The Full Motor Neurons Graft As a Preliminary Study in Rats

AMR MOHAMED ELHUSSINY KHATER, M.D.* and AKIHIKO TAKUSHIMA, M.D.**

The Department of Plastic Surgery, Faculty of Medicine, Mansoura University, Egypt and
*Kyorin University School of Medicine, Tokyo***

Abstract

Background: Conventional nerve grafting was used for treatment of nerve defects. However, the slow rate of nerve regeneration, donor site limitations and morbidities were considered the major disadvantages of this technique.

Objective: In this article, a novel motor neuron nerve allografts were used to accelerate nerve regeneration and were compared to nerve allograft in a rat model.

Methods: We harvested full motor neuron grafts and conventional nerve grafts and used them to reconstruct a ten mm defect in the motor nerve to the Extensor Digitorum communis (EDC).

Results: Histological examination confirmed intact connections between the motor neurons and its axons. The mean of the amplitudes of evoked potentials of neuron graft group and conventional graft group were 12.2±3.6 milliampere and 8.0±6.0 milliampere. However, muscle force, muscle weight and histological examination showed insignificant differences between the two groups.

Conclusion: Full neuron transfer is a new type of nerve grafts in the rat model. As a preliminary report, we investigated the viability of the transferred nerve cells and if they worked as a booster for augmentation of the signals. Electrophysiological examinations showed favorable results and histological studies showed the survival of the motor neurons outside the central nervous system.

Key Words: *Neuron graft, Nerve graft – Wallerian degeneration – Nerve regeneration.*

Introduction

Ever since Sunderland classified nerve injuries [1], numerous researchers have explored various approaches to enhance functional outcomes following nerve repair [2-4]. Improved comprehension of internal nerve topography [5], the application of microsurgical techniques [6], and the use of tissue adhesives [7] have been instrumental in achieving better reformation of transected nerve ends.

Despite advancements in microsurgery, peripheral nerve injuries remain a significant clinical and surgical challenge [8]. Within 24 hours post-injury, Wallerian degeneration initiates. Following the complete clearance of distal nerve debris, Büngner chains, formed by reorganized Schwann cells, serve as conduits for the newly regenerated axons toward their targets. These processes ensure axonal regrowth to the distal stump at a rate of 1mm per day in humans [9].

However, the outcomes of surgical repair for peripheral nerve injuries still fall short of restoring the pre-injury state for many reasons [10-11] that are major contributors to suboptimal nerve recovery despite appropriate surgical intervention.

However, the crucial factor influencing functional outcomes after nerve repair is the state of the denervated muscle following complete nerve recovery [12]. Prolonged nerve recovery may lead to muscle atrophy, emphasizing the importance of expediting the period for nerve regeneration to enhance functional recovery.

Reconstructive surgeons engage in numerous laboratory trials aimed at improving nerve regeneration [13-15]. Our novel concept involves transferring multiple nerve cells into the degenerated nerve. If the transferred nerve cells prove viable, the formation of new synapses and complete axonal regeneration can occur rapidly.

In this article, we describe how to harvest a new model of nerve graft and we use them in reconstruction of nerve injuries in a rat model. We try to harvest the motor neuron, including nerve cells and their axons, and transfer it from one rat to another.

Correspondence to: Dr. Amr Mohamed Elhussiny Khater,
E-Mail: dr_amrkhater@yahoo.com

Material and Methods

We conducted the study at plastic surgery department, Kyorin University, Tokyo, Japan after obtaining approval from the Institutional Animal Care and Use Committee (IACUC) approval. April 1, 2014. The study included ten Wistar rats. We utilized five rats as donor animals and five rats as recipient animals. The average weight was 331.25 grams and average age was 8 weeks.

I- *Harvest of the neuron grafts:*

After induction of general anesthesia and animal positioning, we started by making a Y shaped incision in the neck's midline. Under the surgical microscope, we dissected the salivary glands and suspended them cranially. Then, we detached the infrahyoid muscles and the sternocleidomastoid muscles from the clavicle and retracted them cranially. Afterwards, we ligated the external jugular veins and removed the omohyoid muscle. Next, we did Careful dissection of the carotid artery and the vagus nerve from the underlying muscles. Also, we removed the prevertebral muscles. We repeated the same steps on the contralateral side to expose the bodies of the cervical vertebrae completely.

