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Novel application of CHUANG BI FU biologic protein sponge in promoting peripheral nerve injury repair by regulating vascular regeneration

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Abstract

Background The search for effective drugs that promote regeneration after peripheral nerve injury (PNI) has received widespread attention, but little attention has been paid to the potential secondary damage to nerves during neurosurgical procedures. CHUANG BI FU Biologic Protein Sponge (CHUANG BI FU) is a composite biological sponge loaded with basic fibroblast growth factor (bFGF) that has been effectively applied to clinical wounds, but has never been used for repairing peripheral nerves.

Methods This study used a 3.5-mm nerve defect model in mice, and wrap CHUANG BI FU around the nerve defect site, connecting the distal and proximal ends of the damaged nerve without suturing the nerve. The repair function of the nerve by the wound was tested by detecting mouse footprints, electrophysiology, NF200 staining, CD31 staining, and gastrocnemius muscle Masson staining.

Results It was found that CHUANG BI FU can promote axonal elongation, promote neuromuscular reinnervation function, and promote angiogenesis of regenerating nerves, thereby achieving the function of promoting damaged nerve regeneration. And through cellular level research, it was found that CHUANG BI FU may promote vascular regeneration through the PI3 K–AKT pathway.

Conclusions Using CHUANG BI FU to repair peripheral nerves can promote vascular regeneration through the PI3 K–AKT pathway and it can greatly reduce the technical difficulty and operation time required by surgical operators.

Keywords Peripheral nerve injury, Nerve regeneration, Angiogenesis

Introduction

Peripheral nerve injury (PNI) can cause neuropathy in patients, leading to weakness, paralysis, sensory deprivation, neurogenic pain, and impaired autonomic nervous function [1, 2]. Currently, autologous nerve transplantation or suturing of nerve conduits are commonly used in clinical practice to replace missing nerves [3, 4]. However, autologous nerve transplantation surgery may not only cause sensory or functional loss in the donor site [5], but also require a lot of time and high technical requirements for surgical operators. Mistakes in surgical operation may cause secondary damage to the nerves [6, 7]. Therefore, it is imperative to find a new material and surgical method that can serve as an alternative to autologous nerve transplantation while reducing surgical time and lowering the requirements for surgical techniques.

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In clinical practice, autologous nerve transplantation or the use of nerve conduits to connect the nerves on both sides are commonly used for long-distance defects [8, 9]. During surgery, it is required to align the distal or proximal end of the missing nerve with the graft and perform bundle membrane suturing [10]. This surgery not only takes a long time, but also easily causes secondary nerve damage due to improper surgical operation [11], such as surgical suture needle piercing the nerve, nerve misalignment alignment, increasing the probability of neuroma formation [12]. There are studies using nerve glue as a substitute for nerve suturing [13, 14], but the degree of nerve elongation is limited. Using nerve glue to bond long-distance injuries can easily affect muscle recovery due to excessive nerve tension and exacerbate neuropathic pain. Therefore, we chose CHUANG BI FU Biologic Protein Sponge (CHUANG BI FU) as the autologous nerve graft. CHUANG BI FU has a good adhesive effect. It can adhere to nerves without suturing, greatly saving surgical time and reducing surgical difficulty. CHUANG BI FU is a medically active biomaterial made from a combination of collagen derived from pig brain tissue and basic fibroblast growth factor (bFGF).

Basic fibroblast growth factor (bFGF) is a neurotrophic factor that plays a key regulatory role in neuroprotection and promoting axonal elongation in PNI [15, 16]. Meanwhile, BFGF can promote the proliferation of epithelial cells and facilitate vascular regeneration [17]. Angiogenesis is the initial and critical stage of nerve regeneration, and the regeneration of blood vessels helps promote nerve regeneration [18, 19]. The bFGF contained in CHUANG BI FU can promote the generation of new capillaries and tissue regeneration.

In this study, we performed a transverse surgery on the sciatic nerve of mice to create a 3.5-mm defect, and use wound healing to clamp the nerve on both sides, allowing bFGF to fully wrap around the nerve as the experimental group. Mice with only sciatic nerve transection without treatment and mice with autologous nerve transplantation were used as the control group. Evaluate the impact of simplified application of CHUANG BI FU on nerve injury repair through nerve repair testing, angiogenesis testing, and related molecular mechanism analysis 3 months later [20, 21]. The aim of this study is to explore whether CHUANG BI FU can promote nerve regeneration by promoting axonal elongation and promoting angiogenesis, and to provide new ideas for clinical nerve donor substitutes and simplified surgical methods by reducing surgical time and lowering technical requirements to minimize damage.

Method

Materials

Male C57/B6 mice were provided by Hangzhou Medical College (Hangzhou). Anesthetize mice by intraperitoneal injection of pentobarbital (1 mg/g). CHUANG BI FU Biologic Protein Sponge (CHUANG BI FU) is provided by Harbin Peiqilong Biopharmaceutical Co. LTD. (Harbin). The surgical incision is sutured with absorbable sutures. All the animal experiments followed the guidelines given by the National Institutes of Health (Pub. No. 85 - 23, revised 1996).

In vivo studies

Surgical methods

The animal clinical study used C57/B6 mice (4–5 weeks) as the standard animal model. Mice were anesthetized by intraperitoneal injection of 1% pentobarbital sodium. After 15 male mice were housed in the SPF animal house for 1 week, divide all C57 mice into three groups: autologous nerve transplantation group, transection group, and CHUANG BI FU treatment group. Then scrape off the hair on the right leg and disinfect the skin on the leg. Cut open the skin of the right leg of the rat with a surgical knife and expose the muscle tissue through blunt dissection. Finally, carefully expose the sciatic nerve to the surgical field of view and create a 3.5-mm sciatic nerve defect. Randomly reverse the sciatic nerves of 5 mice and perform microsurgical suturing (Fig. 1B). The sciatic nerve defect sites of the 5 mice were treated with a substitute of autologous nerve transplantation, which was sandwiched on both sides of the damaged nerve, so that the nerve was fully wrapped around the distal and proximal ends of the defect nerve. The nerve was not sutured to the nerve (Fig. 1C, D), and the remaining 5 mice were not subjected to any treatment (Fig. 1E). Finally, suture the muscles and skin.

