Journal of Cranio-Maxillo-Facial Surgery 46 (2018) 898–904

Contents lists available at ScienceDirect

Journal of Cranio-Maxillo-Facial Surgery

journal homepage: www.jcmfs.com

Evaluation of nerve growth factor (NGF) treated mesenchymal stem cells for recovery in neurotmesis model of peripheral nerve injury

Mehrnaz Moattari [a], Homa Mohseni Kouchesfehani [a], Gholamreza Kaka [b, *], Seyed Homayoon Sadraie [b], Majid Naghdi [c]

[a] Department of Animal Biology, Faculty of Biological Sciences, Kharazmi University, Tehran, 15719-14911, Iran
[b] Neuroscience Research Center, Baqiyatallah University of Medical Sciences, Tehran, 19568-37173, Iran
[c] Department of Anatomy, Fasa University of Medical Sciences, Fasa, Iran

ARTICLE INFO

Article history:
Paper received 18 October 2016
Accepted 20 March 2018
Available online 5 April 2018

Keywords:
Histomorphometry
Neurotmesis
NGF
Vessels
Rat

ABSTRACT

Background: Peripheral nerve damages are a relatively common type of the nervous system injuries. Although peripheral nerves show some capacity of regeneration after injury, the extent of regeneration is not remarkable. The present study aimed to evaluate the effect of NGF treated mesenchymal stem cells on regeneration of transected sciatic nerve.

Materials and methods: In this experimental study, forty-two male Wistar.rats (180–200 g) were randomly divided into 6 groups (n = 7) including control, Membrane + Cell (Mem + Cell), NGF group, NGF + Cell group, NGF + Mem group and NGF + Mem + Cell group. Regeneration of sciatic nerve was evaluated using behavioral analysis, electrophysiological assessment and histological examination.

Results: The rats in the NGF + Mem + Cell group showed significant decrease in sciatic functional index (SFI) and hot water paw immersion test during the 2nd to 8th weeks after surgery. ($p < 0.001$). At 8 weeks after surgery, electrophysiological findings showed that amplitude increased and latency decreased significantly in NGF + Mem + Cell group ($p < 0.001$). Measured histological parameters showed that number of nerve fibers, number of vessels and percent of vessel area also increased significantly in NGF + Mem + Cell group ($p < 0.05$).

Conclusion: The present study showed that NGF in accompany with mesenchymal stem cells improved electrophysiological and histological indices.

1. Introduction

Peripheral nerve injury is a prevalent complication. The rate of peripheral nerve injury is assessed to be between 13 and 23 per 100,000 persons per year in developed countries (Asplund et al., 2008). Although these injuries are not life-threatening, they can lead to complete functional loss or permanent impairment, affecting quality of life and imposing economic pressure on the patients and society (Dubový et al., 2011). Nerve damage has been divided into neuraproxy, axonotmesis, and neurotmesis (Cheng et al., 2011). Cell therapy as a nerve repair strategy creates a favorable environment in peripheral nervous system due to properties such as self-renewal, a high proliferation rate, and multipotency (Deuse et al., 2011). A part of the umbilical cord, Wharton's jelly, is a rich source of mesenchymal stem cells. Mesenchymal stem cells from Wharton's jelly of the umbilical cord is considered to be one of the most practical sources for cell-based therapies because they are proliferative, cell collection is not an invasive procedure, their associated ethical problems are few, and they are immunologically compatible (Margossian et al., 2012). On the other hand, researchers look for scaffolds to replace damaged tissue. Chitosan is the N-deacetylated product of chitin. Chitosan is the second most abundant natural polysaccharide next to cellulose, which is embedded in a protein matrix of a crustacean shell or a squid pen (Shahram et al., 2013). Previously, we showed that use of membrane in conjunction with cell therapy had positive effects on transected sciatic nerve (Moattari et al., 2016) On the other hand, numerous therapeutic interventions, mostly pharmacotherapeutic,

* Corresponding author. Neuroscience Research Center, Baqiyatallah University of Medical Sciences, Aghdasie, Artesh Boulevard, Artesh Square, PO Box: 19568-37173, Tehran, Iran. Fax: +98 2126127286.
E-mail address: gh_kaka@yahoo.com (G. Kaka).