Then, we removed the bodies of the cervical vertebrae together with intervertebral discs to expose the meninges by the use of electrical saw and performed a careful separation of the spinal cord with attached rootlets and brachial plexus branches from within the spinal canal as in Fig. (1). Histological examination confirmed intact connections between the motor neurons and its axons Fig. (2). The full motor neuron graft can be defined as a type of nerve grafts that consisted of the motor neuron cell body (which are usually located within the central nervous system) and its axons (which is usually located in the peripheral nervous system) connected to each other. This graft was used as one unit for reconstruction of nerve defects.
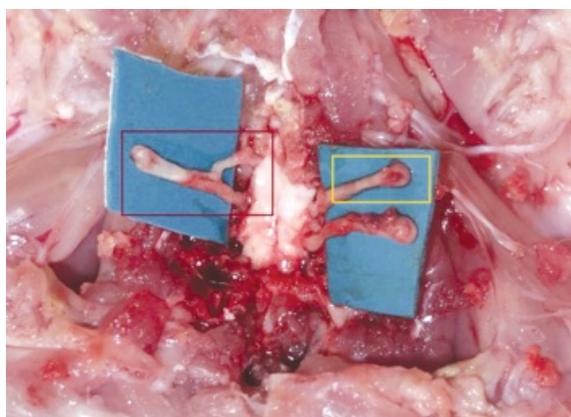

Fig. (1): Intraoperative view after exposure of the cervical spinal cord together with its attached brachial plexus.
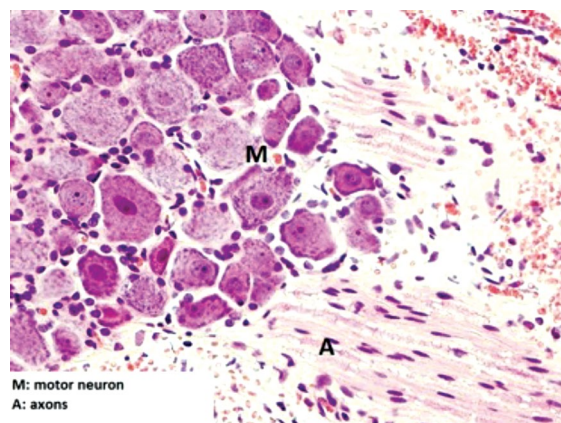

M: motor neuron
A: axons

Fig. (2): Histological examination of the neuron grafts shows intact connections between the neurons and its axons.

II- *Harvest of conventional nerve graft:*

From the same donor rat, we obtained conventional nerve graft from branches of the brachial plexus.

III- *Allo-transplantation of the grafts:*

We incised the lateral aspect of the right thigh and the leg of the recipient rat and identified the Extensor Digitorum communis (EDC) and its nerve supply. Then, we removed a 10mm segment of the nerve and used the neuron graft to repair the nerve defect. After skin closure, we repeated the same steps on the left side using a conventional nerve allografts.

IV- *Testing the efficacy of the neuron graft:*

After 3 months, we used the following tests to compare the efficacy of the neuron graft with that of the conventional nerve graft:

A- *Measurement of the amplitude of the evoked potentials:*

We performed Careful dissection of the sciatic nerve and dividing of all of his branches except that branch that supplies the EDC muscle. Then, we used an electrical stimulus with an intensity of 1 milliampere and 0.2 millisecond duration to induce contraction of the EDC muscle through stimulation of the sciatic nerve proximal to the nerve graft. Later, we recorded the produced potentials from the EDC muscle by Neuropack U EMG device (Nihon Kohden, Tokyo, Japan). After we used 3 consecutive stimuli to induce 3 evoked potentials, we recorded the amplitudes of these potentials and compared with that of the opposite side.

B- *Measurement of the Muscle force:*

We connected the EDC tendon to a power transducer attached to data acquisition system Power Lab 16/30 (ADInstruments, Australia) for recordings of the force of muscle contraction onto a Chart 5.0 for Windows software (AD Instruments, Australia). Then, we used 3 consecutive stimuli to induce 3 muscle contractions. Later, we recorded the

produced muscle contraction force and compared it with that of the opposite side.

C- *Measurement of the Muscle weight:*

We used A and D GR200 analytical balance (A&D Engineering, San Jose, C.A. USA) for the measurement of the weight of the muscles.