Behavioral analysis

In order to evaluate the effect of simplified application of CHUANG BI FU on neurological function recovery, we used the CHUANG BI FU treatment group as the experimental group, complete transverse injury as the negative control group, and clinical gold standard (autologous nerve transplantation) as the positive control group to repair a 3.5-mm sciatic nerve defect. To maintain normal physiological status, mice were freely housed in cages and fed by automatic feeders.

At different timepoints after surgery, the hind limb footprints of mice in the injury group, CHUANG BI FU group, and autologous nerve transplantation group were recorded on blank paper using red ink cartridges. The information from these footprints and

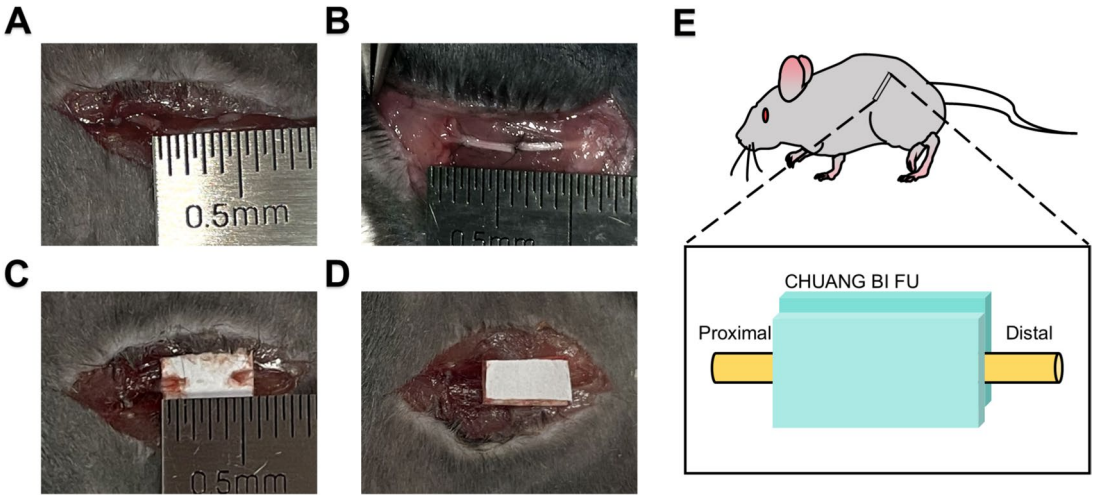

Fig. 1 Implantation of nerve grafts in each group. **A** Neurotransection injury's picture; **B** autograft group's picture; **C, D** in vivo implantation of CHUANG BI FU. **E** CHUANG BI FU utilized for the repair of sciatic nerve defects

the formula in the experimental section were used to: $\mathrm{SFI} = -38.3 * \frac{\mathrm{EPL}-\mathrm{NPL}}{\mathrm{NPL}} + 109.5 * \frac{\mathrm{ETS}-\mathrm{NTS}}{\mathrm{NTS}} + 13.3 * \frac{\mathrm{EIT}-\mathrm{NIT}}{\mathrm{NIT}} - 8.8$ [22, 23]. We can calculate the SFI value to evaluate the recovery of motor function.

PL represents footprint length, which refers to the length from the third toe to the heel of a rat. TS represents the length of the first and fifth toes and IT represents the length of the second to fourth toes. The "N" in NPL, NTS, and NIT represents non-surgical hind limbs. The "E" in EPL, ETS, and EIT means the experimental hind limb.

Electrophysiological examination

Three months after the surgery, the sciatic nerve on the surgical side of the mice was reopened under anesthesia for electrophysiological testing. Record electromyographic activity using an electrophysiological system (RM6240, China). Use 10 mV electrical stimulation to stimulate the sciatic nerve that innervates the gastrocnemius muscle, i.e., the injured nerve, and then place the receiver on the gastrocnemius muscle. Record and analyze compound muscle action potential (CMAP).

Gastrocnemius muscle evaluation

Three months after surgery, the gastrocnemius muscle of the operated hind limb and normal hind limb were removed. Record wet weight and analyze muscle weight recovery rate:

$$\mathrm{Wr}(\%) = \frac{\mathrm{Ws}}{\mathrm{Wn}} * 100.$$

Among them, Wr is the muscle weight recovery rate, Ws in—is the muscle weight of the operated gastrocnemius muscle, and Wn is the normal lateral muscle weight of the gastrocnemius muscle. After weighing and taking photos of the gastrocnemius muscle tissue, it was cut in half and then fixed in a 4% paraformaldehyde solution within 1 day. After embedding the gastrocnemius muscle in paraffin, prepare 5 μm sections and perform Masson's trichrome staining. Collected 3 or more slices for data analysis. Calculate the percentage of collagen fiber area according to the formula:

$$c(\%) = \frac{b}{a+b} * 100.$$

Among them, "*a*" represents the cross-sectional area of muscle fibers, "*b*" represents the area of collagen fibers, and "*c*" represents the percentage of collagen fibers.

Regenerative neurohistological evaluation

Three months after surgery, the regenerated neural tissue of the mice was removed. Fix some of the regenerated nerve tissue in a 4% paraformaldehyde solution for 48 h, dehydrate the regenerated nerve, embed it in paraffin, and slice it into 5 μm. Then perform H&E staining and capture images using digital slide scanning (BX51, OLYMPUS, Japan).

Immunofluorescence staining was performed on the regenerated nerves, and the paraffin embedded regenerated nerves were longitudinally cut, deparaffinized, and antigen recovery was blocked. They were then treated with anti-nf200 (N4142, Sigma Aldrich) or CD31 (AB_10854276, Invitrogen) primary antibody at 4 °C overnight. Slices were incubated with IgGMouse488 (Servicebio, China) and IgGMouse555 (Servicebio, China) labeled secondary antibodies for 1 h. The cell nucleus was

stained with DAPI. Finally, images were collected using digital slide scanning (BX51, OLYMPUS, Japan).

In vitro studies

Cell culture

Obtain HUVECs from American Type Culture Collection (ATCC) Cultivate in DMEM medium (Gibco, USA) and add 10% fetal bovine serum (FBS, Gibco, USA) and 1% penicillin/streptomycin (PS, Gibco, USA). The cells were cultured in a 37 °C incubator (Healforce HF100, USA) in a humidified atmosphere of 95% air and 5% carbon dioxide.

