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Original Article

Efficacy of autologous epineurium small gap coaptation combined with platelet-rich plasma, nerve growth factor, and nerve fragments in the repair of damaged peripheral nerves

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Abstract: Purpose: To explore the efficacy of platelet-rich plasma (PRP) combined with autologous epineurium small gap coaptation in the repair of damaged peripheral nerves. Methods: Fifty rabbits were randomly divided into five groups. The control group was treated using the traditional suture method. The remaining four groups were treated using the autologous epicardial small gap model. The autologous epithelium bridged the small gap in experimental group A, and 0.4 mL of nerve growth factor was injected into the small gap. Growth factor was administered in experimental group B, and an appropriate amount of shredded nerve fragments were added into the small gap in experimental group C. Meanwhile, 0.4 mL of PRP was injected into the small gap in experimental group D. Neuroelectrophysiological and histological assessments were performed 12 weeks after surgery. Results: The nerve conduction velocity in experimental group D was higher than in other groups. Compared to other groups, the nerves of the experimental group had small gaps, as well as no collapse or displacement. The diameter of the regenerative nerve was consistent with that of the normal nerves. In the experimental group, a higher number of myelinated nerve fibers were observed in the small gap, and the arrangement was more regular. Extensive angiogenesis was observed among the nerve fibers. More mature Schwann cells proliferated, and the cytoplasm was clear. Mitochondrial swelling was not obvious, and the epicardium and fascia were intact. Conclusion: PRP can significantly improve the microenvironment in the small gap and promote peripheral nerve regeneration and functional recovery.

Keywords: Autologous epineurium, peripheral nerve injury, platelet-rich plasma (PRP), small gap

Introduction

The repair of peripheral nerve injury is extremely challenging in clinical practice. The traditional suture method cannot achieve accurate and effective docking of the nerve fibers at the distal end of the injured nerve [1], neither can it create a suitable cellular and molecular microenvironment. In some cases, function cannot be completely restored after nerve injury [2]. Several in-depth studies have investigated the mechanism of nerve regeneration, with researchers [3, 4] proposing the use of small gap anastomosis based on the selective regeneration theory. Small gap anastomosis provides a relatively tight seal, allowing peripheral nerve regeneration and preventing the growth of surrounding connective tissues and nerves. Furthermore, the microenvironment created by small gap anastomosis prevents the nerve beam from escaping. Some studies have derived the autologous epithelium required for this procedure from the nerve itself and observed no defects, such as immune rejection. Unfortunately, small gap anastomosis is a costly procedure, but it can be used to bridge small gaps and repair peripheral nerve damage [5] with satisfactory results.

In summary, small gap anastomosis provides a relatively closed microenvironment for peripheral nerve regeneration that prevents the grow-

th of surrounding connective tissue and the escape of nerve bundles. The addition of nerve growth factors (NGFs) and nerve fragments to the small gap significantly promotes peripheral nerve regeneration. However, clinical NGFs are expensive and of a single species, and they are insufficient to repair nerve damage. For this reason, several clinicians have explored multifactor approaches to repairing nerve damage. Platelet-rich plasma (PRP) shows potential in neuroprotective, neurogenic, and neuroinflammatory treatments [6-8], and it accelerates the recovery of sensory and motor nerve-muscle unit functions [9-11] as biological adjuvants for peripheral nerve injuries and neuropathy. Therefore, PRP may be a gold standard treatment for nerve regeneration and neuropathy. The present study aimed to explore the efficacy of PRP combined with autologous epineurium small gap coaptation in the repair of damaged peripheral nerves.

Materials and methods

Animals

Fifty healthy male adult Rex rabbits weighing 2.5-3.0 kg (Animal Experimental Center of Baotou Medical College) were included in the present study. The study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Baotou City, Inner Mongolia, China. All experiments were in accordance with the guidelines of the Chinese Directive 2018, as well as with Chinese national laws. All efforts were made to minimize the suffering and number of animals used.

Experimental equipment and reagents

The following experimental equipment was used: Microdevices (Jiangxi Bangmei Medical Equipment Co. Ltd.), Olympus microscope (Olympus Co., Japan), paraffin slicer (Shanghai Jingxue Scientific Instrument Co. Ltd.), pathological image analyzer (LKB, Swedish), and transmission electron microscope S-3400N (Hitachi High-Technologies Co.).

The following were also utilized: non-invasive microsurgery needle thread (Tangshan Hengchang Medical Equipment Co. Ltd.), 10% chloral hydrate (self-formulation), hematoxylin and eosin (H&E) stain (Beijing Shiji Biological Technology Co. Ltd.), NGF (Sigma-Aldrich), and PRP (preparation obtained from the biochemical center laboratory).

