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Olfactory ensheathing cells seeded muscle-stuffed vein as nerve conduit for peripheral nerve repair: A nerve conduction study

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We evaluated bridging of 15 mm nerve gap in rat sciatic nerve injury model with muscle-stuffed vein seeded with olfactory ensheathing cells as a substitute for nerve autograft. Neurophysiological recovery, as assessed by electrophysiological analysis was faster in the constructed biological nerve conduit compared to that of autograft.

[**Key words:** Olfactory ensheathing cells; Muscle-stuffed vein; Nerve conduction study; Compound motor action potential; Latency]

Peripheral nerve injury is a prevalent devastating complication that is often caused by accidents, removal of cancerous tissues and diseases. The severed peripheral nerve causes the loss of connection between neural circuits and the musculoskeletal system. In many nerve injuries, the nerve ends cannot be directly joined as the nerve gap is too big for tensionless apposition and a nerve graft is needed to bridge the defect. Most patients receiving treatment such as nerve-cable interfascicular autografts for traumatic peripheral nerve injuries do not show measurable signs of recovery or at least, suffer from drastically reduced muscle strength (1). One of the reasons for poor functional restoration is that axon regeneration in peripheral nerves is slow, at an elongation rate of only 1–3 mm per day (2).

Currently nerve autograft is the gold standard in treating nerve trunk defect when the tensionless apposition to suture the severed nerve is not possible. Nevertheless, autograft poses various drawbacks such as need for a second surgical procedure to extract the donor nerve, permanent loss of the donor nerve function, donor site morbidity, limited availability and structural differences between donor and recipient grafts (3,4). Therefore there is an urgent and unmet need to find an alternative approach in bridging the nerve defects and to optimize functional recovery of injured nerve. Allogeneic nerve grafts also have been used in reconstruction, but require systemic immunosuppression. Nerve tissue engineering to promote nerve regeneration has been a promising alternative to nerve autograft. Various natural and synthetic biomaterials have been studied in the construction of artificial nerve conduits. The ideal artificial nerve conduit should have certain biochemical and mechanical properties such as biocompatibility, patency, semi flexibility and easy handling during surgery (5,6). The use of biological conduits, such as veins, and denatured muscles as substitutes to replace damaged nerves, provides a promising approach for the treatment of peripheral nerve injuries. However, each conduit has its own advantages and disadvantages. By combining two conduit materials, the advantages of one may offset the disadvantages of the other. A combination of a muscle-stuffed vein conduit can produce such promising results, where the muscle within the vein prevents collapse of the vein and the basal lamina of the muscle provides a component of the extracellular matrix, allowing the regenerating axons to attach preferentially. Previously, artificial nerve conduits made from various biomaterials filled with neural-differentiated human mesenchymal stem cells (hMSCs) and Schwann cells have been studied for use as nerve grafts (7–9). Besides hMSCs and Schwann cells, olfactory ensheathing cells (OECs) are also potential cells for nerve regeneration as they exhibit properties of both Schwann cells and astrocytes, and are known to improve axonal regeneration and produce myelin after transplantation (10,11). A previous *in vivo* study involving transplantation of OECs on microsurgically repaired rat sciatic nerve following transection showed that the OECs integrated into the repaired nerves and the OEC transplanted group had higher axon count, increased conduction velocity and better functional recovery as measured by sciatic functional index (SFI) (12). In our study, we evaluated the neurophysiological recovery following bridging of 15 mm nerve gap in rat sciatic nerve injury model with acellular muscle-stuffed vein seeded with OECs as a substitute for nerve autograft. The neurophysiological recovery was assessed by electrophysiological analysis.

Animal handling was in accordance to UKM Animal Ethics Committee guidelines and prior ethics approval was obtained for this study (02-01-02 SF0446). Rat vein, muscle and OECs were

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harvested from adult female Sprague–Dawley (SD) rats weighing 250–350 g. Midline laparotomy was performed and veins measuring 15 mm were harvested from inferior vena cava. The muscle was harvested along its fibre from rat hamstring muscle and sectioned into 5 mm × 15 mm pieces. The veins and muscles were washed and decellularized as described previously (13). OEC layer was stripped away from the rest of the bulb through septum portion as reported previously (14). The tissue was minced and then digested with collagenase type I 0.3% solution. Subsequently, the cell suspension was centrifuged and cultured in minimum essential medium alpha (α-MEM) medium until passage 2. Characterization by immunostaining showed that OECs were positive for GFAP, S100b and cytokeratin 18 (14). Three million OECs were seeded longitudinally on the pre-treated muscle and incubated at 37°C and 5% CO_2. After 24 h, the cell seeded muscles were stuffed into the pre-treated vein and kept immersed in α-MEM medium in a CO_2 incubator for 24 h to stabilize the conduit. OECs seeded muscle-stuffed vein will be referred as biological nerve conduit (BNC) hereafter.

Adult female SD rats weighing 250–350 g were used to create sciatic nerve injury model. A total of 35 rats were divided into 3 groups whereby the negative control (defect) group consist of 13 rats, the reversed autograft (RA) group consist of 12 rats and the BNC group consist of 10 rats. The animals were given rat pellet and water *ad libitum*, and were allowed normal cage activities. Saw dust was used as bedding and was changed twice a week.

