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# Ganglioside promotes the bridging of sciatic nerve defects in cryopreserved peripheral nerve allografts

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## Abstract

Previous studies have shown that exogenous gangliosides promote nervous system regeneration and synapse formation. In this study, 10 mm sciatic nerve segments from New Zealand rabbits were thawed from cryopreservation and were used for the repair of left sciatic nerve defects through allograft bridging. Three days later, 1 mL ganglioside solution (1 g/L) was subcutaneously injected into the right hind leg of rabbits. Compared with non-injected rabbits, muscle wet weight ratio was increased at 2–12 weeks after modeling. The quantity of myelinated fibers in regenerated sciatic nerve, myelin thickness and fiber diameter were elevated at 4–12 weeks after modeling. Sciatic nerve potential amplitude and conduction velocity were raised at 8 and 12 weeks, while conduction latencies were decreased at 12 weeks. Experimental findings indicate that ganglioside can promote the regeneration of sciatic nerve defects after repair with cryopreserved peripheral nerve allografts.

***Key Words:*** *nerve regeneration; ganglioside; peripheral nerve; bridge; repair; nerve graft; cryopreservation; nerve allograft; sciatic nerve; neural regeneration*

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## Introduction

Transplantation of autologous nerves is regarded as the optimal method for restoring peripheral nerve defects (Han, 2011; Yuksel et al., 2014). However, the application of autologous nerves is limited due to loss of neurological function, restriction of donor tissue, and deterioration of donor injury (Chen et al., 2012; Li et al., 2012; Luo et al., 2013). Rapid development of tissue engineering technology allows the use of a variety of artificial nerves in the repair of peripheral nerve defects, and these nerve grafts are shown to promote nerve regeneration in animals (Huang et al., 2012; Wang et al., 2012; Xiang et al., 2012; Tang et al., 2013; Krause et al., 2014). Artificial nerve, skeletal muscle, autologous veins, amnion and other materials contribute to the repair of peripheral nerve injury (den Dunnen and Meek, 2001). Nerve allograft transplantation has been widely studied along with the progress and development of transplantation technology. Cryopreservation of nerve grafts is an area of research in neurosurgery that is intensely focused on neurological function and post-transplantation effects.

Gangliosides belong to a class of sialic acid-containing glycosphingolipid that are widely present in the cell membrane of vertebrates, especially in the brain of the fetus and newborn infant. Ganglioside therefore plays a crucial role in neuronal differentiation, neurite outgrowth, and synapse formation (Ning and Chen, 2009). This study aimed to observe the effect of ganglioside on the regeneration of sciatic nerve defects after repair with cryopreserved nerve allograft.

## Materials and Methods

### Experimental animals

Sixty healthy adult New Zealand rabbits, irrespective of sex, at 45–90 days old, weighing 1.0–2.0 kg (mean 1.5 kg), were purchased from the Experimental Animal Center, Qilu Hospital of Shandong University, China (license No. SCXK (Lu) 2004-0013). Experimental disposals were approved by the Ethics Committee, Qilu Hospital of Shandong University, China. Among the 60 rabbits, six were randomly selected as donors and the remaining 54 were the recipients. Recipient animals were randomly divided into ganglioside + nerve allograft group and nerve allograft group.

### Preparation of frozen sciatic nerve allograft

Donor rabbits were anesthetized with intravenous injection of 30 g/L sodium pentobarbital (1 mg/kg) *via* the ear vein, and bilateral sciatic nerves were cut. A midline incision was then made along the thigh skin and subcutaneous tissue was resected. Blunt dissection was then used to expose the sciatic nerve between the biceps femoris and semitendinosus muscles. A segment of 10 mm sciatic nerve was prepared, dried and cryopreserved in the refrigerator (Haier Group, Qingdao, Shandong Province, China). When the freezing temperature was −40°C and drying chamber temperature was −30°C, both the drying rack and nerve tissue were placed in the drying chamber for about 4 hours to allow precooled drying. Cryopreservation was implemented when the vacuum degree reached 1.33 Pa, then the vacuum system was closed and specimens were stored at room tem-