Groups

The rabbits were divided into five groups, each consisting of 10 rabbits, based on the method used to treat the nerves. The sciatic nerves of the rabbits were reattached using different methods. In the control group, traditional detachment of the epicardial anastomosis after sciatic nerve dissection was carried out. In group A, simple autologous epicardial small gap anastomosis was performed. In group B, 0.4 mL of NGF was injected into the small gap. In group C, a nerve bundle was selected, which was then completely removed and cut into nerve fragments; the appropriate nerve fragments were then added to the small gap. In group D, 0.4 mL of PRP extract was injected into the small gap.

Surgery

In total, 10% chloral hydrate was intraperitoneally injected at a dose of 3 mL/kg [12]. After anesthesia induction, the rabbits were placed in the lateral position on a self-made operating table. The animals' skin was prepared and an iodophor was used three times for disinfection. A sterile single was then placed, and the posterior lateral skin was incised. The muscles on both sides were cut open to expose and free the sciatic nerve by about 3 cm. Using a sharp blade, the sciatic nerve trunk was then cut about 2 cm distal to the lower edge of the sciatic nerve to free both the nerve bundle and the nerve at the proximal end of the outer membrane of the severed sciatic nerve trunk. In the same incision, about 1 mm of the epicardium was also freed. Next, the trunk was cut off to prevent damage to the blood vessels on the surface of the epicardium. The same method was used to free the distal epithelium, the nerve bundle, and 3 mm of epithelium, as well as to subsequently cut off the free nerve. The bundle was then inverted to the distal adventitia, and the proximal aneurysm was anastomosed. A 2.0-mm small gap model was established in the autologous epithelium, and six

needles were used similarly during suturing using 9-0 sutures under a surgical microscope. Small needles were used to suture the ends of the small gap and the surrounding tissue. Finally, the muscles and skin were sutured closed. After injecting penicillin for 3 days, one rabbit was kept in a cage and fed daily. The rabbit's cage was cleaned and its litter was changed regularly. The room temperature was controlled at 20°C-25°C. If the animal lost a higher volume of blood than expected during the operation, blood from the ear vein was transfused and intravenous saline was administered to prevent death due to hemorrhagic shock.

Observation indicators and methods

Postoperative assessments revealed autonomic activity in all animals, as well as the condition of the plantar skin. Twelve weeks after surgery, five rabbits were randomly selected from each group. Abdominal anesthesia was induced and the sciatic nerve operation site of the affected limb was exposed. Neuroelectrophysiological examination was performed first, and the conduction speed of the sciatic nerve was recorded. The anastomosis and small gap were observed under the microscope, and the five groups of animals were treated differently. In the control group, the proximal and distal ends of the anastomosis, each measuring 2.5 mm, were cut; in all groups, a 2-mm small gap plus a proximal end and a distal end measuring 1.5 mm were cut. The specimen was fixed using 10% formaldehyde, embedded in paraffin, and subjected to H&E staining to observe nerve regeneration. The remaining animals in each group were then anesthetized. The neuroelectrophysiological test was then performed first, and the conduction velocity of the sciatic nerve was recorded. Next, using immunohistochemistry, the specimen was fixed with glutaraldehyde, and the ultrastructure of ultrathin section uranium lead staining was observed using transmission electron microscopy (EM).

General observations

Postoperative observation was used to record the time of spontaneous recovery of the affected limbs and the healing of the plantar ulcers in each group. The surgical site of the sciatic nerve was exposed, and the regenerative nerves were observed under the microscope.

Electrophysiology detection

Twelve weeks after the operation, the rabbits were placed in left lateral position. The surgical site of the sciatic nerve was fully exposed along the original surgical incision, and a suitable length of nerve was released. The nerve conduction velocity of the sciatic nerve was then measured.

Histological observation

Twelve weeks after the operation, biopsy was performed, and tissue sections were embedded in paraffin. After H&E staining, the density and arrangement of the regenerated nerve fibers were observed under microscope. The regeneration of myelinated nerve fibers, angiogenesis, anastomosis, and small gaps were assessed. Moreover, neuroma-like tissue development and connective tissue proliferation were evaluated.

Transmission electron microscopy

Twelve weeks after the operation, the remaining five animals in each group were assessed via electrophysiological examination, uranium lead staining, and transmission electron microscopy (EM) to observe the shape and thickness of the regenerated nerve fiber myelin sheath, Schwann cell proliferation, vascular proliferation, and mitochondria. No edema was observed, and the nucleoli showed no deformity.