https://doi.org/10.1016/j.jcms.2018.03.015
1010-5182/

have been tested to enhance functional recovery after sciatic nerve injuries (Sun et al., 2009). The identification of neurotrophic factors offers molecular therapy as a potential approach to enhance nerve regeneration. Among the neurotrophic factors, nerve growth factor has a key role (Zhao et al., 2013). It acts as a neuroprotector (Bhang et al., 2007). Since NGF bioactivity will be easily lost in few minutes (half life: 2–4 min), a combination of NGF and fibrin glue, which lasts longer (4–8 weeks), has been used in some studies (Ma et al., 2013b). The combination of NGF and fibronectin, as the main components of fibrin glue, enhance peripheral nerve regeneration by an unknown mechanism (Ma et al., 2013a). Chen and Wang (1995) concluded that NGF improved nerve regeneration in crushed sciatic nerve injury (Chen and Wang, 1995). Li et al. (2015) reported that using nerve growth factor had better functional and structural results 14 days after surgery (Li et al., 2015). Barmpitsioti et al. (2011) reported that NGF improved morphometric and electromyographic results (Barmpitsioti et al., 2011). Kemp et al. (2011) showed that NGF promoted early axonal regeneration and locomotor recovery (Kemp et al., 2011). In the present study, the effect of NGF on mesenchymal stem cells cultured by a membrane of chitosan was investigated for repairing of peripheral nerve injury in a transected sciatic rat model.

2. Materials and methods

In this experimental study, 42 male adult Wistar rats (180–200 g) purchased from Pasture Institute Tehran, Iran, were used. The animals were kept in home cages (n = 4 per cage) at a controlled temperature 23 ± 2 °C and 50% humidity with 12/12-h light/dark conditions and had free access to standard rat chow and tap water. All experiments involving animals and surgical procedures were approved by Ethical Committee of Baqiyatallah University of Medical Sciences.

2.1. Preparation of MSCs

Fresh human umbilical cords were obtained at the time of delivery from women who had experienced no complications during pregnancy or at the time of delivery. Umbilical cords were collected in Hanks Balanced Salt Solution (HBSS) (Gibco, USA) at 4 °C. Following disinfection in 70% ethanol (Sigma, UK) for 30 s, the umbilical cord vessels were removed. The Wharton's jelly was then diced into cubes of about 0.5 cm^3 and centrifuged at 250 g for 5 min. Following removal of the supernatant, the precipitate was washed with serum-free Dulbecco's modified Eagle's medium (DMEM) (Gibco, USA) and centrifuged at 250 g for 5 min. The precipitate was then treated with collagenase at 37 °C for 18 h, washed, and further digested with 0.25% trypsin (Gibco, USA) at 37 °C for 30 min. Fetal bovine serum (FBS) (Hyclone, USA) was then added to the mesenchymal tissue to stop trypsinization. The dissociated MSCs were further dispersed in 10% FBS-DMEM and counted under a microscope with the aid of a hemocytometer. At P3, the cells were identified by CD90 and fibronectin antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and CD45 as a marker for hematopoietic stem cell (Santa Cruz Biotechnology, USA) (Moattari et al., 2018) (Fig. 1).

2.2. Membrane preparation

Membranes were prepared with a mixture of 0.25 g of chitosan and 0.08 g polyethylene glycol dissolved in 50 ml of 1% acetic acid solution. The mixture was stirred for 2 h at 40 °C. The resultant solution was centrifuged at 2500 rpm for 10 min to prevent air bubbles from forming. The mixture was cast into plastic Petri dishes with 75-mm diameter and dried at 25 °C for 24 h. The films were then dried and cut into patches 1 cm × 1 cm in size.