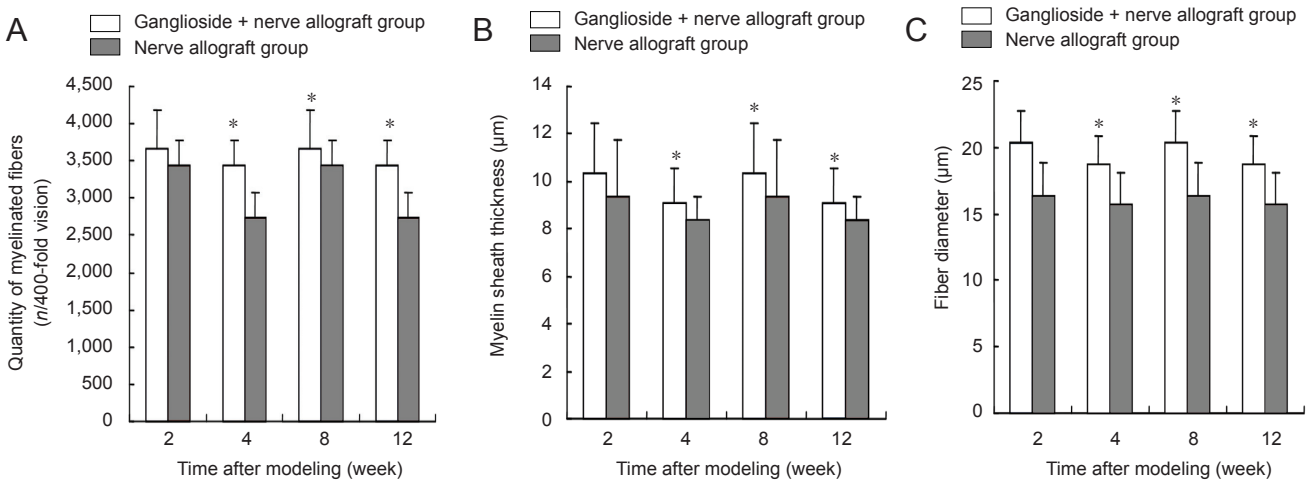


**Figure 4 Effect of ganglioside on nerve fibers in rabbit sciatic nerve repaired with cryopreserved peripheral nerve allograft.**
(A) Quantity of myelinated fibers, (B) myelin sheath thickness and (C) fiber diameter. Data were expressed as the mean ± SD. There were six rats at 2, 4, and 8 weeks and nine rats at 12 weeks in each group. The differences were compared using paired sample *t*-test. ∗$P < 0.05$, *vs.* nerve allograft group.

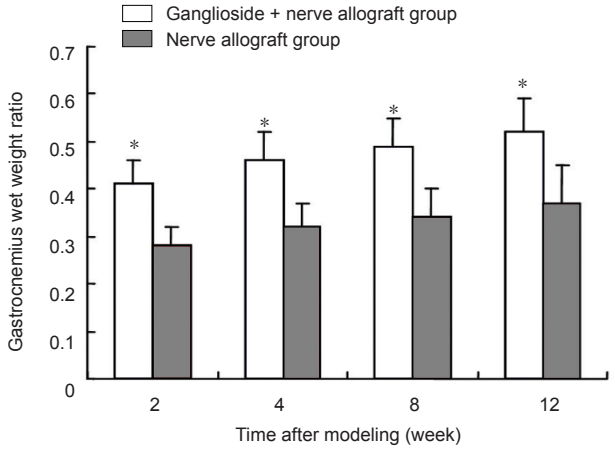


**Figure 3 Effect of ganglioside on gastrocnemius wet weight in rabbit sciatic nerve repaired with cryopreserved peripheral nerve allograft.**
Gastrocnemius wet weight ratio = wet weight of gastrocnemius muscle on the injured side/wet weight of gastrocnemius muscle on the contralateral side. Data were expressed as the mean ± SD. There were six rats at 2, 4, and 8 weeks and nine rats at 12 weeks in each group. The differences were compared using paired sample *t*-test. ∗$P < 0.05$, *vs.* nerve allograft group.

perature for further use.