Statistical analysis

Two independent-sample *t*-tests using the Statistical Package for the Social Sciences (SPSS) software version 20.0 were used for one-way analysis of variance. *P*-values < 0.01 were considered statistically significant; data were expressed as $X \pm S$ and were plotted using Microsoft Excel 2003 software.

Method of euthanasia and humane endpoints

Immediately after the end of the experiment, the condition of each experimental animal was examined. If the animal had lost any body parts or was suffering enough to affect its quality of life, and it was impossible to relieve the animal's pain or distress using drugs or other means, euthanasia was performed. Specifically,

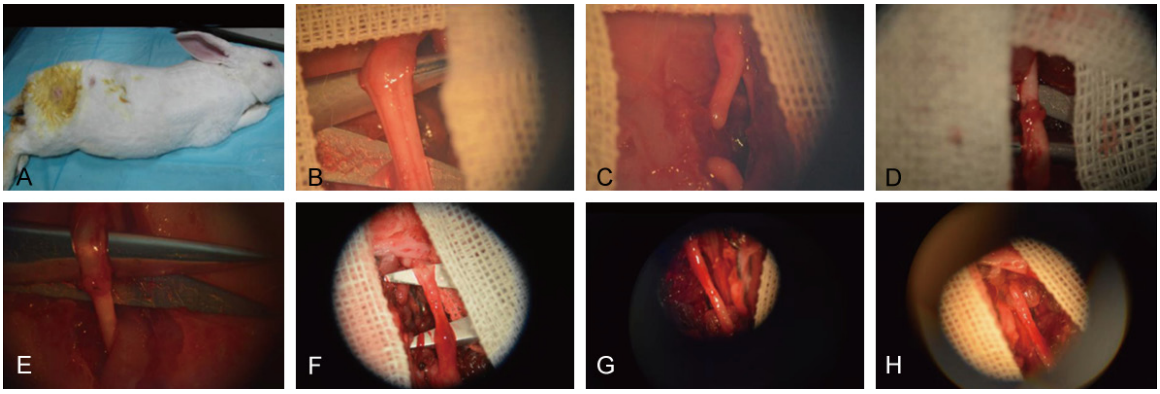

Figure 1. General observations. A: Preoperative skin preparation, disinfection; B: Revealing the sciatic nerve; C: Disconnected sciatic nerve; D: Control group 3 months after surgery; E: Group A 3 months after surgery; F: Group A 3 months after surgery; G: Group A 3 months after surgery; H: Group A 3 months after surgery.

Table 1. Comparison of neurophysiological examination 12 weeks after operation

Group	N	Conduction velocity (m/s)	F	P
Control	10	8.38±2.04	93.66	< 0.01
A	10	12.81±1.78		
B	10	19.63±3.19		
C	10	24.66±2.91		
D	10	29.61±3.68		

The homogeneity test of variance: the variance is homogeneous (P=0.304). One-way ANOVA results: F=93.66, P < 0.01 (significant difference).

the animal was euthanized using carbon dioxide (CO_2) gas perfusion in a high-pressure box. Before the animals were placed in the euthanasia box, CO_2 was injected for 20-30 seconds (the flow rate of CO_2 ranged between 10% and 30% chamber vol/min). The CO_2 was then turned off and the animal was placed in the box. The box was then reperfused with CO_2 for ≥ 10 minutes. It was then confirmed that the animal did not move or breathe, that its pupils did not dilate, and that its heart did not beat. The CO_2 was then turned off, and the animal died after 2 minutes of observation.

Results

General observations

After surgery, the Rex rabbits were paralyzed in the right lower extremity and unable to walk with weight, and they could only walk on the ground. Healing of the plantar ulcer and recovery of the limbs in group D were faster than those in groups A, B, and C. In group D, the foot ulcers healed in about 5 weeks, and the rabbits were able to perform spontaneous activities with the affected limbs. Groups A, B, and C were able to perform such activities after about 6 weeks. Moreover, their ulcers healed, and they were able to perform autonomic activities with the affected limbs. The foot ulcers healed after about 8 weeks in the control group, and the rabbits were able to resume their autonomic activities with the affected limbs at that time. Twelve weeks after surgery, the tumor was observed under microscope. The nerve anastomosis had swelled in the control group, and it had clearly adhered to the surrounding connective tissue. A neuroma developed, and no angiogenesis was observed at the superficial site. In the experimental group, there was no obvious adhesion to the surrounding tissue, and the small gap was continuous. Neither collapse nor displacement was noted. The visible proximal regenerative nerve fibers grew at the distal end, and the regenerative nerve diameter was consistent with that of normal nerves. Moreover, no neuroma developed, and no superficial visible blood vessels were observed (**Figure 1**).