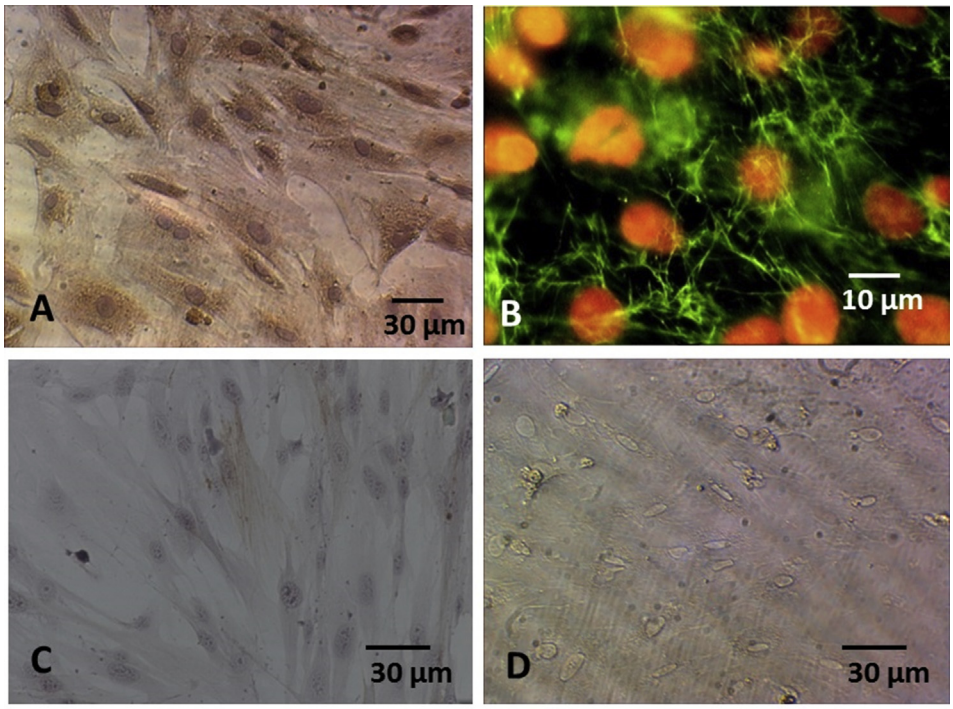

Fig. 1. Immunocytochemical photomicrographs of undifferentiated mesenchymal stem cells in Wharton's jelly. They were incubated with (A) anti-CD90, (B) anti-fibronectin, and (C) anti-CD45 as primary antibodies, followed by the secondary antibody conjugated with streptavidin-biotin horseradish peroxidase for CD90 and FITC for fibronectin (D) negative control. Magnifications × 1000.

2.3. Animal grouping

A total of 42 rats were randomly divided into 6 groups (n = 7) as follows: Control group (transected sciatic nerve rats without any intervention); Membrane + Cell group (transected sciatic nerve rats treated with a membrane and transplantation of 300,000 MSCs around the injured nerve); NGF group (transected sciatic nerve rats treated with NGF was injected at the site of the damaged nerve); NGF + Cell group (transected sciatic nerve rats treated with transplantation of 300,000 MSCs and NGF injected at the site of the damaged nerve); NGF + Membrane group (transected sciatic nerve rats treated with membrane and NGF at the site of the damaged nerve); and NGF + Membrane + Cell group (transected sciatic nerve rats treated with transplantation of 300,000 MSCs, membrane, and NGF at the site of the damaged nerve).

2.4. Surgical procedure

Animals were anesthetized by intraperitoneal injection of 80 mg/kg ketamine hydrochloride (Alfasan, Netherlands) and 5 mg/kg xylazine hydrochloride (Alfasan, Netherlands). To dissect sciatic nerve, the right hind limb was shaved, and a longitudinal cutaneous incision was made on the posterolateral side of the thigh at a length of 3 cm to expose the sciatic nerve. Then the right sciatic nerve was transected midway by a sharp surgical knife. The epineurium was sutured with 7.0 Prolene sutures, and the muscle fascia and skin were then sutured with 4.0 nylon sutures.