### Cryopreserved nerve allograft bridging of sciatic nerve defects

All recipient rabbits were anesthetized with intravenous injection of 30 g/L sodium pentobarbital (1 mg/kg) *via* the ear vein, and a midline incision was made along the skin of the left thigh and subcutaneous tissue was resected, exposing the sciatic nerve between the biceps femoris and semitendinosus through blunt dissection. A segment of 10 mm sciatic nerve was cut, as previously in the donor, and a sciatic nerve defect was produced at 5 mm above the sciatic nerve bifurcation. Subsequently, the donor tissue was immersed in saline for 3–4 hours and was used to repair sciatic nerve defects through bridging (Yang et al., 2011). Rabbits were given oral antibiotics to prevent infection post-operation.

### Ganglioside injection

Three days after modeling, rabbits in the ganglioside + nerve allograft group were subcutaneously injected with 1 mL ganglioside solution into the right hind legs every day. Ganglioside injection (1 g/L) was provided from Qilu Hospital of Shandong University in China.

### Electrophysiological detection

At 2, 4, 8, and 12 weeks after modeling, rabbits in the two groups were detected using EMG evoked potentials (Nanjing Repson Biotechnology Co., Ltd., Nanjing, Jiangsu Province, China). Stimulation electrodes were placed on the proximal and distal ends of the left sciatic nerve allograft, and recording electrodes were placed in the tibialis anterior muscle belly. Sciatic nerve was stimulated at 60 mV, 200 Hz, to determine potential amplitude, conduction velocity and conduction latencies.

### Ultrastructural observation

Nerve segments in each group were sutured and fixed in glutaraldehyde solution, and sliced into ultrathin sections (0.2 mm thick) for electron microscopy observation. Paraffin sections were observed under transmission electron microscopy (JEOL), and the quantity of myelinated fibers, myelin thickness and fiber diameter were determined.

### Muscle wet weight

After all rabbits were killed, bilateral gastrocnemius were harvested and wet weight was measured using an EL204 electronic balance (Mettler-Toledo Instruments, Greifensee, Switzerland). The wet mass ratio was calculated according to the formula: the ratio = wet weight of gastrocnemius from the injury side/wet weight of gastrocnemius from the contralateral side.

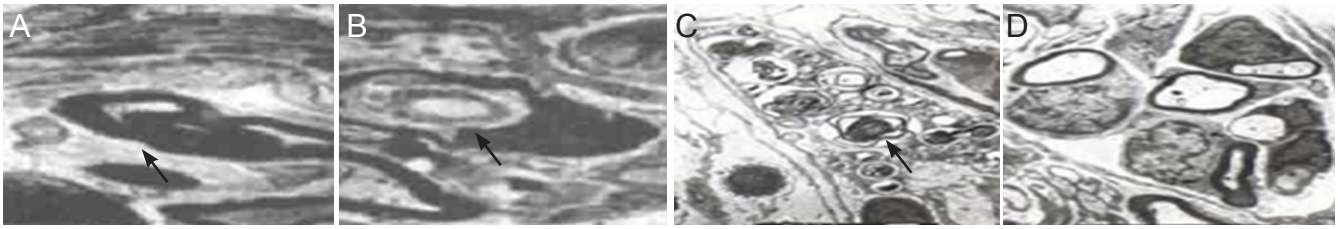


**Figure 1 Effect of ganglioside on the morphology of sciatic nerve defects repaired with cryopreserved peripheral nerve allograft (electron microscopy, × 10,000).**
(A–C) At 2, 4, and 8 weeks after modeling, myelinated nerve fibers (arrows) appeared disintegrated in the ganglioside + allograft nerve group. (D) At 12 weeks after modeling in the ganglioside + nerve allograft group, myelinated nerve fiber damage was reduced.