Electrophysiology detection

The conduction velocity of the sciatic nerve in groups A, B, C, and D was significantly better than that in the control group (P < 0.01). Meanwhile, the conduction velocity of the sciatic nerve in group D was better than that in groups B and C (P < 0.01 in both cases) (**Table 1**).

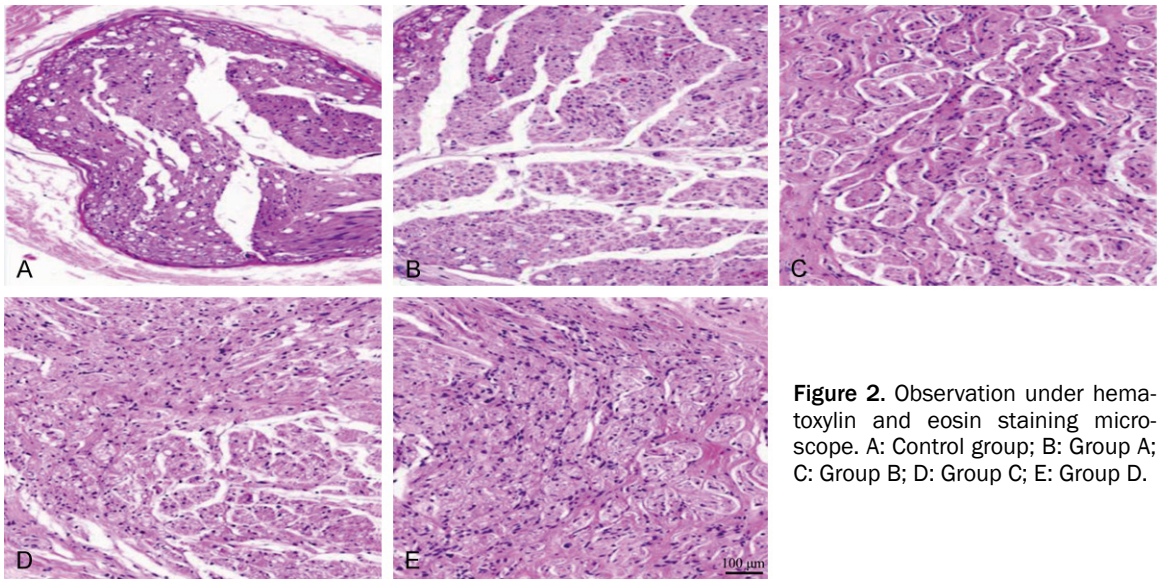

Figure 2. Observation under hematoxylin and eosin staining microscope. A: Control group; B: Group A; C: Group B; D: Group C; E: Group D.

To compare between groups, two independent samples *t*-tests were used. The following results were obtained: control group vs. experimental group A, $P < 0.01$; control group vs. experimental group B, $P < 0.01$; control group vs. experimental group C, $P < 0.01$; control group vs. experimental D group, $P < 0.01$; experimental group A vs. experimental group B, $P < 0.01$; experimental group A vs. experimental group C, $P < 0.01$; experimental group A vs. experimental group D, $P < 0.01$; experimental group B - experimental group C, $P < 0.01$; experimental group B vs. experimental group D, $P < 0.01$; and experimental group C vs. experimental group D, $P < 0.01$.

Histological observation

Twelve weeks after surgery, the tissue of each group was sectioned and observed under a microscope. After H&E staining, in the control group, the myelinated nerve fibers were sparse and irregularly arranged within the anastomosis. Moreover, scar hyperplasia was noted on the cut surface, and there was more extensive connective tissue and less angiogenesis between the nerve fibers than in the experimental groups. The other four groups with small gaps had a higher number of myelinated nerve fibers, with more regular arrangement, as well as extensive angiogenesis between nerve fibers. The regenerated nerve fibers in the small gap of group D were the most abundant, evenly distributed, and regularly arranged. The myelin sheath was thicker, and numerous capillary formations were observed (**Figure 2**).

In the control group, the regenerated nerve fibers were sparse, with less myelinated nerve fibers, irregular arrangement, and discontinuity. The diameter of the regenerated nerve was smaller than that of the normal nerves. The myelin sheath of the myelinated nerve fibers was thin, and there was extensive connective tissue but few blood vessels between the nerve fibers. In each experimental group, several regenerated nerve fibers were observed in the small gap, with large density as well as regular, continuous, and complete arrangement. Furthermore, the diameter of the regenerated nerve was closer to that of the normal nerves than in the control group; the myelin sheath of the myelinated nerve fibers was thick, as was the connective tissue. Less tissue proliferation was observed, with more visible capillary proliferation.