D- *Biopsy:*

At the end of the experiment, we performed histological examinations of the biopsied muscle and the nerve using immunohistochemical staining (Anti-Neurofilament antibody, Abcam Inc).

Results

One of the recipient rats showed nerve rupture secondary to local infection and we excluded this rat from the study. Four rats continued in the study. After 3-4 months, we reexamined the recipient rats under general anaesthesia. Then, we explored the muscles and its nerve supply and obtained the following results:

1- *Measurement of the amplitude of the evoked potentials:*

The mean of the amplitude of evoked potentials of the neuron graft group and the conventional

graft group were 12.2±3.6 milliampere and 8.0±6.0 milliampere. Using the paired sample *t*-test, there was a significant difference between the two groups (*p*-value: 0.035).

2- *Measurement of the muscle force:*

The mean of the force of contraction of the neuron graft group and the conventional graft group were 0.063±0.036 Millinewtons and 0.046±0.036 Millinewtons. Using the paired sample *t*-test, there was an insignificant difference between the 2 groups (*p*-value: 0.33).

3- *Measurement of the muscle weight:*

The mean of the Muscle weight of the neuron graft group and the conventional graft group were 0.77±0.12 grams and 0.72±0.13 grams. Using the paired sample *t*-test, there was an insignificant difference between the 2 groups (*p*-value: 0.2) Table (1).

4- *Biopsy:*

Nerve biopsy showed a denser configuration of the regenerating axons in the motor neuron group Fig. (3). However, the change was not statistically significant.

Table (1): Shows the measurement of the amplitude of the evoked potentials Milliampere (mA), Muscle force in Millinewtons (mN) and muscle weight in grams (g) of motor neuron graft group and the conventional nerve graft group.

| Animal Number	Amplitudes of the evoked potentials in Milliampere (mA)		Muscle force in Millinewtons (mN)		Muscle weight of both groups in grams (g)	
	Motor neuron graft group	Conventional nerve graft group	Motor neuron graft group	Conventional nerve graft group	Motor neuron graft group	Conventional nerve graft group
1	15.54	17.45	0.05	0.04	0.66	0.56
	12.71	12.35	0.07	0.04		
	10.04	7.45	0.05	0.06		
2	13.58	17.78	0.04	0.09	0.7	0.77
	7.98	12.18	0.04	0.1		
	7.98	1.68	0.05	0.11		
3	17.28	1.9	0.03	0.02	0.79	0.69
	12.47	1.3	0.03	0.01		
	7.27	1	0.04	0.01		
4	18.06	9.57	0.11	0.03	0.92	0.87
	13.71	7.61	0.12	0.02		
	9.73	5.77	0.13	0.02		
Mean	12.2	8	0.063	0.046	0.77	0.72
Standard Deviation	3.6	6	0.036	0.036	0.12	0.13
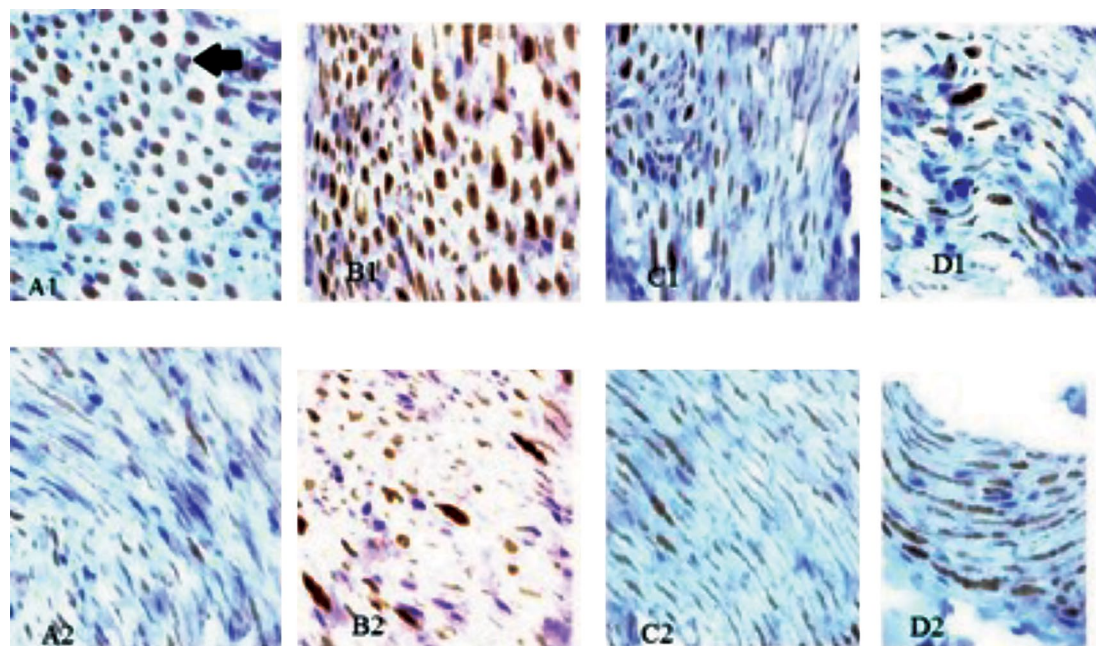
Fig. (3): Histological examinations were done using immunohistochemical staining (Anti-Neurofilament antibody, Abcam Inc) of the motor neuron grafts (A1, B1, C1, D1) and the conventional nerve grafts (A2, B2, C2, D2).