The rat surgery and post-surgery care was performed as previously described (15). Briefly, the right sciatic nerve of the rats under deep anaesthesia was exposed through a skin incision extending from the greater trochanter to the midway distally followed by an intermuscular plane deep dissection. After nerve mobilization, a transection injury of 15 mm was performed (neurotmesis) using straight microsurgical scissors. The nerve was cut just above the terminal nerve ramification. The transected nerve was repaired using the resected portion of sciatic nerve for the 12 rats in the RA group and using the BNCs that were produced in this study for the 10 rats in BNC group. BNC or reversed nerve autograft was anastomosed to the proximal and distal stumps with three sutures (10/0 Ethilon) at each junction. Nerve defects created in control group (13 rats) were left alone without any graft. Following the implantation, the intermuscular plane was closed and Chloramphenicol cream was applied to sutured skin area. After the surgery, all the animals were nursed in individual ventilated cages (IVC) and closely monitored for three days; there was no post-operative mortality. All the animals were monitored for 6 weeks before euthanized for further analysis.

Nerve conduction study (NCS) was performed on fifteen normal SD rats, in order to establish a mean and standard error mean (SEM) for normal SD rats NCS baseline, in terms of amplitude (compound motor action potential) and latency (distal motor latency). For the experimental rats, NCS was performed at the interval of 2, 4, and 6 weeks after defect creation and implantation. Under anaesthesia (ketamine 80 mg/kg, xylazine 10 mg/kg; intravenous), a recording needle electrode was positioned in the right anterior tibialis muscle and the right sciatic nerve was stimulated at 98 mA by two electrodes connected with DC electrical stimulator. The stimulus duration was 0.1 ms. The ground electrode was placed in adjacent muscle tissue.

Data is expressed as mean ± standard error mean (SEM). Electrophysiological data with inter-group differences were tested by one-way analysis of variance (ANOVA) using SPPS 14.0 software.

Nerve conduction study (NCS) was used to assess neurophysiological recovery. The compound motor action potential (CMAP) at 2, 4, and 6 weeks post implantation (PI) were compared to each other (Fig. 1). All the groups showed incremental CMAP across the three time points.

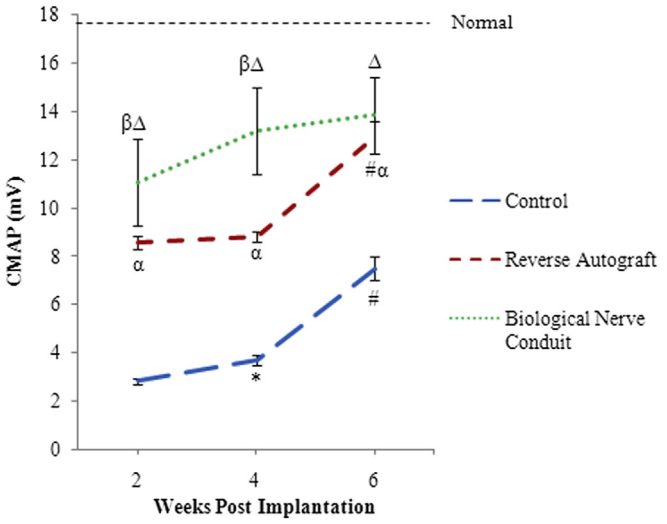

FIG. 1. The mean CMAP ± SEM of the various nerve defect treatment groups at 2, 4 and 6 weeks post implantation. Asterisk, significant difference between week 2 and week 4 ($P < 0.05$). Hatch, significant difference between week 4 and week 6 ($P < 0.05$). Alpha, significant difference between control and reversed autograft at same time point ($P < 0.05$). Beta, significant difference between reversed autograft and biological nerve conduit at same time point ($P < 0.05$). Triangle, significant difference between control and biological nerve conduit at same time point ($P < 0.05$).

CMAP of BNC group at week 2 was highest compared to that of control and RA groups ($P > 0.05$) and achieving 62.7% (11.10 ± 1.80 mV) of the normal baseline values (17.68 ± 1.58). CMAP values further increased from week 2 to week 6 PI. However, due to the high variance of the CMAP values of the samples within the group, this increase was not statistically significant. Nevertheless, CMAP of BNC group remained the highest among the three groups throughout the 3 time points.

CMAP in the RA group at week 2 (8.59 ± 0.27 mV) was lower than that of BNC group (11.10 ± 1.80 mV) but was significantly higher than that of control group (2.83 ± 0.10 mV) ($P < 0.05$). CMAP values continued to increase from week 2 to week 6 with a sharp rise from week 4 to week 6. By week 6, RA group achieved a CMAP value (12.95 ± 0.66) comparable to that of BNC group (13.85 ± 1.60).

CMAP in the control group at week 2 showed steady and significant increase in nerve conduction amplitude from week 2 (2.83 ± 0.10 mV) to week 4 (3.70 ± 0.20 mV), and from week 4 to week 6 PI (7.50 ± 0.48 mV), P value < 0.05. However, CMAP of control group remained far lower than the CMAP of RA group and BNC group at all the 3 time points.

By week 6, CMAP of the RA group and BNC group showed neurophysiological recovery of 73.2% and 78.3% respectively, compared to normal baseline value whereas the neurophysiological recovery in the control group was only 42.4%.

The comparison of latency showed that latency of the control group throughout the 6 weeks PI (1.3–1.4 ms) remained above the latency of the normal rats (1.12 ± 0.07 ms) (Fig. 2). The latency of the RA group showed an irregular trend where the latency at week 2 was 1.22 ± 0.09 ms and it reduced to 1.08 ± 0.01 ms at week 4 before increasing again at week 6 to 1.20 ± 0.12 ms. The latency of BNC group was below the latency of normal rat sciatic rat and it showed a decreasing trend where the latency decreased from 0.96 ± 0.15 ms on week 2 to 0.79 ± 0.13 ms on week 6 PI. However, these changes in latency at week 2, 4 and 6 PI were statistically insignificant for all the studied groups.

Zhang et al. (16) have reported that vein grafts caused less donor site morbidity compared to nerve grafts, and vein grafts provided metabolically supportive environment for the regenerating axons. The wall of the vein graft also acted as a barrier against scar