Transmission electron microscopy

According to the experimental design and materials for each group, uranium lead staining was carried out after tissue sectioning, and the ultrastructure was observed using transmission electron microscope. Traditional distal endocardial anastomosis (group A) presented with distal medullary dysplasia, irregular shape, less proliferation of Schwann cells, immature nerves, neuroma-like tissue formation, nucleo-

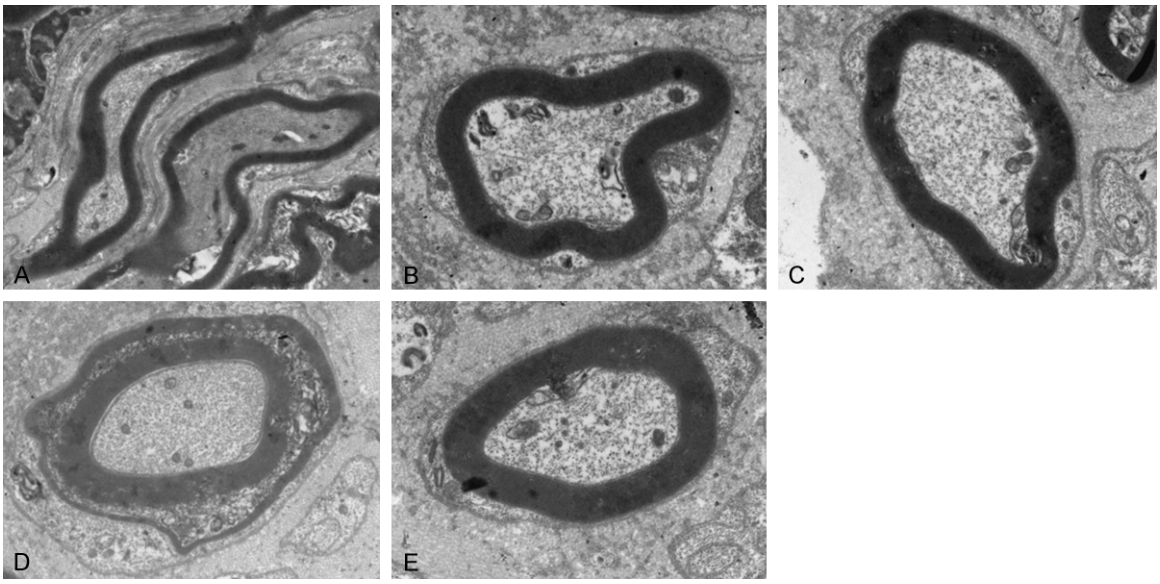

Figure 3. A: Traditional distal endocardial anastomosis group (control group) showing distal nerve myelin dysplasia, irregular shape, less Schwann cell proliferation, neurological immaturity, neuroma-like tissue formation, nucleolar malformation, mitochondrial swelling, and vascular hyperplasia (TEM × 4000). B: In the simple autologous epicardial small gap group (group A), the myelin sheath was mature, with no neuroma-like tissue, and acceptable vascular proliferation had taken place (TEM × 4000). C: The nerve growth factor combined with autologous epicardial small gap group (group B) had a more mature myelin sheath, Schwann cell proliferation was more apparent, there was no mitochondrial swelling, and acceptable vascular proliferation had taken place (TEM × 4000). D: The autologous nerve fragments combined with the autologous epicardial small gap group (group C) had mature myelin sheath, no neuroma-like tissue, and Schwann cell proliferation was apparent. There was no mitochondrial swelling, the epicardium and fascia were intact, and there was more vascular proliferation (TEM × 4000). E: The PRP combined with autologous epicardial small gap group (group D) showed mature myelin sheath, no neuroma-like tissue, Schwann cell proliferation, and clear cytoplasm. There was no mitochondrial swelling, the neuroepithelium and tunica were intact, and blood vessel hyperplasia had taken place (TEM × 4000).

lar malformation, mitochondrial swelling, and less vascular proliferation. PRP combined with an autologous epicardial small gap was administered in group D. Group C received autologous nerve fragments combined with autologous epicardial small gap. Meanwhile, NGFs combined with autologous epicardial small gap and simple autologous tissues were provided to group B. In the extraneural small gap group (group A), the myelin sheath had matured. No neuroma-like tissue was observed. Schwann cell proliferation was noted. The cytoplasm was clear, and no mitochondrial swelling was apparent. The neuroepithelium and tunica were intact, and vascular hyperplasia was observed. With regards to the regenerated nerve myelin maturity, the PRP plus autologous epicardial small gap group (group D) was superior to the autologous nerve fragments plus autologous epicardial small gap group (group C), followed by the NGF plus autologous nerve outer membrane gap group (group B), and finally by the simple autologous epicardial small gap group (group A) (**Figure 3**).