Cell scratch assay

To verify the cell migration ability, we chose the cell scratch assay. Cells were seeded into culture plates and allowed to form a dense monolayer. A straight scratch was then made on the cell layer surface using a sterile pipette tip or scratch tool. The dislodged cells were washed away with PBS, and the medium was replaced with serum-free medium to inhibit proliferation. Images were captured under a microscope at 0 and 24 h, respectively, to record the migration distance of cells from the scratch edge toward the blank area. The migration rate was quantitatively calculated using imageJ software to evaluate the effects of different treatments on cell migration ability.

Transwell migration

The cells were digested with trypsin and centrifuged for 5 min, after which the supernatant was discarded. The cells were resuspended in serum-free medium, and the cell density was adjusted to 1×10^5 cells/mL. The Transwell chambers were placed into a 24-well plate, and the lower chambers were filled with medium containing CHUANG BI FU as a chemotactic stimulus. Subsequently, the cell suspension was added to the upper chambers, and the chambers were gently shaken to ensure even distribution of the cells. The plate was incubated at 37 °C with 5% CO_2 for 24 h. After incubation, the Transwell chambers were removed, and both the upper and lower chambers were gently washed with PBS to remove non-migrated cells. The cells were fixed with 4% paraformaldehyde for 20 min, washed with PBS, and then stained with crystal violet solution for 15 min. After staining, the Transwell chambers were photographed under an inverted microscope, and the cells were counted.

Endothelial tube formation test

To evaluate the ability of CHUANG BI FU to promote angiogenesis, Matrigel basement membrane matrix (BD, 356234, USA) was used to induce the formation of HUVEC tubes on 96-well tissue culture plates. Firstly, co-culture CHUANG BI FU with HUVECs in a culture dish for 3 days, and prepare three sets of cell suspensions after digestion. All experimental consumables, including pipette tips, culture plates, and ice plates, were pre frozen at − 20 °C Matrigel was also thawed overnight at 4 °C and then added to a pre cooled 96 well plate (50 μL/well) per well. After incubating at 37 °C in a culture dish for 1 h, 10^4 HUVECs were evenly spread in each air. Then the culture plate is placed in the incubator. After incubation for 4 h, capture the cells with an optical microscope and calculate the average value of tube formation in 3 wells using ImageJ v1.8.0 software.

Western blot

After co-culturing Chuanbifu and HUVEC for 3 days, collect HUVEC cells treated with Chuanbifu and control cells, and prepare protein extracts using a total protein extraction kit. Use gel electrophoresis to separate the total protein, transfer it to PVDF (USA Millipore), block it with 5% skimmed milk (Beyotime, China), use TBST [0.15 M NaCl, 0.05% Tween- 20, 10 mM Tris HCl (pH8.0)] to dissolve it at 25 °C for 30 min, and then use the following primary antibody to incubate at 4° C overnight: anti P-PI3 K antibody (AB_2816326,1:500 dilution, Invitrogen) anti-PI3 K antibody (AB_10984433, diluted 1:500, Invitrogen), Anti-AKT antibody (10176 - 2-AP, diluted 1:500, proteintech), Anti-P-AKT antibody (66444 - 1-Ig, diluted 1:500, proteintech), Anti-β-actin antibody (GB1101, diluted 1:2000, Servicebio). Dilute the membrane with horseradish peroxidase (HRP) labeled secondary antibody (GB23303, 1:5000). After incubating with Servicebio for 60 min, the WB signal was detected using an enhanced chemiluminescence (ECL Western Blotting Substrate, Pierce, USA) system. Measure the density value using Image J software and normalize the target gene to β-actin or total protein.

Statistical analysis

All results are presented as mean ± SEM uses one-way ANOVA and post hoc testing to compare the mean values of two or more samples. A *P*-value less than 0.05 is considered statistically significant.

Results

Recovery of neurological function

A complete injury to the sciatic nerve results in significant walking difficulties, including symptoms like foot drop on the affected side, where the foot is dragged due to an inability to lift it properly. This is accompanied by a loss of plantar flexion at the ankle and reduced ability to spread the ankle [24]. As the damaged section of the sciatic nerve begins to heal and regenerate, some

degree of motor function may be restored, though typically only partially. The SFI value is a quantitative indicator for evaluating the nerve dysfunction and recovery [23]. The trend of nerve recovery within 3 months post-surgery is illustrated in the figure. At 14 days post-surgery, the SFI of the injury group was −94.96, the SFI of the CHUANG BI FU group was −90.8, and the SFI of the autograft group was −90.35. The SFI value of the CHUANG BI FU group was higher than that of the injury group after 14 days, showing a significant difference, indicating that the CHUANG BI FU sponge protein can effectively promote the recovery of motor function. At 3 months post-surgery, the walking footprints of the three groups of mice on the surgical side and the affected side are shown in Fig. 2A, and the walking footprints of the three groups are different. The SFI of the injury group was −67.77, the SFI of the CHUANG BI FU group was −52.4, and the SFI of the autograft group was −43.06. These results indicate that the CHUANG BI FU group can effectively promote nerve repair, but the recovery of motor function is inferior to that of the autograft group.

Electrophysiological detection of nerve action potentials is further used to monitor the recovery of the nervous system [25]. Figure 2A shows representative CMAPs records of the surgical limbs in the injury group, the treated group, and the autograft group. The average latency of CMAPs in the CHUANG BI FU group was 2.6 mV, while in the autograft group it was 2.3 ms. The average peak period of CMAPs in the.

CHUANG BI FU group was 1.87 mV, while in the autograft group it was 2.73 ms. CAMPs signals could not be recorded in the injury group (Fig. 2C, D). The electrophysiological results of the CHUANG BI FU group were higher than those of the negative control group at each time point. Therefore, bFGF can help improve the recovery of neural action potentials, but the recovery ability is not as good as that of the autologous nerve transplantation group.

