

## Effect of Vascular Endothelial Growth Factor (VEGF) Loaded on Gelatin Sponge on Transected Facial Nerve Regeneration

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### Abstract

**Objective:** To evaluate the local effect of vascular endothelial growth factor (VEGF) loaded on gelatin sponge on transected facial nerve regeneration.

**Methods:** Twelve male white SD rats were divided into two groups randomly (n=6): VEGF group and PBS (phosphate buffer saline) group. In the VEGF group the left facial nerve was transected and the stump was connected by silicone conduit filled with gelatin sponge loaded with VEGF. In PBS group the defect was connected by silicone conduit filled with gelatin sponge loaded with PBS. Functional assessment and morphological evaluation were studied 8 weeks after operation.

**Results:** Functional study of facial nerve, Electrophysiological assessment and morphometric indices confirmed a faster recovery of regenerated axons in VEGF group than in PBS group (P<0.01).

**Conclusion:** Local administration of nerve conduit consisting of VEGF and gelatin sponge will improve functional recovery and morphometric indices of facial nerve.

**Keywords:** Facial nerve; Vascular endothelial growth factor (VEGF); Nerve regeneration

### Introduction

Currently, facial nerve regeneration is a major clinical problem. There is a short-term and long-term experience of pain followed by the facial nerve injury [1-4]. During the acute phase of facial nerve injury, patients often suffer of asymmetrical facial movement, incomplete eye closure, nose collapse and other clinical manifestations. Long-term complications include flaccid facial paralysis and incomplete functional recovery. Motor nerve fibers scattered growth will lead to facial synkinesia. In addition to the patient face and nerve functions, facial paralysis will affect the patient's interaction seriously, which resulting in emotional expression in patients with psychological problems [5,6]. There is a common method of autologous nerve transplantation to treat nerve defects [7,8]. However, autologous nerve transplantation requires donor of nerve injury. Autologous nerve transplantation clinical application is not ideal [9,10] because of limited sources of donor. Therefore, searching for a new method for facial nerve regeneration becomes a hot topic.

In this study, we used silicone tube as nerve conduit connecting nerve ends to guide the growth of nerve. Early studies showed that there is a good effect of silicone tube connected to nerve ends to repair nerve damage. In addition, more inert silicone tube does not form scar in vivo easily [11]. Vascular endothelial growth factor (VEGF) was applied on injured facial nerve to improve the nerve regenerative capacity. Facial nerve transection model made first in rats with VEGF incubating on the gelatin sponge. Then gelatin sponge was filled in the

silicone tube connected nerve ends. The behavior of rats, as well as electrophysiology, regenerative nerve myelin sheath thickness, was analyzed to evaluate nerve regeneration in rats in each group after eight weeks post-operation.

### Materials and Methods

#### Reagents and materials

VEGF was purchased from peprotech company. Silicone tube purchased from Shanghai Metal Rubber Co., Ltd. Gelfoam were purchased from Guangzhou Express Kang Medical Devices Co., Ltd. Male SD rats were purchased from Experimental Animal Slack Limited.

#### Experimental design

Twelve white SD rats, weighing about 200g, were randomly assigned to two groups: VEGF group and PBS group (n=6). During the experiment, rats breeding environment temperature (23 ± 3)°C, air humidity stable, rats had easy accessed the animal feed and drinking water.

#### Methods

Experimental operating strictly in accordance with the relevant provisions of the Ministry of Science in September 2006 issued "Guidance for the Care of Laboratory Animals," experimental rats were intraperitoneal anesthetized by 4% pentobarbital sodium (30 mg/kg), following skin incision, separating the muscle, exposing the buccal

branch of the facial nerve. Nerve defects of 5 mm long were made from cutting section of the facial nerve buccal branch. In the VEGF group, 2 micrograms of VEGF was hatched on the gelatin sponge and then gelatin sponge was filled into the silicone tube. In the PBS group, 2 micrograms of bFGF was incubated on gelatin sponge and then gelatin sponge filled into the silicone tube. 10/0 nylon was used to suture silicone tubing and nerve ends. Sutured muscle, skin. Rats were sacrificed by cardiac perfusion of 4% paraformaldehyde after 8 weeks post-operation.

### Motor function score

Tentacles motor scores in the eighth post-operative week. Normal rats tentacles forward cocked and rhythmic blowing. After the facial nerve injury, namely caudal tentacles hang down, and the loss of blowing capacity. Tentacles movement can be divided into the following five levels [12]: 0 level - no moving tentacles; 1 level - tentacles trace observable movement; 2 level - a small amount of movement tentacles; 3 level - tentacles asymmetric movement; 4 level - tentacles symmetrical movement.