Discussion

The repair of peripheral nerve injury is extremely challenging in clinical practice. The traditional repair method cannot achieve accurate and effective docking of the nerve fibers at the distal end of the injured nerve [1], and functional recovery is usually unsatisfactory. After injury to the peripheral nervous system, Wallerian degeneration (WD) occurs at the distal end, and axonal degeneration distal to the anastomosis occurs in the axons and myelin at both ends after injury [13-15]. Unlike the central nerves, the peripheral nerves can regenerate after injury, leading to WD [16, 17]. Numerous studies have found that nerve damage plays a key role in regulating the activity of Schwann cells and in promoting axonal regeneration by releasing a large number of regeneration-relat-

ed factors, such as cytokines, growth factors, and chemokines [17, 18]. Therefore, the key factors regulating degeneration and regeneration in the peripheral nervous system after injury must be fully elucidated [19-22]. Similarly, the molecular mechanisms regulating WD are not fully understood. In this regard, understanding the factors that regulate rapid response during WD may reveal the supportive mechanisms of nerve repair and regeneration [23]. Following in-depth studies into the mechanism of nerve regeneration, some researchers [3, 4] have proposed a small gap anastomosis method based on the selective regeneration theory. Introducing substances that promote nerve regeneration into a small gap can significantly improve the regeneration of peripheral nerves. In particular, PRP is rich in various growth factors, such as epidermal growth factor, vascular endothelial growth factors (VEGF), platelet-derived growth factor (PDGF), insulin-like growth factor 1 (IGF), transforming growth factor-β (TGF-β), etc. [24]. These can act in a single or synergistic manner to nourish neurons and promote nerve regeneration and functional reconstruction.

Feasibility and superiority of bridging small gaps in autologous epicardium

Some investigators have attempted to repair peripheral nerve damage using the autologous epithelium because there is no immune rejection to autologous tissue and because expensive defects must be created to achieve peripheral nerve repair using other methods [25, 26]. The results of this process have been satisfactory. In the present experiment, we used the autologous epicardium to bridge the small gap. After surgery, we observed irregularities in the animals' limbs; after 12 weeks, we carried out neurophysiological examination, gross morphology observation, and histological examination to explore the feasibility of this method in promoting nerve regeneration. After the procedure, the right lower extremity of the Rex rabbits was paralyzed, so the animals were unable to walk with weight, and could only walk on the ground. The foot ulcers in group A had healed after about 6 weeks, and the rabbits were able to perform autonomic activities using the affected limbs. Conversely, in the control group the foot ulcers had healed after about 8 weeks, with the rabbits being able to perform autonomic activities in the affected limbs at that point. The simple autologous epithelium bridged the small gap to repair the damaged peripheral nerve, and the recovery of limb autonomic activity was significantly better in the experimental group than in the control group.

Twelve weeks after surgery, observation under the microscope revealed that the nerve anastomosis of the control group swelled and clearly adhered to the surrounding connective tissue. A neuroma developed. However, no vascular formation was observed on the superficial site. No small adhesion was noted between the small and the surrounding tissue gap in the group A. The small gap was continuous. No collapse was noted. Displacement was observed. Moreover, visible proximal regenerative nerve fibers grew at the distal end, and the regenerative nerve diameter was consistent with that of the normal nerves. A neuroma did not develop, and superficial angiogenesis was noted. A small gap provides a confined space for peripheral nerve regeneration, which does not only prevent the escape of nerve bundles and the formation of neuroma but also prevents the growth of connective tissue. The electrophysiological examination of each group of sciatic nerves 12 weeks after surgery showed that the conduction velocity of the sciatic nerve in group A was significantly better than that in the control group ($P < 0.01$). This result indicated that this method was beneficial in nerve regeneration and in repairing peripheral nerve injury. Twelve weeks after surgery, in the control group, H&E staining showed that the myelinated nerve fibers were sparse and irregularly arranged and had a scar hyperplasia on the cut surface and a large amount of connective tissue and a small amount of angiogenesis between the nerve fibers. Meanwhile, in the other groups, there were numerous myelinated nerve fibers in the four small gaps, and the arrangement was relatively regular. There was a large number of angiogenesis between the nerve fibers. Among them, the regenerated nerve fibers were the most distributed in the small gap in group D, and they were evenly distributed. Moreover, the arrangement was regular, the myelin sheath was thick, and numerous capillary formations were noted. Based on ultrastructural observations made using transmission electron microscopy, the distal end in the control group had poor myelin dysplasia,

neurological immaturity, neuroma-like tissue formation, distortion, nucleolar malformation, mitochondrial swelling, and less vascular proliferation. The neuromyelin sheath of group A was mature and had no neuroma-like tissue. The proliferation of Schwann cells was evident, and the cytoplasm was clear. Moreover, no mitochondrial swelling was apparent, and the epicardium and fascia were intact. More vascular proliferations were observed than in the control group.