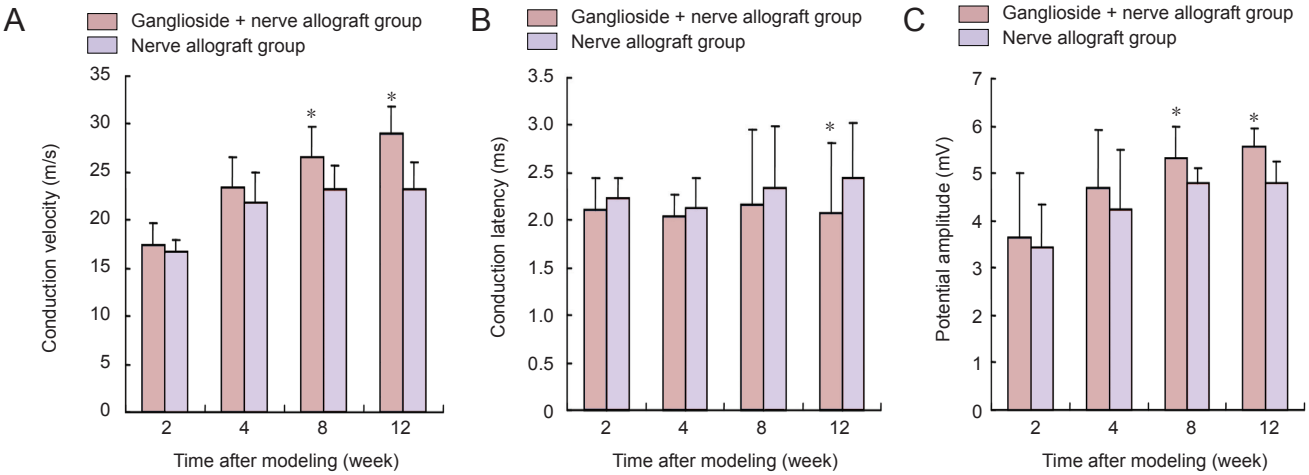


**Figure 2 Effect of ganglioside on the functions of sciatic nerve defects repaired with cryopreserved peripheral nerve allograft.**
(A) Conduction velocity, (B) conduction latency, and (C) potential amplitude. Data were expressed as the mean ± SD. There were six rats at 2, 4, and 8 weeks and nine rats at 12 weeks in each group. The differences were compared using paired sample *t*-test. ∗$P < 0.05$, *vs.* nerve allograft group.

### Statistical analysis

Data were analyzed using SPSS 18.0 software (SPSS, Chicago, IL, USA) and were expressed as the mean ± SD. The differences were compared using paired sample *t*-test and a $P < 0.05$ level was considered a significant difference.

## Results

### Ganglioside improved the morphology of sciatic nerve defects repaired with cryopreserved peripheral nerve allograft

At 2 weeks after modeling, myelinated nerve fiber began to disintegrate and Schwann cell proliferation was not obvious in the nerve allograft group. The ganglioside + nerve allograft group showed a slower disintegration of myelinated nerve fibers and more obvious proliferation of Schwann cells compared with the nerve allograft group. At 4 and 8 weeks after modeling, the transplanted sciatic nerve was not changed when compared with 2 weeks. At 12 weeks, a small amount of myelinated nerve fibers was found in the nerve allograft group. Blood capillaries between the perineurium proliferated and scarring nerve fibers were obvious. In the ganglioside + nerve allograft group, there were a large number of myelinated nerve fibers in the rabbit sciatic nerve. Only a few nerve fibers were scarred and blood capillaries between the perineurium were clearly visible (**Figure 1**).

