## Materials and methods

### *Ethical statement and animals*

All the protocols involving animals were reviewed and approved by the institutional ethic review board and performed in accordance with the Provincial Guidelines on Animal Experimentation. Male SD rats (160-200 g) were purchased from Shanghai s & p-shall kay laboratory animal co., LTD (Batch No. 2008001645435) and hosted in the animal center of Shanghai City Public Health Center with food and water provided.

All rats were randomly divided into 5 groups: Group A-E. Group A-C: rats with sciatic nerve injury were treated with type I collagen-derived artificial tubular implants (Group A), autologous common carotid artery implants (Group B), or autologous sciatic nerve implants (Group C). Group D (mock injury group: same surgery was performed on the rats without generation of nerve injury) and Group E (normal rats without surgery) was served as controls.

### *Surgical procedure*

All animals were anaesthetized by 10% chloral hydrate solution (300 mg/kg) before surgery. For generation a sciatic nerve defect, a skin incision and underlying muscle splitting were made in the right lateral thigh. A segment of sciatic nerve (about 20 mm) was resected and left a 5.0 mm long defect following retraction of the nerve ends. Subsequently, the nerve defect was bridged by type I collagen-derived artificial implant (Group A), autologous common carotid artery implants (Group B), or autologous sciatic nerve implants (Group C). For the generation of a mock injury (Group D), same surgical procedure was performed to isolate the sciatic nerve but without the introduction of nerve defect.

### *General observation*

After surgery and corresponding treatments, all rats were monitored for changes in their posture and gait, claw reflection, food drop, toe swollenness and skin ulcer.

### *Motor Nerve Conduction Velocity (MNCV) measurement*

The MNCV of the injury sciatic nerve was measured on week 2, 4, 8 and 12 using a noninvasive procedure in the sciatic posterior tibial conducting system in a temperature controlled environment, as previously described [15]. In brief, rats were first anaesthetized with 10% chloral hydrate solution (300 mg/kg) and then the right sciatic nerve was stimulated by single 0.2 ms supra maxial (8 V) pulses at the sciatic notch and then at the Achilles tendon using a myoelectricity evoked potential equipment (Dantec Keypoint). The evoked potentials and proximal latency were recorded. MNCV was calculated by the following formula: MNCV (m/s)=the distance between the two electrodes (in meters)/transduction time (in seconds).

### *Histological assessments*

At week 2, 4, 8 and 12, the adhesion of sciatic nerve to the surrounding tissue was monitored and graded as follows: Grade 0, no apparent adhesion; Grade 1, mild adhesion (adhesion was observed but could be easily separated) and Grade 2: severe adhesion (adhesion was observed and hard to be separated).

At week 2, 4, 8 and 12, gastrocnemius muscles from both sides were harvested and wet weight was measured. The gastrocnemius muscle weight loss was calculated as the weight difference in percentage between the surgery side and healthy side. After weighting, the gastrocnemius muscles were fixed by 10% formalin, embedded in paraffin and cut into 5μm slides. Then HE staining was performed and the sectional areas as well as the diameter of the muscle cells were measured.

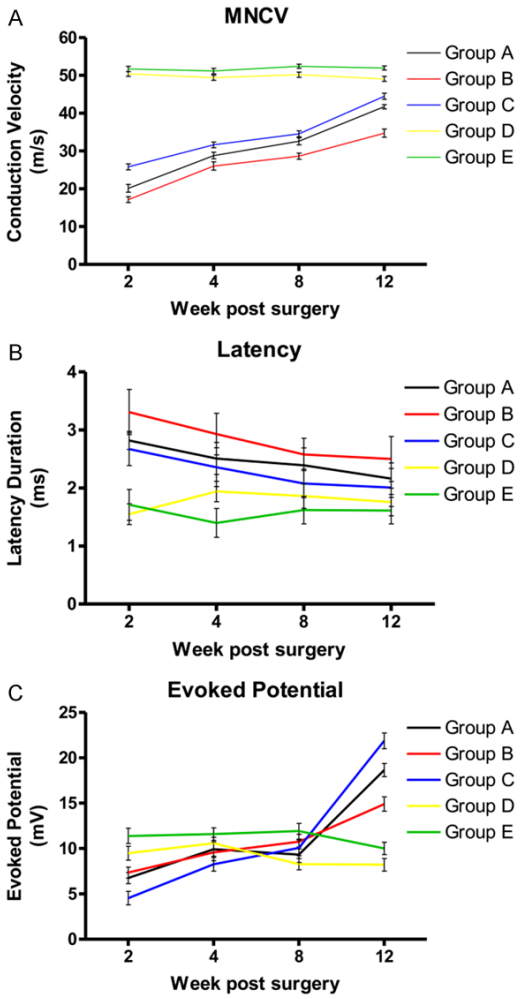


**Figure 1.** MNCV of sciatic nerves after surgery. (A) MNCV, (B) Latency and (C) evoked potential of rat sciatic nerves after surgery at week 2, 4, 8 and 12 were measured. Data shown are mean ± SD of three independent experiments (n=12).