In summary, the present experiment showed that the small gap in the autonomic aneurysm bridge provides a free interference from the peripheral nerves for peripheral nerve regeneration. It can prevent the escape of nerve bundles, the formation of neuroma, and the growth of surrounding connective tissues within a good microenvironment.

Properties of PRP associated with nerve injury repair

PRP is rich in a variety of growth factors. PRP liquid or gel can be made into an injectable stent and administered *in vivo*. It can also be used as a matrix-like viscous and malleable structure to be wrapped around the injured nerve space [11]. The tissue fibrinolysis around this area stimulates the release of cellular signaling molecules, such as neurotrophic factor (NGF), brain-derived neurotrophic factor, IGF-1, PDGF, VEGF, hepatocyte growth factor, and neurotrophic factors (fibrin, fibronectin, and vitronectin) [27].

PRP confers neuroprotection and apoptosis prevention. Several studies have verified that several growth factors in PRP, either alone or in combination with mesenchymal stem cells (MSCs), neurons, stromal cells (SCs), or human neural stem cells, have protective effects in nerves and against apoptosis [28, 29]. PRP fibrin scaffolds are rich in NGF, bone derived growth factor (BDGF), and retinoic acid, and they are loaded with bone marrow stromal cells (BMSCs), which enhance the survival of PRP fibrin scaffolds and encourage their differentiation into a neural phenotype [30]. In addition, when this PRP scaffold was transplanted into the brain, the viability and biological activity of allogeneic BMSCs increased [31]. The long-term use of PRP, which induces neuroprotection in a mouse model of Alzheimer's disease, may activate the apoptotic PIEK/Akt-mediated signaling pathway [32].

PRP stimulates angiogenesis. Borselli et al. [33] used an injectable scaffold loaded with VEGF and IGF-1 to accelerate the regeneration and angiogenesis of injured neuromuscular junction in a rodent model of ischemic limb, with neuromuscular junction innervation loss. In terms of enhancement, in a rat model, repair of the sciatic nerve defect with a vein graft filled with PRP for 10 mm showed more pronounced early angiogenesis than repair with autologous nerve graft alone. It follows that fibrin may be the key component in PRP. Elements that provide extracellular matrix tissue with a robust and licensed 3D matrix for angiogenesis [34].

PRP promotes axonal regeneration. As a cell carrier, two studies of the acute nerve injury model of guinea pigs and rabbits have combined PRP with inoculation of cell-free carriers using either MSCs or SCs. Physiological parameters, such as postoperative axon number, myelination, and nerve electricity, significantly improved [35, 36]. Zheng et al. [37, 38] used PRP as a filler in acellular nerve allograft to significantly promote neuronal cell proliferation. Their results showed that the thickness of the myelin sheath, the number of axons, and neurophysiological indicators significantly increased. Kim et al. [39] used both autologous vein grafting and PRP combined with autologous vein grafting to bridge the sciatic nerve space in the rat model. They found that, when using PRP as a filler, the number of myelinated axons, axonal diameter, and the thickness of the myelin sheath significantly increased.

PRP has anti-inflammatory effects. Anitua et al. [32] reported that PRP completely inhibits nuclear factor-kB (NF-kB)-mediated expression of β- amyloid and pro-inflammatory cytokines in astrocytes. In a mouse model of Parkinson's disease, Anitua et al. [7] found that microglia-mediated neuroinflammatory processes reduced, and motility improved, with NF-kB activation. Nitric oxide, cyclooxygenase, and tumors were strongly correlated with a marked decrease in tumor necrosis factor expression.

PRP inhibits denervated target muscle atrophy. Sanchez et al. [11] used PRP to repair nerve damage in sheep and found that PRP was associated with earlier electrophysiological recovery

and lower muscle atrophy, indicating that PRP can inhibit target muscle atrophy. Anitua et al. [40] found that intramuscular injection of PRP 24 hours after limb ischemia in mice reduced fibrosis and muscle atrophy.