### Ganglioside improved the function of sciatic nerve defects repaired with cryopreserved peripheral nerve allograft

At 2 and 4 weeks after modeling, sciatic nerve conduction velocity and conduction latency were similar to potential amplitude in the two groups ($P > 0.05$). At 8 and 12 weeks after modeling, sciatic nerve potential amplitude and conduction velocity in the ganglioside + nerve allograft group were higher than that in the nerve allograft group ($P < 0.05$). The ganglioside + nerve allograft group had a lower conduction latency than the nerve allograft group at 12 weeks only ($P < 0.05$; **Figure 2**).

### Ganglioside increased gastrocnemius wet weight in rabbit sciatic nerve repaired with cryopreserved peripheral nerve allograft

At 2–12 weeks after modeling, gastrocnemius wet weight ratio in the ganglioside + nerve allograft group was higher than that in the nerve allograft group ($P < 0.05$; **Figure 3**).

### Ganglioside improved the morphology of nerve fibers in sciatic nerve repaired with cryopreserved peripheral nerve allograft

At 2 weeks after modeling, the quantity of myelinated fibers, myelin thickness and fiber diameter in the regenerated sciatic nerve showed no difference between the ganglioside +

nerve allograft group and nerve allograft group ($P > 0.05$). At 4, 8, and 12 weeks after modeling, the ganglioside + nerve allograft group had greater myelinated fibers, myelin thickness and fiber diameter than the nerve allograft group ($P < 0.05$; **Figure 4**).

## Discussion

Growing evidence from previous studies has shown that pre-implantation of Schwann cells contributed to repairing long nerve defects and improving nerve regeneration (Zhang, 2011; Yu et al., 2012). The results of this study demonstrated that ganglioside injection attenuated myelin disintegration in myelinated nerve fibers and promoted the proliferation of Schwann cells. An increase in blood capillaries between the perineurium was also clearly visible. Nerve injury is highly associated with the deterioration of neurological function, especially nerve conduction velocity, conduction latency and potential amplitude (Ji and Qi, 2012). The present study showed that, ganglioside-treated rabbits had a higher potential amplitude and conduction velocity than the non-treated rabbits at 8 and 12 weeks after modeling. Previous studies revealed that nerve damage causes muscle atrophy because the nerve provides nutrition for the muscle and can secrete hormones and substances that promote and maintain muscle activity (Han, 2011). In this study, gastrocnemius wet weight ratio in the ganglioside-treated rabbits was higher than that in the model rabbits at 2, 4, 8, and 12 weeks after modeling. This evidence indicates that ganglioside solution enhances the nutrition effect on rabbit nerves, and is conducive to maintaining and promoting the survival and function of muscles. After nerve injury occurs, the nerve has self-repair potential in humans and animals (Cao et al., 2013; Chen and Guo, 2014). Regenerated nerve fibers will continuously grow to minimize the inconvenience caused by nerve injury (Liu et al., 2012; Yu, 2013). Ganglioside solution increased the quantity of myelinated fibers, myelin sheath thickness and fiber diameter significantly in the regenerated sciatic nerve of rabbits after transplantation of peripheral nerve allograft.

In summary, ganglioside promotes the regeneration of peripheral nerve allograft after transplantation. Ganglioside can also accelerate nerve conduction velocity, increase the potential amplitude, shorten conduction latency, promote nerve fiber remodeling, and repair peripheral nerve defects. The same drug may exert significantly different effects at different time points after peripheral nerve injury (Hou et al., 2009). Therefore, further studies are needed to explore the exact durations of ganglioside injection for promoting the restoration of peripheral nerve allograft, so as to provide theoretical evidence for the choices made in the clinical setting.

**Author contributions:** *Wang YD and Liu YG designed the study and were responsible for the article. Wang YD, Liu YG and Liu Q implemented the experiment. Liu YG evaluated the study. Liu YG and Liu Q collected experimental data. Wang YD drafted the manuscript. Liu YG revised the manuscript. All authors approved the final version of the manuscript.*
**Conflicts of interest:** *None declared.*

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