2.5. Sciatic functional index assessment

The sciatic functional index (SFI) was determined using an apparatus as follows. The SFI apparatus was made from wood with 60 × 7 × 20 cm (L, A, and H, respectively) dimensions, and its floor was covered with white paper. Functional recovery was assessed at the second, fourth, sixth, and eighth weeks after surgery. Before the test, the rat's paw was painted with a water-soluble blue ink; then the rats were permitted to walk through the apparatus and their footprints were tracked. The lengths of the third toe to the heel (PL), the second toe to the fourth toe (IT), and the first toe to the fifth toe (TS) were measured on the contralateral normal side (N) and the experimental side (E). SFI was computed by the following modified formula:

$$SFI = |-38.5\,(EPL\text{-}NPL/NPL) + 109.5\,(ETS\text{-}NTS/NTS) + 13.3\,(EIT\text{-}NIT/NIT) - 8.8|$$

In this study, SFI oscillates around 0 in the case of normal nerve function and around 100 SFI in the case of motor sciatic nerve dysfunction (Sadraie et al., 2016).

2.6. Hot water paw immersion test

A hot water foot immersion test was performed using a hot water bath (DID SABZ Co., Iran). The water temperature was set at 50 ± 1 °C. Measurement of reaction time in hot water paw immersion test was done at the second, fourth, sixth, and eighth weeks after surgery. The paw immersion procedure was as follow: each rat was gently held by the experimenter and one of its feet (i.e. intact or experimental) was immersed into the water up to its paw. The time at which the rat withdraws its paw from the water was recorded and expressed as the reaction time.

2.7. Electrophysiological evaluation

At 8 weeks after surgery, rats were anesthetized and the sciatic nerves were exposed. Electric stimulation was used at the proximal site of the injured nerve. The compound muscle action potential amplitude (CAMP) and latency were recorded in the gastrocnemius muscle with a needle electrode and a reference cap electrode inserted on the knee joint. The stainless steel needle used as the ground electrode was inserted into the tail skin (Bain et al., 1989).

2.8. Histological analysis

The morphological assessments were performed at 8 weeks after the sciatic nerve injury. After harvesting, the distal segment of sciatic nerves (~2 cm) at the injury sites were fixed in 10% formaldehyde for 72 h. The segments were embedded in paraffin, and transverse sections (5 μm) were cut and stained with hematoxylin and eosin. The number of nerve fibers, number of vessels, and percentage of the vessel area were evaluated by using Motic software from at least five randomly selected fields.

2.9. Statistical analysis

All results are expressed as mean ± SD. Statistical analysis was performed using SPSS version 22.0 software (SPSS Inc., Chicago, IL, USA). The data were evaluated for statistical significance by one-way analysis of variance followed by a post hoc Tukey least significant difference test. A P value < 0.05 was considered statistically significant.

3. Results

3.1. Immunocytochemistry of MSCs

Immunocytochemical results showed that the cells expressed CD90 and fibronectin as mesenchymal stem cell markers but did not express CD45, a hematopoietic stem cell marker (Fig. 1).

3.2. SFI evaluations

At 8 weeks after surgery, the SFI values increased significantly in the experimental groups compared to the Control group (P < 0.001). SFI decreased significantly, and improvement was found in the NGF + Mem + Cell group compared to the control group (P < 0.001) (Fig. 2).

3.3. Hot water paw immersion test

At 8 weeks after surgery, the reaction time in t he hot water paw immersion test significantly decreased in the therapeutic groups,

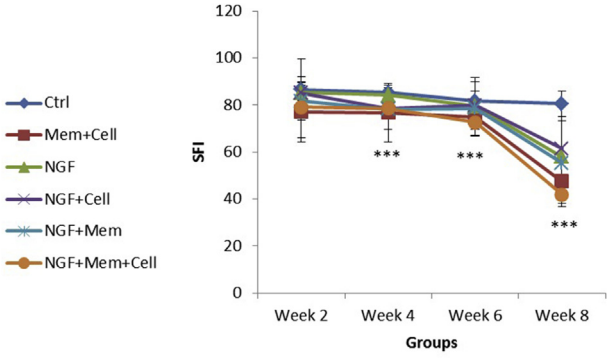

Fig. 2. Comparison of the mean functional recovery of SFI (mean ± SD). At 8 weeks after surgery, SFI decreased significantly in NGF + Mem + Cell group compared to control group (P < 0.001).