Improvement of small gap microenvironment by PRP

The mechanism of peripheral nerve regeneration is a complex process involving molecular and cellular levels, and small gaps alone are insufficient to repair the intensity of peripheral nerve injury. Therefore, adding substances that promote nerve regeneration can significantly improve the regeneration of peripheral nerves. Numerous studies have found that Schwann cell localization to injured nerves significantly improves the healing process. However, the source of Schwann cells is limited, and local vaccination cannot guarantee the activity of cells. Moreover, Schwann cells are difficult to isolate and culture *in vitro*, so they cannot be widely used in clinical practice. Qin et al. [41], corroborating other studies, found that early regeneration of Schwann cell division, proliferation, and addition of exogenous NGF to the damaged microenvironment can promote nerve regeneration. However, most growth factors on the market have a limited variety, and their effects for promoting nerve regeneration are limited. In recent years, PRP, which is rich in a variety of growth factors that are favorable for cell growth and differentiation, has been widely studied. It is derived from autologous plasma, is simple to prepare, and does not cause immune rejection. Zhang et al. [42], corroborating other researchers, found that PRP can compensate for the deficiency of exogenous growth factors, and the regeneration effect of PRP on sciatic nerve injury in rabbits is significantly stronger than that of growth factors. In the present experiment, we injected PRP extract into the small gap of the autogenous nerve outer membrane bridge to create a suitable microenvironment that can promote nerve regeneration. After extensive observation, the healing of the plantar ulcer and the recovery of the limbs were faster than those of the other groups. In group D, the foot ulcers healed in about 5 weeks, and the affected limbs resumed activity. Meanwhile, the foot ulcers in groups A, B, and C groups healed in approximately 6 weeks. The plantar ulcer healed, and the affected limb resumed its previous activities. Twelve weeks after surgery, no significant difference was observed between group D and the other experimental groups. However, the neurophysiological examination results were better in groups C and D than in group B ($P < 0.01$). In the histological examination carried out 12 weeks after surgery, the following results were obtained via H&E staining under light microscope: the regenerated nerve fibers were denser in group D than in group C, there were more myelinated nerve fibers, the arrangement was regular, the Schwann cells had clearly proliferated, and the size of the capillary was large. There was vascular hyperplasia and the myelin sheath was thicker; groups B and C had more regenerated nerve fibers, more myelinated nerve fibers, higher density, regular arrangement, capillary proliferation, and thicker myelin sheath; compared with group A. Group B had regularly arranged, dense regenerated nerve fibers, with more myelinated fibers and thicker myelin sheath. From the ultrastructure of transmission electron microscopy, the rejuvenation of myelin sheath was better than that in group B.

In summary, the present experiment showed that PRP can significantly improve the microenvironment in small gaps and promote peripheral nerve regeneration and functional recovery better than nerve fragments and NGFs.

Limitations of this experiment

In the present study, the outer wall of the neuroepithelium was thin, and it lacked support after the nerve bundle was released. Therefore, it could easily collapse; when it did so, the small gap did not survive and therefore could not provide an effective space for selective regeneration of peripheral nerves. However, axon formation occurs from the proximal end to the distal side, which is a challenge in nerve ending regeneration.

The length of the small gap was also a factor affecting the regeneration of peripheral nerves. When the small gap was extremely short, it could not provide an effective space for selective regeneration of peripheral nerves. Meanwhile, if it was extremely long, the proximal nerve fibers could not grow into the distal end. Numerous experimental studies [43-45] have

found that a 2-mm small gap is the most appropriate, and that the peripheral nerve has the most proliferative ability. However, most such experiments have been carried out in rats. In the present experiment, Rex rabbits were used. To assess inconsistencies in the length of the small gaps suitable for nerve regeneration, accounting for differences in race, thickness of nerve fibers, and diameter of small gaps, further research must be conducted. Moreover, there is no consensus regarding whether any platelet-activating agent must be added to the PRP extract. Some researchers have added a mixture of thrombin and calcium gluconate to PRP to prepare a gel [46], and the repair effects have been satisfactory. In any case, the choice of PRP activator affects the timing and level of growth factor release [47]. However, other studies have claimed that directly applying PRP without activating platelets achieves better results [48]. In the present experiment, the PRP extract was directly added to the activator, and the effect for repairing peripheral nerve damage was better than that of adding an activator alone. However, further studies must be carried out to validate these results.

Conclusion

The effect of autonomic aneurysm to bridge small gaps on peripheral nerve damage was significantly better than that of the traditional epicardial suture.

Substances that promote nerve regeneration, such as PRP, nerve fragments, and NGFs, are added to the small gap to form a microenvironment suitable for nerve regeneration, which promotes the repair of damaged nerves.

Compared with nerve fragments and growth factors, PRP can significantly improve the microenvironment in small gaps and promote peripheral nerve regeneration and functional recovery.

The effect of PRP combined with autologous epicardial small gap for peripheral nerve damage repair was evident, thereby providing a theoretical basis for the clinical application of PRP to repair peripheral nerve damage.

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Disclosure of conflict of interest

None.

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