Discussion

In this study, we established a new model of nerve grafts in the rats. We called it the full neuron transfer. Electrophysiological examinations showed favorable results. Histological examinations showed the survival of the functioning motor neurons outside the central nervous system.

Several studies have investigated the possibility of nerve regeneration [16-18]. In the peripheral nervous system, it was possible to achieve nerve regeneration. However, it was impossible to achieve it in the central nervous system. Also, several studies have used the nerve grafts from the peripheral nervous system to bridge nerve defects in the central nervous system [19], but in this study, we transferred the motor neuron, within the central nervous system, together with its axons to bridge nerve defects in the peripheral nervous system.

Previous reports described dorsal exposure of the spinal canal in the lumbar region of the rat for studying of nerve regeneration [20]. In this study, we used a ventral exposure of the spinal canal at the cervical region to get a part of the spinal cord with its attached rootlets and spinal nerves as one unit.

We called this unit "the full neuron transfer" and we used these structures as a nerve graft. This approach allows direct exposure of these structures in one surgical field. It also allowed non-traumatic harvest of these structures as one unit. Grafted nerve offers neuron bodies and axons in its continuity, being as proper for transplantation experiments.

Several researchers chose rat model to investigate nerve regeneration [21,22]. Sciatic nerve [23,24], facial nerve [25] and nerves of the forelimbs [26] were selected as models to investigate the nerve recovery. We chose the Sciatic nerve because of easy exposure to the nerve and the supplied muscle and easy assessment of the outcome of nerve graft.

Since introducing nerve grafts to treat nerve injuries, several studies have focused on enhancing the process of nerve regeneration using tissue adhesive [27], vascularized nerve grafts [28], nerve conduits [29] and stem cells [30].

However, in this study, we tried a new non-synthetic nerve grafts. We think that it is possible for nerve regeneration to occur with this graft by forming of new synapses between the end of the transected nerve and the motor neuron allowing a more rapid rate of nerve regeneration in comparison with the slow Wallerian degeneration process. With the evolving technologies of regenerative medicine, immune compatible motor neuron graft may be available, which would facilitate rapid recovery of the injured nerve. However, further studies are needed to investigate these events.

The limitations of this study include that the full neuron grafts cannot be applied as an autograft because the donor rat was sacrificed during the harvest of the grafts. This rat model is not suitable as an auto model because we could not harvest a motor neuron and used it as an autograft in the same rat. In addition, this is a preliminary study cannot be applied to human being in the current state. Also,

further studies are needed to compare these various types of nerve allografts that include viable cells.

We think that this study offer the base for a new type of nerve grafts. However, clinical application of the motor neuron graft must overcome the limited availability of the donor site and recipient rejection. Further studies are needed on a large scale of animal individuals and the use of xenografts after immunomodulation and removal of major compatible antigens are key researches for the clinical application of the full motor neuron graft.

To conclude, full neuron transfer is a new type of nerve grafts in the rat model. As a preliminary report, we investigated the viability of the transferred nerve cells and if they worked as a booster for augmentation of the signals. Electrophysiological examinations showed favorable results and histological studies showed the survival of the motor neurons outside the central nervous system. Although it is impossible to harvest the nerve cells in the clinical setting, the immuno-compatible motor neuron will enable this concept.

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