*Statistical analysis*

Measurement data were expressed as mean ± SD in this study. Statistical comparisons were performed using One-way ANOVA plus SNK post hoc. A $P < 0.05$ was considered as statistically significant. All statistical analyses were performed with SPSS17.0 (SPSS. Inc).

## Results

*Microscopic observation*

Due to the sciatic nerve defect, all rats from Group A, B and C exhibited paralysis symptoms including foot drop and disappeared claw reflection. Neurological malnutrition of the skin was observed in all rats from Group A to C since day 12 after surgery and no statistical difference was monitored among these three groups (data not shown). The average claw reflection recovery time for Group A, B and C were 48.05, 58.08 and 42.00 days, respectively (**Table 1**). These data indicated that rats received artificial nerve conduits implantation had faster claw reflection recovery than rats with autologous common carotid artery implantation, but slower than rats with autologous nerve implantation. Since rats in Group D and E had no sciatic nerve injury, no abnormalities were observed.

*Electrophysiological assessment*

At week 2, 4, 8 and 12, the nerve conduction velocity of the surgical repaired sciatic nerves were tested and MNCV, latency as well as evoked potentials were recorded. Rats from Group D and E had the fastest MNCV and shortest latency time and no apparent difference was observed between the two groups, indicating that surgical procedure had little impact on the nerve function (**Figure 1A** and **1B**). Aside from Group D and E, Group C had the fastest MNCV and shortest latency duration, followed by Group A and then Group B (**Figure 1A** and **1B**). The evoked potentials of Group D and E remained relatively constant while the other three groups exhibited gradual increase over time. Among Group A, B and C, Group C showed the highest evoked potential and Group A next while Group B was the lowest of the three (**Figure 1C**). These data implied that sciatic nerve function was better restored in rats with artificial nerve tubular implantation than in autologous common carotid artery implantation. But still, autograft possessed the best repair efficiency among the three.

*Evaluation of gastrocnemius muscle*

At week 2, 4, 8 and 12, the wet weight, cell diameter as well as sectional area were measured on gastrocnemius muscle on both sides of the thigh. As shown in **Table 2**, Group D and E showed no apparent weight loss in gastrocnemius muscle over time while the other three groups showed significantly loss since surgery. Of notice, rats from Group A, B and C all started to gain weight on gastrocnemius muscle since week 4. However, Group C exhibited the fastest weight-gain, which was followed by Group A and then Group B. Similar results were observed

**Table 2.** Gastrocnemius muscle wet weight loss (n=12)

| Group | Week 2 (%) | Week 4 (%) | Week 8 (%) | Week 12 (%) |
|---|---|---|---|---|
| A | 43.65±2.67 | 33.41±2.31 | 57.33±2.61[a] | 65.31±2.48[a] |
| B | 41.39±2.31 | 33.46±2.11 | 50.49±2.09 | 54.37±2.62 |
| C | 44.82±3.31 | 33.43±2.63 | 60.14±2.79[b] | 71.96±2.81[b] |
| D | 97.61±2.91[c] | 98.05±2.73[c] | 98.36±2.85[c] | 97.73±2.48[c] |
| E | 99.37±2.74[c] | 100.06±2.1[c] | 99.84±2.81[c] | 100.03±2.4[c] |

[a], $P < 0.05$ (vs. Group B); [b], $P < 0.05$ (vs. Group A and B); [c], $P < 0.001$ (vs. Group A, B and C).

**Table 3.** Diameter of gastrocnemius muscle cells (n=4)

| Group | Week 4 (μm) | Week 8 (μm) | Week 12 (μm) |
|---|---|---|---|
| A | 28.23±1.47[a] | 24.30±1.33[a] | 21.54±1.79[a] |
| B | 29.19±1.37 | 23.75±1.62 | 18.45±1.63 |
| C | 31.39±1.54[b] | 25.45±1.27[b] | 22.39±1.22[b] |
| D | 46.67±1.73[c] | 47.05±1.63[c] | 48.50±1.39[c] |
| E | 47.02±1.62[c] | 49.62±1.63[c] | 49.97±1.47[c] |

[a], $P < 0.05$ (vs. Group B); [b], $P < 0.05$ (vs. Group A and B); [c], $P < 0.001$ (vs. Group A, B and C).

