Negative Pressure Therapy in the Regeneration of the Sciatic Nerve Using Vacuum – Assisted Closure in a Rabbit Model

Background: The aim of this study was to investigate the effects of negative pressure therapy in the regeneration of the rabbit sciatic nerve using vacuum assisted closure (VAC).

Material/Methods: Thirty male New Zealand white rabbits underwent surgical injury of the sciatic nerve, followed by negative pressure therapy using vacuum assisted closure (VAC), in three treatment groups: Group A: 0 kPa; Group B: –20 kPa; Group C: –40 kPa. At 12 weeks following surgery, the following factors were studied: motor nerve conduction velocity (MNCV); the number of myelinated nerve fibers; the wet weight of the gastrocnemius muscle. Gastrocnemius muscle and sciatic nerve tissue samples were studied for the expression of S100, and brain-derived neurotrophic factor (BDNF) using Western blot.

Results: At 12 weeks following VAC treatment, the MNCV, number of myelinated nerve fibers, and wet weight of the gastrocnemius muscle showed significant differences between the groups (p<0.05), in the following order: Group B > Group A > Group C. The sciatic nerve at 12 weeks following VAC in Group B and Group C showed a significant increase in expression of S100 and BDNF when compared with Group A; no significant differences were detected between Group B and Group C results from Western blot at 12 weeks.

Conclusions: The findings of this study, using negative pressure therapy in VAC in a rabbit model of sciatic nerve damage, have shown that moderate negative pressure was beneficial, but high values did not benefit sciatic nerve repair.

MeSH Keywords: Contingent Negative Variation • S100 Proteins • Wound Healing

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/906696
Background

The first use of the technique of vacuum-assisted closure (VAC) in wound repair was reported in 1993 by Fleischman [1,2]. Currently, the dressing materials used in VAC consist of polyvinyl alcohol (PVA) or polyurethane (PU) foam dressings; each wound is covered with an appropriately-sized dressing that is covered with adhesive [3]. A pad is then applied to an evacuation tube attached to the equipment to provide controlled negative pressure and to discharge exudate from the wound [3]. The procedure of VAC has now been widely used to promote wound healing by promoting cytokines and growth factors that promote wound healing [4,5].

Repair and regeneration of peripheral nerve defects remain a challenge in orthopedic surgery and neurosurgery. Several methods have been reported to improve nerve repair, but autogenous nerve grafting has been regarded as the gold standard [6]. However, autogenous nerve grafting has some disadvantages as it may cause damage to healthy nerves, sensory disturbance, and painful neuromas [7,8].

In 2010, Sharula et al. compared the effect of nerve-lengthening and autogenous nerve grafting in the repair of the sciatic nerve defect with a direct gradual lengthening of the proximal and distal nerve stumps in rabbits [9]. The results of this previous study showed that the rabbits in the nerve-lengthening group, achieved by long-term nerve stretching, showed improved results when compared with the autografting groups, including the electrophysiological index as well as improved changes on histological examination [9]. In 2015, a study by Hu and colleagues explored the influence of VAC on the injured nerve in an animal model, and the results showed that VAC improved repair the injured peripheral nerve [10].

However, high-intensity traction may damage recently-sutured peripheral nerve [11]. The use of VAC with the normal value of negative pressure may benefit peripheral nerve growth when the negative pressure value ranges from –125 to –200 Hg (1 kPa=7.5 mmHg). However, the effects of a high value of negative pressure on peripheral nerve growth remain unknown.

The aim of this study was to investigate the effects of negative pressure therapy, including 0 kPa, –20 kPa, and –40 kPa, in the regeneration of the rabbit sciatic nerve using VAC.

Material and Methods

Animals and experimental groups

Thirty male New Zealand white rabbits, with a weight that ranged from 3.5–4.5 kg, age range from 3.5–6.0 months were obtained from the Animal Experimental Center of Suzhou University, Suzhou, China. All animal experiments were approved by the Institutional Animal Care and Use Committee of Suzhou University (Suzhou, China).

Thirty rabbits were randomly assigned following the surgical injury of the sciatic nerve, and negative pressure therapy using vacuum assisted closure (VAC), into three treatment groups: Group A: 0 kPa; Group B: –20 kPa; Group C: –40 kPa. The weight of the gastrocnemius muscle, the motor nerve conduction velocity (MNCV), and the number of myelinated fibers at 12 weeks after the application of VAC were conducted for the three different groups.

Surgical procedure

The surgical operator identified the rabbit ear vein and then injected a 3% solution of sodium pentobarbital (30 mg/kg). A 2.5cm incision was made on the right side of the thigh to expose the sciatic nerve, which was then cut and sutured back with 8-0 surgical suture. The injured nerve was covered with peripheral muscle and then covered with a dressing and VAC was used (Group A: 0 kPa; Group B: –20 kPa; Group C: –40 kPa) with Intramuscular injection of penicillin (400,000 U/Ci), which was given for three days after surgery (Figure 1).
**Motor nerve conduction velocity (MNCV)**

The motor nerve conduction velocity (MNCV) measurements were performed at 12 weeks postoperatively, at the same nerve site. The MNCV was defined as the distance between the recording electrode and the stimulating electrode (m)/the operating time (s). The MNCV was measured in triplicate, and the average value was recorded [12].