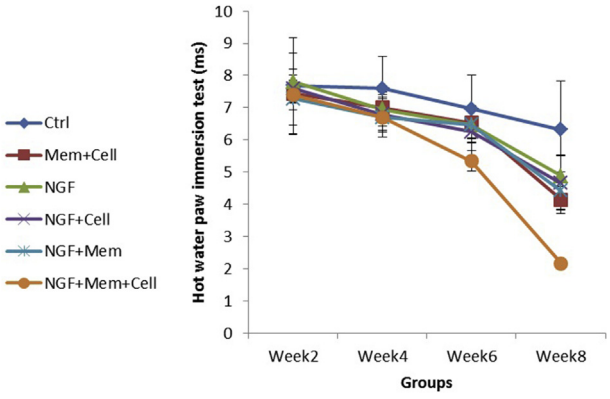

Fig. 3. Results of hot water paw immersion test (mean ± SD). At 8 weeks after surgery, the reaction time in the hot plate test increased significantly in NGF, NGF + Cell, NGF + Mem, and NGF + Mem + Cell groups compared to control group ($P < 0.001$, $P < 0.05$, $P < 0.001$, and $P < 0.001$, respectively).

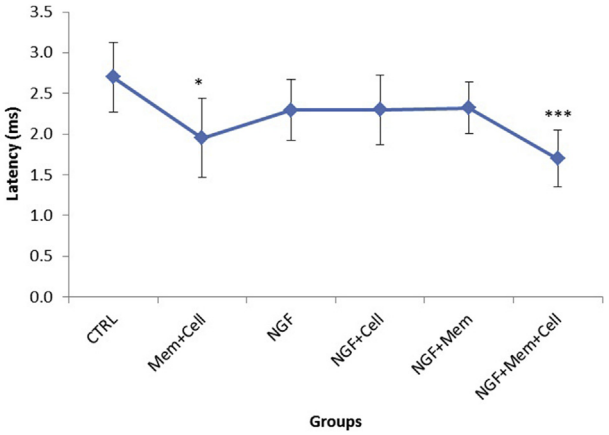

Fig. 5. Comparison of mean latency (ms) analysis in all experimental groups (mean ± SD). *,***Significant differences between Mem + Cell and NGF + Mem + Cell groups compared to control ($P < 0.05$, $P < 0.001$, respectively).

especially in the NGF + Mem + Cell group compared to the control group ($P < 0.001$) (Fig. 3).

3.4. Electrophysiological studies

At 8 weeks after surgery, the mean amplitude (mV) increased significantly in the experimental groups compared to the control group ($P < 0.05$). There were significant increases between the Mem + Cell group and NGF, NGF + Cell, and NGF + Mem groups ($P < 0.001$, $P < 0.05$, and $P < 0.05$). Amplitude was significantly improved in the NGF + Mem + Cell group compared to the other groups ($P < 0.001$) (Fig. 4). The mean latency (in milliseconds) decreased in the Mem + Cell and NGF + Mem + Cell groups compared to the control group ($P < 0.05$ and $P < 0.001$) (Fig. 5).

3.5. Histological and morphometric findings

At 8 weeks after surgery, the number of nerve fibers (measured in 10,000 μm^2), increased significantly in the experimental groups compared to the control group ($P < 0.001$). The number of nerve fibers increased significantly in the NGF + Mem + Cell group compared to the other experimental groups ($P < 0.001$) (Figs. 6 and 7).

Statistical evaluations revealed that the mean number of nerve fibers increased significantly in the Mem + Cell and NGF + Mem + Cell groups compared to the control group ($P < 0.001$). There was a significant increase between the Mem + Cell group and the other experimental groups ($P < 0.001$). In addition, the number of vessels increased significantly in the NGF + Mem + Cell group compared to the other experimental groups ($P < 0.001$) (Fig. 8).