### Nerve conduction velocity measurements

Experimental rats were anesthetized of 4% sodium pentobarbital (30 mg/kg) in the eighth week post-operation, following up skin and muscle incision, then New-born facial nerve exposed. Regeneration of nerve conduction velocity was detected electrophysiological. The facial nerve stimulation electrode was placed proximal end, the receiving electrode placed in the distal end of facial nerve, regeneration of nerve conduction velocity detected to evaluate the facial nerve function recovery. Stimulation intensity of 5 V, pulse width of 0.2 ms, frequency 1 Hz.

### TEM

The rats were perfused and sacrificed in the eighth week post-operation, with neurogenesis exposed, silicone tube discarded. The neurogenesis was put quickly into the electron microscopy fixative and then myelin observation followed.

**Fixed:** Fixed for 2-4 hrs under 4°C, rinsed for 3 times by 0.1 M phosphate buffer solution PBS (PH 7.4), each time 15 min.

**After fixation:** Fixed for 2 hrs by 1% osmium tetroxide • 0.1M phosphate buffer PBS (PH 7.4) at room temperature (20°C). Rinsed for 3 times by 0.1M phosphate buffer solution PBS (PH 7.4), each time 15 min.

**Dehydration:** Dehydrated on alcohol sequentially organized into 50%, 70%, 80%, 90%, 95%, 100% and 100%, each time 15 min.

**Infiltration:** Acetone ratio 812 embedding medium of 1 to 1 mixture permeate filtrate, pure 812 embedding medium penetration filtrate.

**Embedding:** Polymerization for 48 hrs under 60°C.

**Slice:** 60-80 nm thin slices by microtome.

**Stain:** Uranium-lead double staining (2% uranyl acetate saturated aqueous lead citrate, each stained for 15 min), dried at slice chamber overnight.

**Observation:** Transmission electron microscope observation and image acquisition analysis.

### Statistical analysis

Experimental data were expressed as mean ± standard deviation. In this study, statistical methods for two independent samples 't' test using statistical software SPSS 13.0 line two samples were compared, P<0.05 was considered statistically significant.

### Results

#### Tentacles motor function

Normal rats tentacles forward cocked and rhythmic blowing. After the facial nerve injury, namely caudal tentacles hang down, and the loss of blowing capacity. Among tentacles motor function scores, higher scores indicates better functional recovery, 0 level represents complete loss of motor function, 4 level indicates motor function fully restored. Statistics showed that VEGF group was significantly higher than the mean score PBS group, and the difference was statistically significant (P<0.01) (Table 1) in the eighth week post-operation.

Group	Sample size	Score
VEGF	6	3.17 ± 0.37
PBS	6	1 ± 0.58
t		7.05
p		<0.01

Table 1: Tentacles motor function score (mean ± standard deviation).

#### Nerve conduction

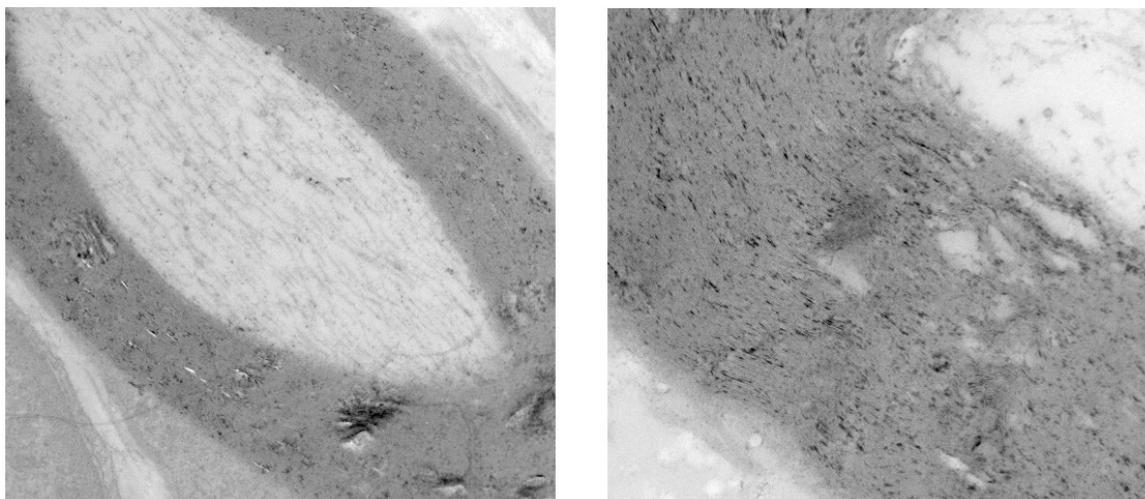
Rats facial nerve regeneration can be reflected by nerve conduction velocity. The faster nerve conduction velocity, the better representation of neural regeneration. Two groups of rats electrophysiological test in the eighth week post-operation showed nerve conduction velocity in HGF group were significantly higher than PBS group rats (P<0.01) (Table 2).