Analysis of gastrocnemius muscle

The gastrocnemius muscle, innervated by the tibial nerve, can indirectly indicate nerve regeneration through its recovery. To analyze the effects of various treatment

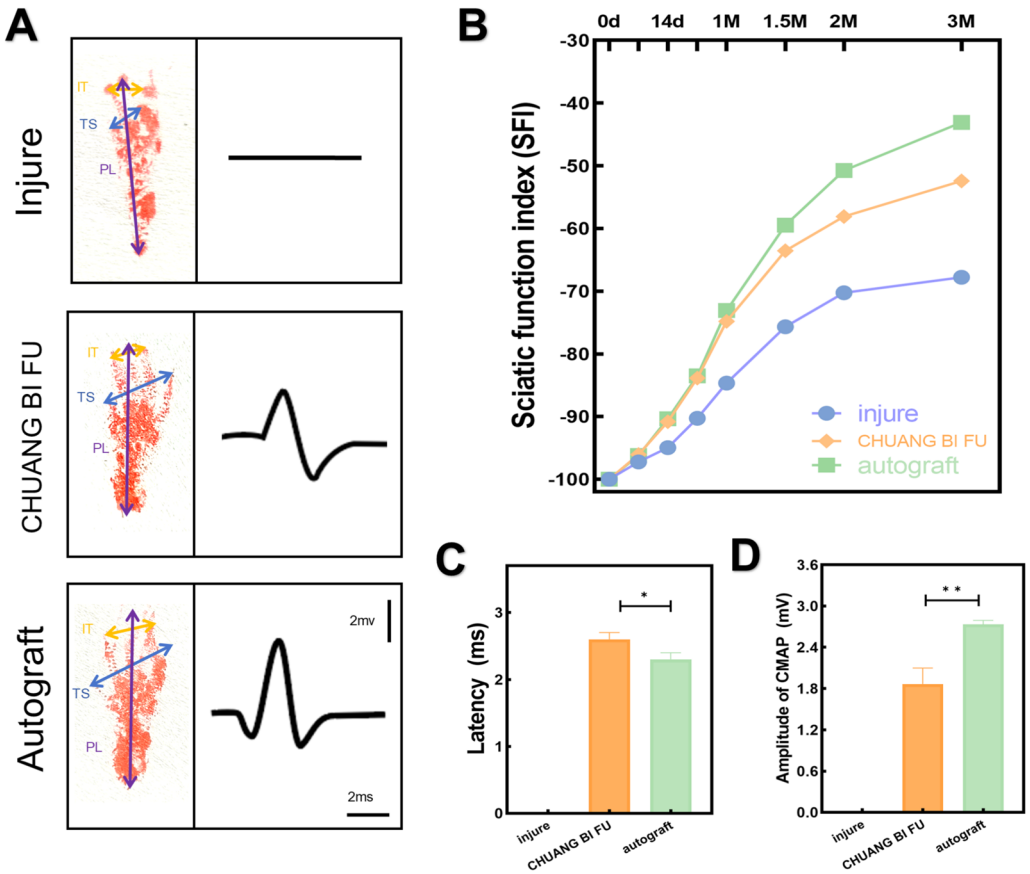

Fig. 2 Neurological function recovery analysis. **A** Representative images of walking track footprints and CMAPs recorded on the repaired nerve. **B** Analysis of SFI values, peak amplitude of CMAPs (**D**) and latency (**C**), $n = 3$, $P < 0.05$

methods on the reinnervation of gastrocnemius muscle (GM) by regenerated nerves, the GM of hind limbs from each group was taken. Record the degree of atrophy of each group of GMs with a camera (Fig. 3A) and weigh them, and perform Masson staining on the GMs to observe and analyze the cross-sectional areas of muscle fibers and collagen fibers (Fig. 3B). The GM of the right hind limb in each group was smaller than that of the left hind limb. The cross-sectional area of three groups of GM muscle fibers and the percentage of collagen fibers are shown in Fig. 3C. The percentage of collagen fiber area in the CHUANG BI FU group and the autologous nerve transplantation group was 5.46% and 3.35% (Fig. 3C). Both of them were significantly lower than the 20.52% in the injury group. Compared with the injury group, the cross-sectional areas of muscle fibers in the CHUANG BI FU group and the autologous nerve transplantation group were 9223.15 square micrometers and 257.57 square micrometers, respectively, which were significantly larger than the 94.99 square micrometers in the injury group (Fig. 3D).The average muscle recovery rate of the CHUANG BI FU group was 77.49%, the injury group was 29.44%, and the autograft group was 75.61% (Fig. 3E). The CHUANG BI FU sponge could significantly elevate the degree of muscle recovery. Both of them were significantly lower than the 20.52% in the injury group. The results indicate that CHUANG BI FU biological sponge protein can effectively prevent muscle atrophy caused by peripheral nerve injury, and promote muscle dominance and nutritional function of regenerated nerves.

Analysis of regenerated neural tissue

HE staining showed that the morphological results of regenerated nerves are shown in Fig. 4A. It can be clearly observed that there is nerve rupture and the formation