The percentage of vessel area increased significantly in the experimental groups compared to the control group except for the NGF group ($P < 0.001$). Application of one-way analysis of variance showed a significant increase in the Mem + Cell group compared to the other groups ($P < 0.001$). There was a significant increase in the NGF + Cell group compared to the NGF group but not the NGF + Mem group ($P < 0.001$). The percentage of vessel area increased significantly in the NGF + Mem + Cell group compared to the other groups ($P < 0.001$) (Fig. 9).

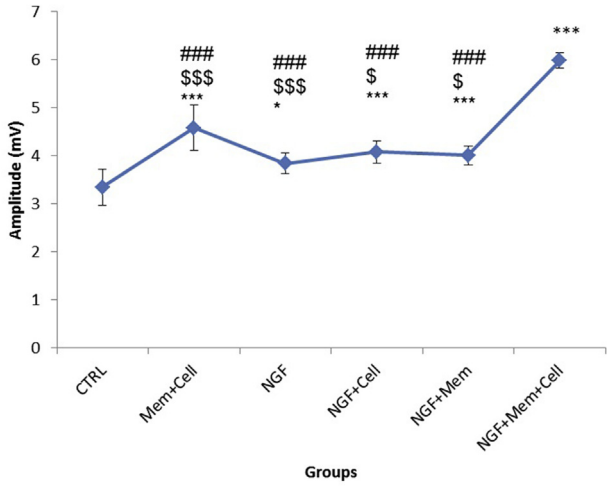

Fig. 4. Comparison of mean amplitude (mV) analysis in all experimental groups (mean ± SD). *, ***Significant differences between experimental groups and control group ($P < 0.05$, $P < 0.001$, respectively). NGF + Mem + Cell compared to NGF + Mem, NGF + Cell, NGF, and control ($P < 0.001$). $, $$$Significant differences between Mem + Cell group compared to NGF, NGF + Cell, and NGF + Mem groups ($P < 0.001$, $P < 0.05$, and $P < 0.05$, respectively). ###Significant differences between NGF + Mem + Cell group compared to other groups ($P < 0.001$).

4. Discussion

The present study showed that the use of NGF may promote regeneration of transected sciatic nerve in a rat model. In this

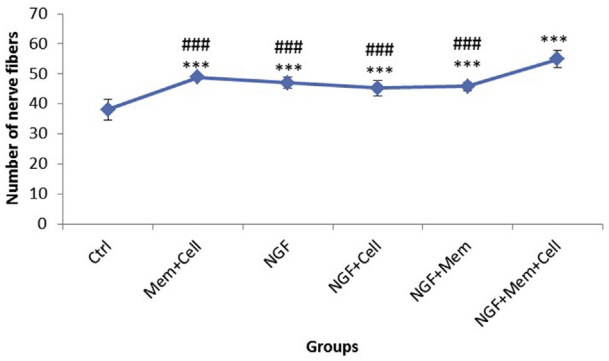

Fig. 6. At 8 weeks after surgery, number of nerve fibers (mean ± SD) increased significantly in NGF + Mem + Cell group. ***Significant increase in experimental groups compared to control group. ###Significant increase in NGF + Mem + Cell group compared to other experimental groups ($P < 0.001$).

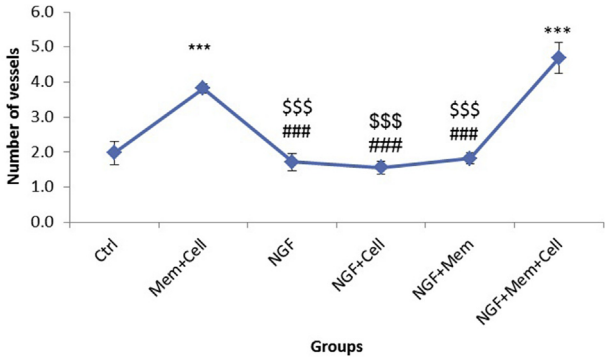

Fig. 7. At 8 weeks after surgery, number of vessels (Mean ± SD) increased significantly in NGF + Mem + Cell group. ***Significant differences among NGF + Mem + Cell and Mem + Cell compared to control group ($P < 0.001$). $^{\$\$\$}$Significant differences between Mem + Cell group compared to other experimental groups ($P < 0.001$). $^{\#\#\#}$Significant differences between NGF + Mem + Cell, compared to other experimental groups ($P < 0.001$).