**Table 4.** Sectional area of gastrocnemius muscle cells (n=4)

| Group | Week 4 ($\mu m^2$) | Week 8 ($\mu m^2$) | Week 12 ($\mu m^2$) |
|---|---|---|---|
| A | 695.96±19.87[a] | 426.33±23.25[a] | 346.41±22.68[a] |
| B | 698.93±19.86 | 411.17±22.82 | 308.76±23.19 |
| C | 775.58±25.34[b] | 436.93±22.72[b] | 386.49±22.58[b] |
| D | 1818.18±21.53[c] | 1881.36±22.82[c] | 1863.39±26.68[c] |
| E | 1928.04±31.86[c] | 1921.58±33.28[c] | 1928.18±31.84[c] |

[a], $P < 0.05$ (vs. Group B); [b], $P < 0.05$ (vs. Group A and B); [c], $P < 0.001$ (vs. Group A, B and C).

when the muscle cell diameter and sectional area of gastrocnemius muscle were determined (**Tables 3** and **4**).

The regenerated nerves as well as tissue inflammation were determined by HE staining. As shown in **Figure 2**, Group D and E had the most nerve fiber cells and these cells were arranged in order without obvious infiltration of inflammation cells. Contrary to the above two groups, the other three groups all demonstrated certain degree of nerve fiber loss and inflammation. Among the three groups, Group A had more properly arranged nerve fiber cells and less inflammation cells than Group B, which, however, still slightly inferior to Group C.

*Adhesion of regenerated nerve to surrounding tissue*

No adhesion was observed in Group E since no surgical procedure was performed. On the other hand, different degrees of adhesion were monitored in the other four groups including the mock surgery group (Group D). The adhesion rates of Group A to D were 50.0%, 91.7%, 66.7% and 41.7%, respectively (**Table 5**). These data indicated that treatment with artificial nerve tubular implants exhibited slightest adhesion of nerve to surrounding tissue (Supplementary).

## Discussion

Autologous nerve implantation has been considered the primary therapy for peripheral nerve injury and it exhibits about 50% functional nerve repair in patients [16, 17]. However, it also has some limitations including allograft availability, donor site morbidity, and adhesion of nerve to surrounding tissue [18]. Consequently, considerable effort has been made to develop alternative treatments and the adoption of artificial nerve grafts using bioresorbable materials has been one of them. A big variety of bioresorbable materials have been investigated for nerve tissue engineering applications, such as chitosan, alginate, polyglycolic acid, poly-3-hydroxybutyrate and type I collagen [19-21]. In the current study, we examined the feasibility of a type I collagen-derived artificial nerve tubular implants for nerve defect treatment. Our results have showed that the adhesion of nerve to surrounding tissue was significantly decreased with use of this artificial nerve conduit. However, the nerve defect repair efficacy of the novel artificial conduit was still not as good as autologous nerve implants.

Growth factors are capable of promote cell growth, proliferation and differentiation. It has been reported that certain growth factors, such as nerve growth factor (NGF), insulin-like growth factor (IGF-I) and IGF-II, could promote nerve regeneration [22]. In addition, longitudinal biomaterial filaments have also been used into artificial nerve tubular implants to guide nerve regeneration [23, 24]. Although beyond the scope of the current study, it would be very interesting to investigate whether the combina-