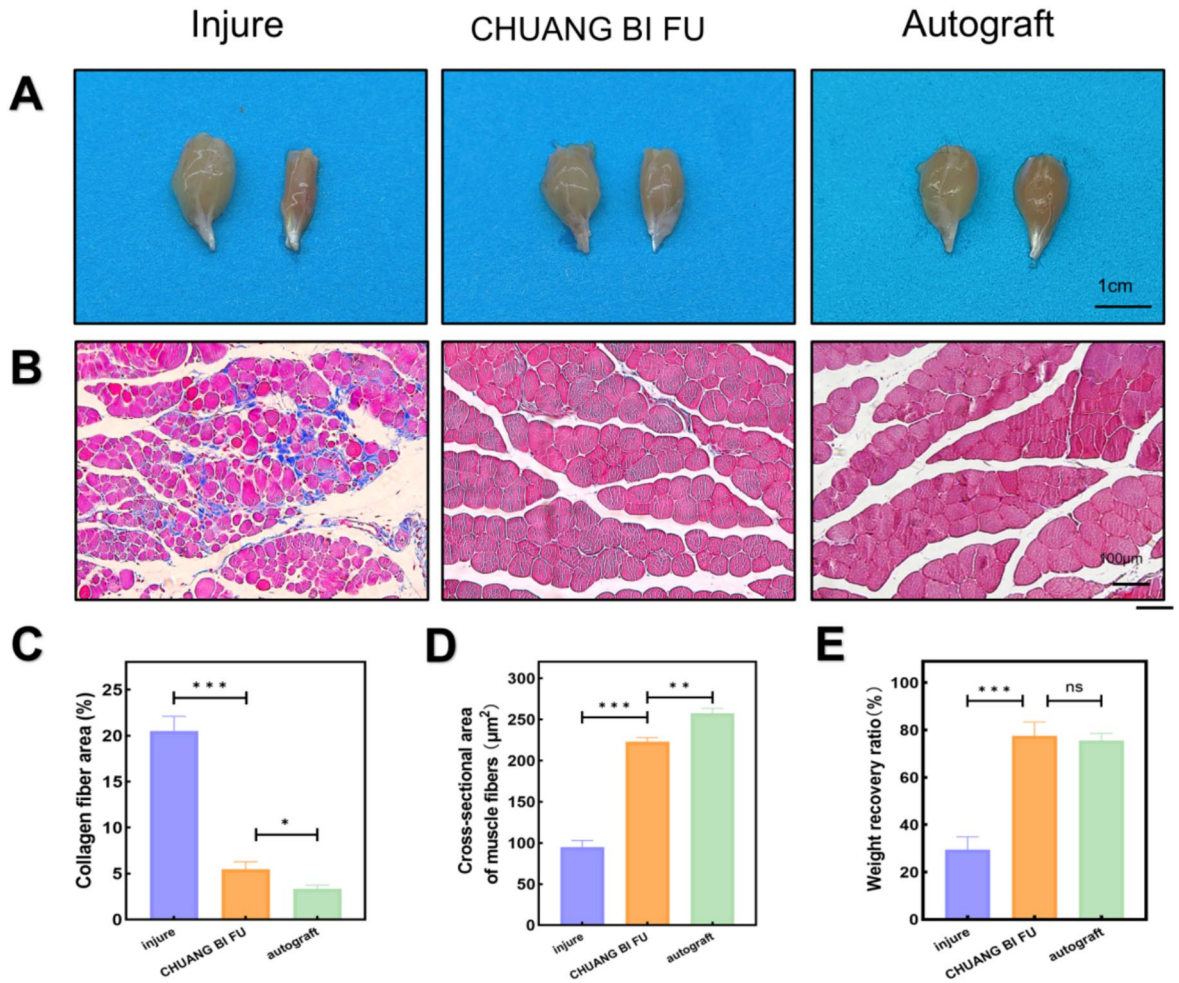

Fig. 3 Histological assessment of gastrocnemius muscles. **A** Images of gastrocnemius muscle of the operative side. **B** Masson's trichrome staining of cross sections of gastrocnemius muscle. **C** The statistical results of the average percentage of collagen fiber area. **D** The statistical results of the cross section area of muscle fibers; **E** the statistical results of the gastrocnemius weight recovery ratio. $n = 3$, $P < 0.05$

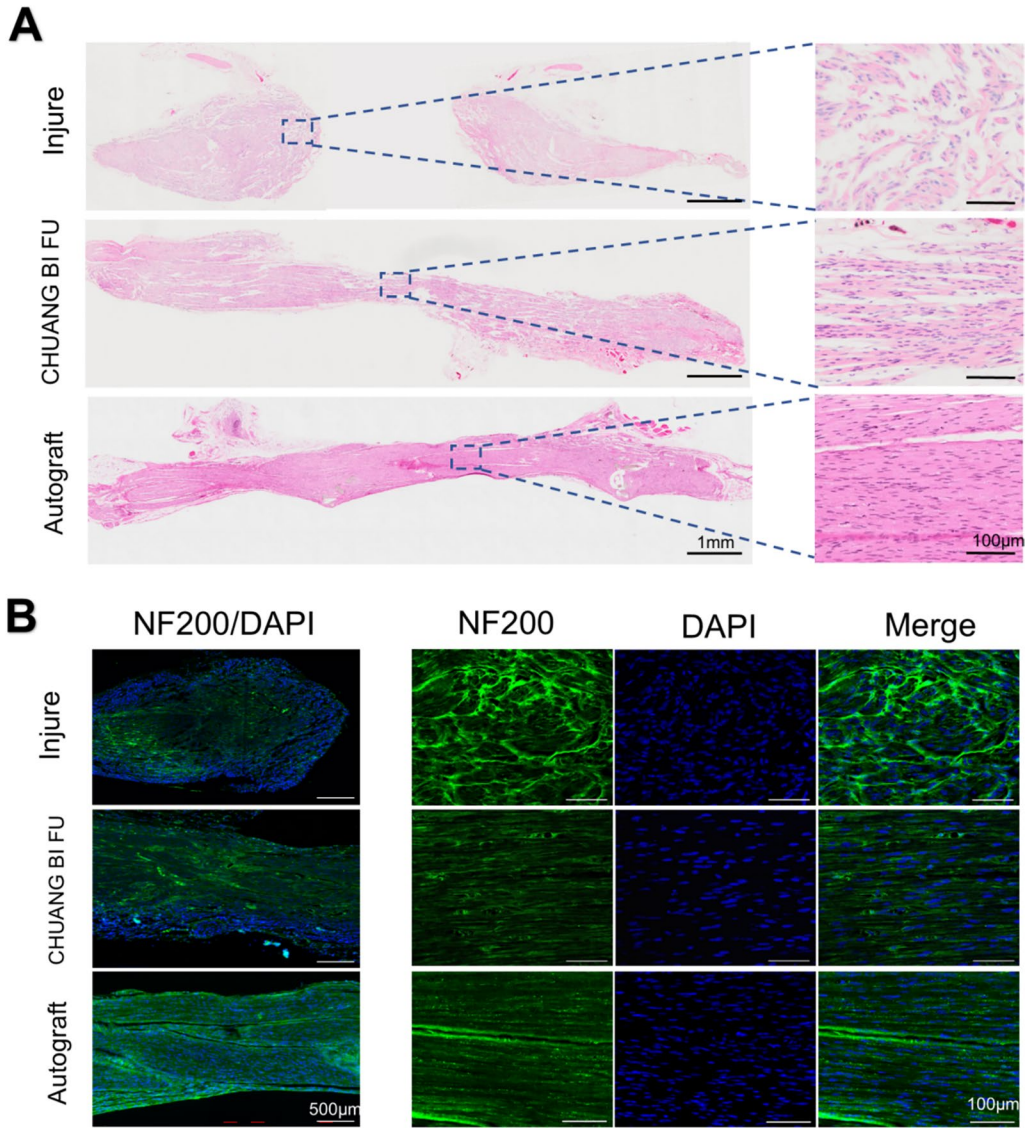

Fig. 4 Immunofluorescence analysis of the regenerated nerves. **A** Images of HE-stained longitudinal sections of the regenerated nerve; **B** representative images of NF200 immunofluorescence staining, along with magnified views