**Number of myelinated fibers**

The number of myelinated fibers at ×400 magnification on light microscopy were measured at 12 weeks after the application of VAC to the sciatic nerves. The sciatic nerve anastomoses from the three groups of rabbits (A, B, C) were fixed in 10% formaldehyde, embedded in paraffin wax before sectioning and histochemical staining with 1% toluidine blue. Scoring of nerve regeneration of myelinated fibers was performed by light microscopy at ×400 magnification.

**Wet weight of the gastrocnemius muscle**

At 12 weeks, both gastrocnemius muscles (right and left) were harvested from each rabbit and immediately weighed on an electronic precision scale. The data was expressed as a percentage of contralateral values (ipsilateral/contra-lateral ×100%).

**Western blot**

Sciatic nerve tissue was collected from the areas of the surgical defects at 12 weeks and homogenized to explore the expression of Schwann cell marker S100 and brain-derived neurotrophic factor (BDNF) by Western blot. The homogenates were transferred to centrifuge tubes and centrifuged at 1,000×g for 15 min at room temperature. Proteins were separated (50 µg) by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes following the dissolution of aliquots in Laemmli buffer (Haoran Bio, Shanghai, China). The membranes were blocked using 5% dried milk powder for 10 min and incubated overnight at 4°C with primary rabbit polyclonal antibodies to S100 (Cat. No: bs1012R; dilution, 1: 200) and BDNF (Cat. No: bs0014R; dilution, 1: 10,000) (Beijing Biosynthesis Biotechnology Co., Ltd., Beijing, China). The blots were rinsed several times with Tris buffered saline (TBS) containing 0.05% Tween 20. Positive signals were observed after the blots were incubated with enhanced chemiluminescence reagent (Elabscience Biotechnology Co., Ltd, Beijing, China) for 12 min.

**Immunofluorescence**

For immunofluorescence staining, cells were incubated with anti-S100 and anti-BDNF antibodies (dilution 1: 200) (Santa Cruz Biotechnology) at 4°C overnight following fixation in 4% paraformaldehyde. Sections were then incubated with secondary antibody (1: 100 dilution) for one hour; 4',6-diamidino-2-phenylindole (DAPI) was used to counterstain nuclei (blue). The cells were incubated for 5 min with chromogen and examined by microscopy.

**Statistical analysis**

Statistical analysis of data was performed using SPSS version 21.0 (SPSS Inc., Chicago, Ill, USA). The data were expressed as the mean ±SD. The MNCV, the wet muscle weight, the number of myelinated fibers, and Western blot data were compared using one-way ANOVA (analysis of variance) and q-test. A value of p<0.05 was considered to be significant between groups.

**Results**

**Motor nerve conduction velocity (MNCV)**

The mean motor nerve conduction velocity (MNCV) in the Group A was 24.64±6.35 m/second; the MNCV in the Group B was 30.26±7.9 m/second; the MNCV in Group C was 20.04±5.0 m/second, at 12 weeks following VAC to the sciatic nerves. There were significant differences between the three groups: Group B >Group A >Group C (p<0.05) (Figure 2).

**Number of myelinated fibers**

At 12 weeks, the number of myelinated fibers was 12.1±2.63×10³ in Group A; the number of myelinated fibers was 15.26±4.21×10³ in Group B; the number of myelinated fibers was 9.10±2.69×10³ in Group C, at ×400 magnification. There were significant differences between the three groups: Group B >Group A >Group C (p<0.05) (Figure 3).

**Wet weight of the gastrocnemius muscle**

At 12 weeks, the mean wet weights of the gastrocnemius muscles were 30.0±5.0 g (Group A), 35.9±4.6 g (Group B), and 49.65±5.9 g (Group C). There were significant differences between the two sides of the rabbits were 55.9±8.2 g (Group A), 60.2±6.9 g (Group B), and 49.65±5.9 g (Group C). There were significant differences between the three groups: Group B >Group A >Group C (p<0.05) (Figure 4).

**Expression of S100 and brain-derived neurotrophic factor (BDNF)**

A 12 weeks, Group B and Group C showed significantly increased expression of S100 and BDNF when compared with
Group A (p<0.05); no significant differences were found between Group B and Group C groups (p>0.05) (Figures 5–7).

**Discussion**

The effects of negative pressure therapy using vacuum-assisted closure (VAC) was studied in the sciatic nerve of rabbits during a 12-week period. In previous studies, VAC technology has shown promise in nerve repair and regeneration, by enhancing the vascularization of wounds, inhibiting infection, and promoting the regeneration of peripheral nerve growth when compared with other therapeutic methods [13,14]. Although the present study showed that the use of VAC might help to repair damaged peripheral nerve, the optimal negative pressure therapy remains to be determined.