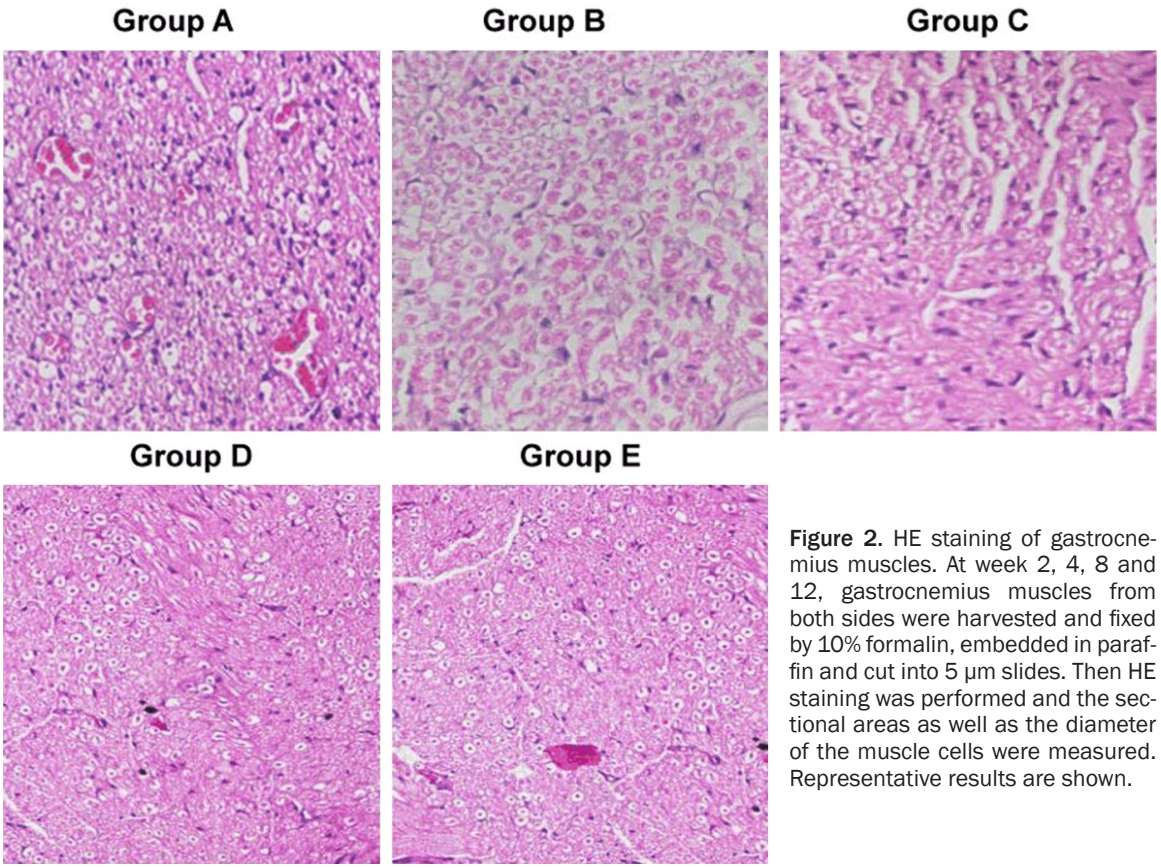


**Figure 2.** HE staining of gastrocnemius muscles. At week 2, 4, 8 and 12, gastrocnemius muscles from both sides were harvested and fixed by 10% formalin, embedded in paraffin and cut into 5 μm slides. Then HE staining was performed and the sectional areas as well as the diameter of the muscle cells were measured. Representative results are shown.

**Table 5.** The adhesion grade of sciatic nerve to the surrounding tissue (n=12)

| Group | Grade 0 | Grade 1 | Grade 2 | Average adhesion (%) |
|---|---|---|---|---|
| A | 6 | 4 | 2 | 50.0 |
| B | 1 | 4 | 7 | 91.7 |
| C | 4 | 4 | 4 | 66.7 |
| D | 7 | 2 | 3 | 41.7 |
| E | 12 | 0 | 0 | 0 |

tion of type I collagen-derived artificial nerve implants with growth factors and/or longitudinal biomaterial filaments could offer better results in nerve regeneration.

Motor nerve defect could lead to denervation of a target muscle and consequently result in muscle fiber decrease in size and weight and atrophy. This situation could be stopped if the muscle is reinnervated [10, 25, 26]. Therefore, measurement of muscle weight loss and size is an effective way to determine nerve regeneration. In our study, the size and weight of the gastrocnemius muscle was measured to represent sciatic nerve regeneration efficiency. Our results showed that artificial nerve conduit promoted the weight gain of the gastrocnemius muscle, but it was still slower than using autologous nerve implants.

In conclusion, our findings in the current study revealed that type I collagen-derived artificial tubular implantation is a feasible way for sciatic nerve regeneration, but its efficacy is still not as good as autologous nerve implantation. Future improvements on this artificial nerve conduit are required before its use in preclinical and clinical studies.

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## Disclosure conflict of interest

None.

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## Supplementary

Beside the conventional autologous and allogeneic nerve implantation techniques, artificial nerve implants have offered an alternative option with promising results. Artificial nerve grafts, usually in the form of nerve tubes or conduits, can be made of many types of materials, including type I collagen, chitosan and polyglycolic acid etc. These types of artificial implants are made of bioresorbable materials that can be biodegraded in the body after its temporary function as nerve scaffolds. Moreover, their unique structure could allow sufficient nutrition infiltration into the inner cavity of the implants. Their characteristics in biocompatibility and biodegradation render these artificial nerve implants very strong candidates for peripheral nerve regeneration.

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