of neuroma in the injured group. The damaged nerves in both the autograft group and the CHUANG BI FU group are connected across the injured area. NF200 is a specific biomarker for neurofilaments [26], and the distal portion of regenerated nerves is subjected to immunofluorescence staining, as shown in Fig. 4B. The results of HE staining and NF200 immunofluorescence showed that, 12 weeks after surgery, axonal growth was observed in all three groups, but there were differences in the density and direction of regenerated fibers. Compared with the injury group, the regeneration axon arrangement of the CHUANG BI FU group and the autograft group was more regular and abundant. In summary, CHUANG BI FU biological sponge can promote axonal regeneration during nerve injury, thereby playing a repairing role in damaged nerves.

The impact of CHUANG BI FU on angiogenesis

Angiogenesis is a critical component of neurogenesis. To evaluate the angiogenesis of each group, $CD31^+$ immunofluorescence staining was performed on the nerve longitudinal sections of each group. CD31, also known as PECAM- 1, it is a key biomarker of blood vessels [27]. The $CD31^+$ immunofluorescence staining image is shown in Fig. 5A. The number and fluorescence intensity of $CD31^+$ stained cells in the repair group and autograft group were

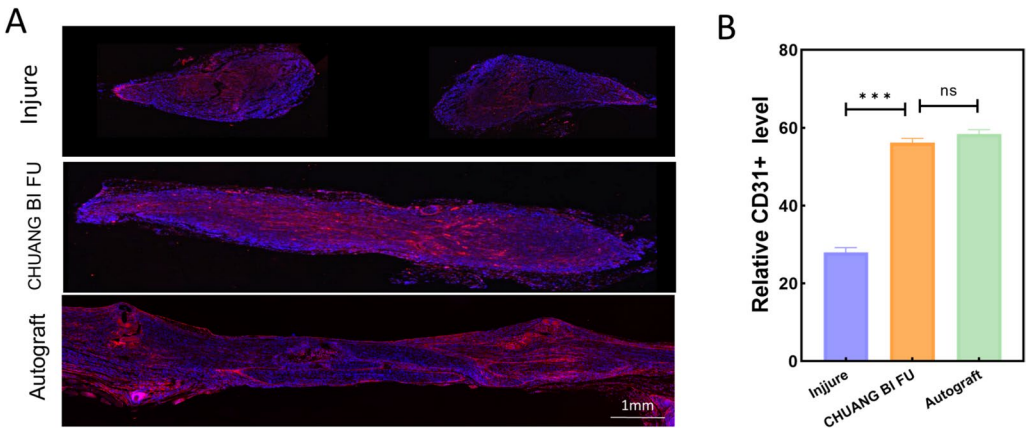

Fig. 5 Angiogenesis in mice tissue. **A** Longitudinal slice CD31$^+$ IF staining image of regenerating nerves. **B** Analysis of CD31$^+$ fluorescence intensity in mice tissue. $n = 3$, $P < 0.05$

higher than those in the injury group (Fig. 5B). Therefore, CHUANG BI FU affects the fiber direction and regeneration speed of peripheral nerve regeneration by promoting angiogenesis.

Possible molecular mechanisms of angiogenesis

Vascular regeneration is crucial for nerve injury regeneration, so we further explore the molecular mechanism of CHUANG BI FU promoting angiogenesis at the cellular level. HUVEC cells are human umbilical vein endothelial cells, which can be used to simulate the formation of blood vessels in vivo [28]. The cell scratch assay evaluates cell migration ability by simulating the wound healing process. The results of the cell scratch are shown in Fig. 6A. The results showed that the CHUANG BI FU group exhibited greater cell migration distance and area compared to the control group (Fig. 6E, F). The transwell migration experiment further demonstrated that CHUANG BI FU promotes the migration of HUVEC, thereby promoting angiogenesis (Fig. 6B).

We conducted a study on human umbilical vein endothelial cells (HUVEC cells) treated with clotrimazole and found that at the same time, in the vascular catheterization experiment (Fig. 6C), the number of vascular connections (Fig. 6J) and the area of vascular formation in the clotrimazole-treated group were higher than that in the untreated group (Fig. 6H).

Total protein was extracted for Western blot analysis from HUVEC samples that were never cultured with CHUANG BI FU and HUVEC samples co-cultured with CHUANG BI FU (Fig. 6D). The p-PI3 K/PI3 K (Fig. 6L) and P-AKT/AKT (Fig. 6K) both relative expression levels in CHUANG BI FU group was significantly higher than in control group. These results indicate that CHUANG BI FU can effectively promote the activation of PI3 K and AKT signaling pathways, which may be one of the important mechanisms for promoting angiogenesis during sciatic nerve repair (Fig. 7).

Discussion

bFGF biological protein sponge (CHUANG BI FU) is a medical active material made from collagen and basic fibroblast growth factor (bFGF) protein, which has been proven to have good effects in promoting wound healing in most clinical studies. According to reports, bFGF, alkaline fibroblast growth factor, can effectively promote axonal elongation [29, 30]. bFGF has been proven to repair long defects in peripheral nerves [31]. Although bFGF can be synthesized and secreted endogenously by neurons and SCs innervated by denervated nerves, its low expression level cannot induce the survival and regrowth of damaged nerves [32]. Therefore, exogenous supplementation of BFGF has become a solution. As protein drugs, bFGF and NGF are easily inactivated under physiological conditions, and their bioavailability varies significantly in tissues and body fluids [33]. Therefore, seeking a safe carrier for bFGF is considered to be helpful for the repair of nerve damage. As a biological sponge protein, CHUANG BI FU can effectively release bFGF at the site of nerve damage. Although previous studies have indicated that bFGF may promote fibroblast proliferation under certain conditions, our in vivo experiments showed that the regenerated tissue exhibited less collagen deposition compared to the negative control group. This suggests that the positive effects of CHUANG BI FU on neural regeneration outweigh its potential negative impacts. Further exploration will be conducted in subsequent studies.