**Figure 2.** The histogram shows the motor nerve conduction velocity (MNCV) measurements (m/second) in the three groups of rabbits (A, B, C) at 12 weeks. Group A: Vacuum-assisted closure (VAC) at 0 kPa. Group B: VAC at −20 kPa. Group C: VAC at −40 kPa. * Indicates P<0.05 between the groups.

**Figure 3.** The histogram shows the number of myelinated nerve fibers in the three groups of rabbits (A, B, C) at 12 weeks. Group A: Vacuum-assisted closure (VAC) at 0 kPa. Group B: VAC at −20 kPa. Group C: VAC at −40 kPa. * Indicates P<0.05 between the groups.

**Figure 4.** The histogram shows the wet weight of the gastrocnemius muscle in the three groups of rabbits (A, B, C) at 12 weeks. Group A: Vacuum-assisted closure (VAC) at 0 kPa. Group B: VAC at −20 kPa. Group C: VAC at −40 kPa. * Indicates P<0.05 between the groups.

**Figure 5.** Western blot for S100 and brain-derived neurotrophic factor (BDNF) protein expression levels in the three groups of rabbits (A, B, C) at 12 weeks. Group A: Vacuum-assisted closure (VAC) at 0 kPa. Group B: VAC at −20 kPa. Group C: VAC at −40 kPa. BDNF – brain-derived neurotrophic factor.
Figure 6. Immunofluorescence shows the S100 and brain-derived neurotrophic factor (BDNF) protein expression in the three groups of rabbits (A, B, C) at 12 weeks. (A) Immunofluorescence staining for S100. (B) Immunofluorescence staining for brain-derived neurotrophic factor (BDNF). Group A: Vacuum-assisted closure (VAC) at 0 kPa. Group B: VAC at −20 kPa. Group C: VAC at −40 kPa.
pressure value and the mechanism of action of negative pressure therapy using VAC remain unclear. The results of this study showed that VAC could be used as a method for the regeneration of peripheral nerve defects. However, the too high a value of negative pressure may not be beneficial.

The repair of nerve injury is a complex process, involving molecular and cellular mechanisms [15]. In many cases, nerve regeneration and growth cannot occur, as some inhibitory factors for axons regeneration exist [16,17]. However, the nerve repair associated with VAC could be promoted in several ways. Firstly, VAC could increase the quality and quantity of capillaries in the surrounding environment, with the improved microcirculation providing improved oxygenation, which could benefit to the damaged nerve [18,19]. Several cell factors have been confirmed to accelerate nerve repair, including substance P, vascular endothelial growth factor (VEGF), and platelet-derived growth factor (PDGF) [20–22]. These and other nerve growth factors could be used to improve the effects of VAC [23–27]. Secondly, VAC could also reduce local inflammation, relieve local hyperemia and edema, to stabilize the surrounding environment and benefit nerve regeneration. However, the limitation of VAC is that it can cause nutrient loss from the wound, and its excessive use may be costly. Therefore, the indications for the use of VAC should include repair of acute wounds, coverage of refractory wounds, and control of infected wounds [17].

Schwann cell proliferation and differentiation indicate regeneration of the peripheral nerve [28,29]. Furthermore, S100 protein detection is commonly used to identify Schwann cells [28,29]. This study showed that more S100 was detected as the value of the negative pressure increased, so it is possible that VAC could promote the regeneration of peripheral nerve by increasing the expression of S100.

Brain-derived neurotrophic factor (BDNF) is expressed in multiple regions of central neurons and peripheral nerves and is a protein that is considered to be closely related to the activity and regeneration of neurons [30]. A previous study has shown that BDNF promotes axonal regeneration in vitro and in vivo, as soon as peripheral nerves become injured [31]. Therefore, based on the findings from this study, it might be possible that VAC can promote rabbit sciatic nerve repair by increasing the expression of BDNF following nerve injury.

According to the electrophysiological index used in our study, the usual value of negative pressure used in wound treatment (–20 kPa) could also be of benefit to the repair of peripheral nerves. However, too high a value of negative pressure (–40 kPa) can cause nerve damage or inhibit nerve repair, possibly by causing excessive traction to the injured nerve, which results in further damage.

**Conclusions**

The findings of this study, using negative pressure therapy in VAC in a rabbit model of sciatic nerve damage, have shown that moderate negative pressure was beneficial, but high values did not benefit sciatic nerve repair.

**Competing interests**

None.

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**Figure 7.** The histogram shows the S100 and brain-derived neurotrophic factor (BDNF) protein expression in the three groups of rabbits (A, B, C) at 12 weeks. (A) Immunofluorescence staining for S100. (B) Immunofluorescence staining for brain-derived neurotrophic factor (BDNF). Group A: Vacuum-assisted closure (VAC) at 0 kPa. Group B: VAC at –20 kPa. Group C: VAC at –40 kPa. BDNF – brain-derived neurotrophic factor. ** Indicates P< 0.01 between the groups.
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