Group	Sample size	NCV(m/s)
VEGF	6	47.33 ± 7.65
PBS	6	24.33 ± 4.57
t		5.77
p		<0.01

Table 2: Nerve conduction velocity in HGF group.

#### Morphology

The middle of the regenerated nerve was observed through transmission electron microscopy in the eighth week post-operation. In VEGH group, nerve fiber bundles are packaged by thin perineurium, which contains a large amount of regeneration of nerve fibers and blood vessels. It showed a large of nerve fibers and myelin maturation on cross-section of the middle nerve under high magnification. Myelin thickness in VEGH group was significantly higher than PBS group of rats, the difference was statistically significant (P<0.01) (Table 3 and Figure 1).



**Figure 1:** The electron microscopy of rat myelin regeneration surface morphology after facial nerve injury in each group.

Group	Sample size	Value $\mu$ m
VEGF	3	0.82 $\pm$ 0.08
PBS	3	0.4 $\pm$ 0.03
t		8.7
p		<0.01

**Table 3:** Myelin sheath thickness (mean  $\pm$  standard deviation).

## Discussion

We used gelatin sponge as VEGF carrier in the present study. Gelatin sponge is used clinically of a biological material. Gelatin sponge is widely used because of the following [13]: (1) Good biodegradability. (2) Good biocompatibility in vivo. Gelfoam in vivo has certain physiological functions and there are cellular interactions between Gelfoam and its surrounding, such as bleeding, as well as filling injured tissue. (3) Low immunogenicity. It is widely used in clinical practice because of very low immunogenicity after implant into body. In addition to the above advantages, the gelatin sponge as a carrier can also act as trophic factors or drugs. Therefore, we used gelatin sponge as carrier of VEGF.

In this study, we used VEGF in partial area of facial nerve injury which was hatched on the gelatin sponge and then filled in silicone tubes in order to promote nerve regeneration. The silicone tube and gelatin sponge were used as a scaffold to limit the rapid spread of VEGF. Being the facial nerve dominates orbicularis muscle, it grows up from the proximal to the distal to dominate orbicularis muscle movement dropped again during facial nerve regeneration, which directly related to tentacle movement. The results shows that VEGF group of rats tentacles movement score is significantly higher than PBS group of rats, which reflects the VEGF indirectly contributes to reinnervation of end-organ. There is close relationship between nerve conduction velocity, which reflects the maturity, and myelination of nerve fiber regeneration. Fast conduction of nerve fibers means the better degree of integration of myelin [14]. Results shows that nerve

conduction velocity of VEGF group is significantly higher than of PBS group, the difference is statistically significant. For morphological study transmission electron microscopy shows that the thickness of the myelin sheath of nerve fibers regenerated in VEGF group is significantly higher than in PBS group. Owing to the functional and morphological findings in VEGF group better than in PBS group, nerve conduit using VEGF and gelatin sponges partial can promote nerve regeneration.

The results shows there is many factors which is related to VEGF promoting effectively facial nerve regeneration. It has shown that VEGF can promote angiogenesis according to numerous studies [15-17]. New blood vessels provide nutrients and oxygen for nerve regeneration, so as to promote nerve regeneration [18]. Recent studies have shown that VEGF can protect damaged neurons or nerve after cerebral ischemia, spinal cord injury [19,20]. Nerve damage leads to reduced oxygen content in turn causes nerve edema through reducing blood vessels of nerves. Nerve tissue edema increases volume of nerve tissue and enlarges nerve sheath tension, causing further aggravate microcirculation disorders and oppression to normal nerve tissue, which ultimately loses of nerve function [21]. Another study reported that blood vessels of epineurium dilate after compression relieved of the nerve sheath when the facial nerve compression, nerves changing from white to red, facial nerve function gradually regenerating [22]. Pathology and nerve conduction velocity prompt that angiogenesis can alleviate pathological damage and improve motor function after nerve injury.

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