Other bFGF carriers have been widely studied for the administration of injured areas [34], but most of

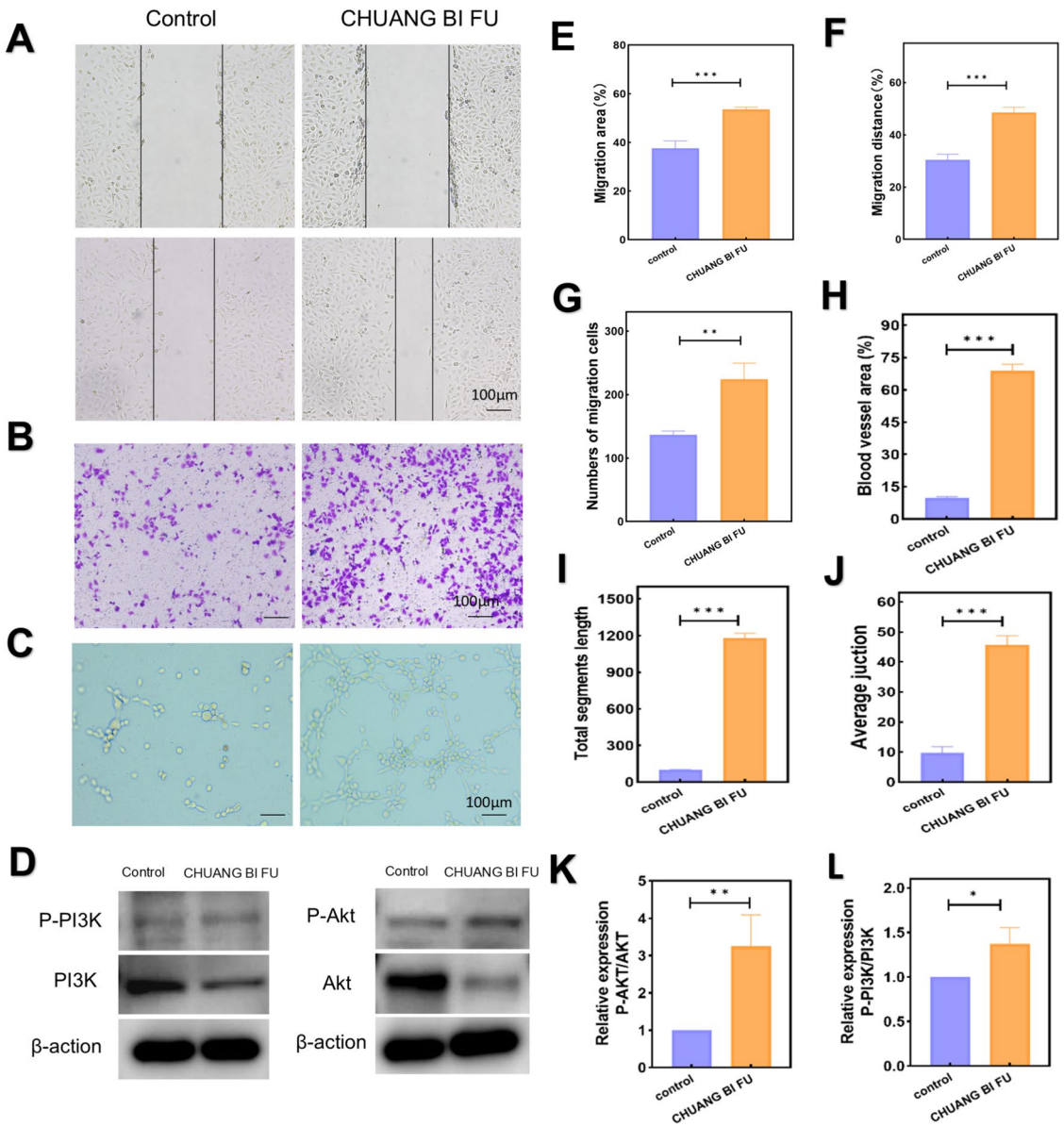

Fig. 6 Evaluation of angiogenesis. **A** Representative images of the cell scratch; **B** representative images of the transwell migration experiment; **C** representative image of HUVEC tube formation after co-culture of d cells and CHUANG BI FU for 6 h; **D** representative images of co-cultured cell Western blot; **E** area of cell migration; **F** the average number of migration diatance; **G** the average number of migration cells **H** percentage of vascular formation area; the vascular density (**I**) and average number of connections in **J** in images; **K L** analysis of Western blot; $n = 3$, $P < 0.05$

the studies focus on the use of nerve conduits or nerve conduits combined with hydrogels as carriers [35, 36]. Nerve conduit or nerve conduit combined with hydrogel requires nerve suture, which requires long operation time and requires high difficulty. Improper suture operation will cause secondary nerve injury, which is not conducive to the repair of nerve injury. Therefore, it is crucial to avoid secondary damage to the nerves.

This study proposes a simplified application of CHUANG BI FU as a new repair strategy for peripheral nerve defects using autologous transplantation as a substitute. CHUANG BI FU, as an adhesive material, can effectively wrap around damaged nerves without falling off, avoiding suturing operations and reducing surgical difficulty. At the same time, the average 30 min required for autologous transplantation suturing is reduced to 15 min, greatly reducing surgical time and minimizing secondary nerve damage. The experimental results showed that after 3 months of HE and NF200 staining of animal nerve tissue, it was evident that the nerve fibers in the

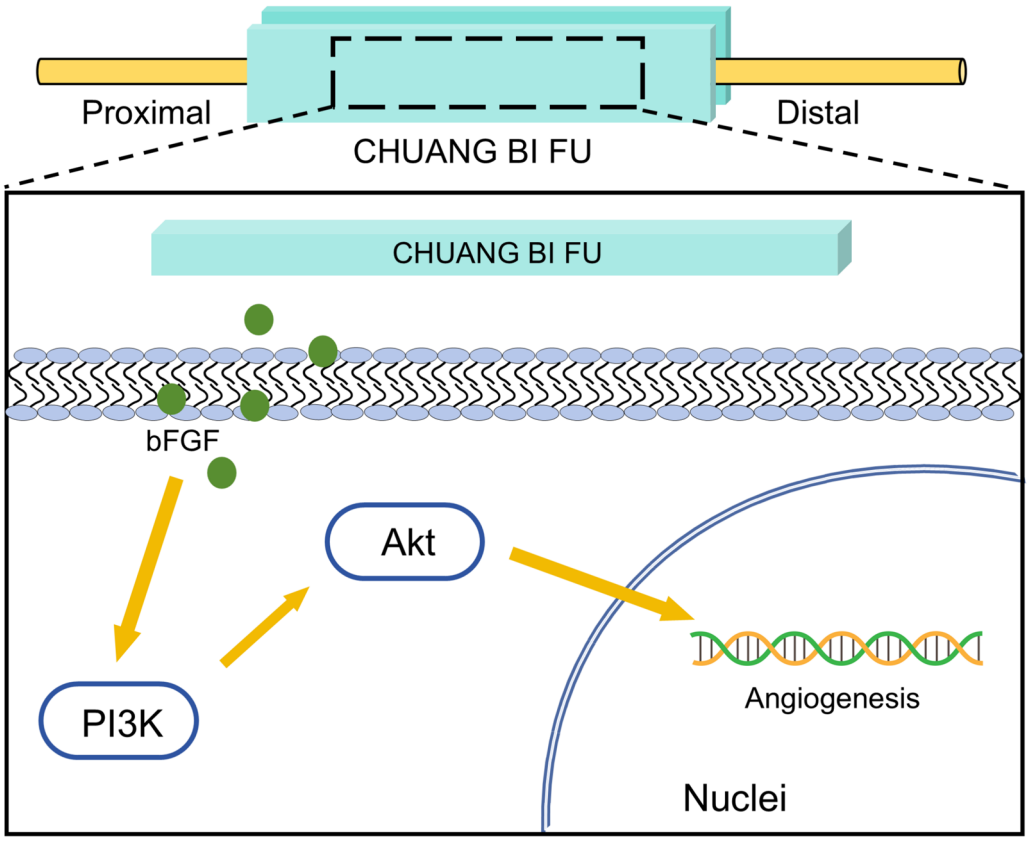

Fig. 7 Schematic diagram of possible mechanisms by which CHUANG BI FU promotes angiogenesis

autograft group and the wound repair group were more orderly and tightly arranged compared to the injury group, and the nerve fibers in the injury group had a significant formation of neuroma at the injured end. This indicates that the action of CHUANG BI FU on peripheral nerve transection injury can effectively promote the growth of nerve fiber axons. The muscle recovery rate and footprint of the autograft group and the CHUANG BI FU group were significantly better than those of the injury group, and there was no significant difference between the two groups. This proves that CHUANG BI FU not only promotes the growth of nerve fiber axons, but also prevents muscle atrophy, promotes nerve reinnervation, and nutritional function. Although the efficacy of CHUANG BI FU in nerve recovery remains inferior to that of the autologous graft group, considering the challenges in finding suitable autologous nerves for long nerve defects in clinical practice, as well as the sensory and motor impairments at the donor site, CHUANG BI FU holds promising clinical application prospects.

During the process of nerve regeneration, angiogenesis, myelin sheath regeneration [37], and axonal regeneration occur sequentially, with angiogenesis being the initial and critical stage of nerve regeneration [38, 39]. Doi et al. demonstrated that the effect of bFGF on the number of vascular arterioles, tissue perfusion and vascular density increased in a dose-dependent manner by designing the bFGF hydrogel sustained-release system [40]. bFGF is believed to effectively promote the proliferation of fibroblasts and epithelial cells, and can act as a chemical inducer to induce vascular regeneration [41, 42]. In this study, it was found that the CD31 fluorescence intensity in the nerve longitudinal section of the CHUANG BI FU treatment group and the autologous nerve transplantation group was significantly higher than that of the injury group, and the neatly arranged capillary network could be clearly seen. The results of this study, combined with literature reports, suggest that bFGF can promote vascular regeneration through the damaged area. During the process of nerve injury repair, Schwann cells migrate along blood vessels, and the shape of blood vessels is consistent with that of nerve fibers [43, 44]. Therefore, while promoting vascular regeneration, bFGF indirectly promotes the regeneration of transverse nerves. In order to explore the possible mechanism of promoting angiogenesis, we conducted a study on HUVECs treated with

clotrimazole and found that the number of vascular connections and vascular formation area in the treated group were higher than those in the untreated group. The P-PI3 K/PI3 K levels in the treatment group of CHUANG BI FU were significantly higher than those in the control group, indicating that CHUANG BI FU can promote PI3 K phosphorylation through bFGF AKT is a downstream molecule regulated by the PI3 K signaling cascade [45], and the phosphorylation of AKT in the CHUANG BI FU group was significantly higher than that in the control group. Therefore, it is speculated that CHUANG BI FU may regulate vascular regeneration during nerve regeneration through the PI3 K–AKT signaling pathway. The above mechanism is a potential pro angiogenic molecular mechanism in neural regeneration. In future research, efforts will be made to identify key molecular mechanisms controlling angiogenesis, which is of great significance for peripheral nerve regeneration.

Conclusion

In this study, we extended the application of CHUANG BI FU to new clinical scenarios and developed a new suture free surgical strategy. Based on the adhesive properties of the CHUANG BI FU sponge, it avoids secondary damage to nerves caused by surgical sutures, and greatly reduces the surgical time and technical requirements for operators. The effect of CHUANG BI FU on nerve regeneration was evaluated in vivo, and the results showed that the regenerated sciatic nerve had good functional and morphological recovery. This may be due to the promotion of angiogenesis by CHUANG BI FU through the PI3 K–AKT pathway, and the promotion of nerve regeneration by guiding axonal elongation through vascular regeneration. Therefore, the combination of CHUANG BI FU biological sponge and its simplified application can effectively guide nerve regeneration, providing a new comprehensive surgical strategy for peripheral nerve regeneration and offering new ideas for the difficult problem of peripheral nerve injury regeneration in clinical practice.

Supplementary Information

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Supplementary Material 1

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Author contributions
YC did so and collected all the data. ZW and PW conceived and designed the experiments. JL, RH, ZM and KY analyzed the data. XL and JW contributed reagents/materials/analysis tools. All authors reviewed the manuscript.

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Availability of data and materials
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent of publication
Not applicable.

Competing interests
The authors declare no competing interests.

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