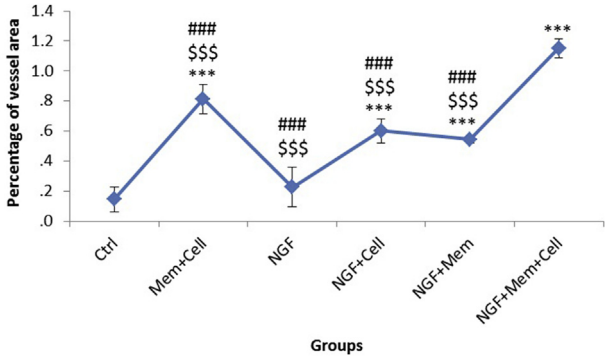

Fig. 8. At 8 weeks after surgery, vessel area percentage (Mean ± SD) increased significantly in NGF + Mem + Cell group.***Significant increase in experimental groups compared to control group except for NGF group ($P < 0.001$). $^{\$\$\$}$Significant increase in Mem + Cell group compared to NGF, NGF + Cell, and NGF + Mem groups ($P < 0.001$). $^{\#\#\#}$Significant differences between NGF + Mem + Cell group compared to other experimental groups ($P < 0.001$).

regard, behavorial analyses (sciatic functional index, hot water paw immersion test) and electromyographical evaluation and histomorphometric assessments (number of nerve fibers and number of vessels in 10,000 μm^2 and percentage of vessel area) were performed on transected sciatic nerve 8 weeks after surgery.

Our results showed that behavorial analyses and electromyographical evaluation improved significantly in the NGF + Mem + Cell group. Chen et al. (2009) reported that electromyographic evaluation (nerve conduction velocity, amplitude, and latent period) were better in the NGF group in their study (Chen et al., 2009). Kemp et al. (2011) showed that there was no significant difference among the treated groups but that amplitude increased in the NGF group compared to the other transection and repair groups (Kemp et al., 2011). Wang et al. (2012) indicated that using an NGF-loaded, chitosan-based nerve conduit had a positive effect on peripheral nerve repair (Wang et al., 2012).

Histomorphometric findings of our study showed the number of nerve fiber and number of vessels in 10,000 μm^2 and percentage of vessel area increased in the NGF + Mem + Cell group at 8 weeks after surgery. Santos et al. (1991) reported that no improvement in nerve regeneration was identified in the NGF group with respect to the number of myelinated axons (Santos et al., 1991). Yu et al. (2009) reported that histological analysis including axon number was better in the NGF-treated group at 16 weeks postoperatively (Yu et al., 2009). Yu et al. (2008) stated that with histological analysis, axon diameter and myelin thickness were larger in this group at 16 weeks postoperatively (Yu et al., 2008). Nunes e Silva et al. (2012) found that there were no differences in the number of regenerated axons among the treatment groups (Nunes e Silva et al., 2012). Santos et al. (1991) showed increased angiogenesis in chitosan prepared with NGF in that group compared to other groups (Santos et al., 1991). Hsu et al. (2013) reported that in the poly-(D,L-lactide) scaffold and Tremella fuciformis polysaccharide (TF) group, angiogenesis increased. In this group, improvement in the sizes of blood vessels was observed. The authors suggested that TF has a function like NGF and promotes neurite outgrowth (Hsu et al., 2013).

In fact, mesenchymal stem cells from umbilical cord participate in regeneration of peripheral nerves by expressing neurotrophins. The neurotrophins NGF has indirect and positive effects on neuronal survival, angiogenesis, Schwann cell viability, and proliferation during development and regeneration of peripheral nerves. Furthermore, NGF guarantees survival and differentiation of sensory and sympathetic neurons (Truzzi et al., 2008). Taniuchi et al. (1986) showed that after axotomy, expression of NGF receptors increase in Schwann cells distal to the lesion. It was demonstrated that NGF significantly exerted synergistic effects on peripheral nerve regeneration by inducing the expression of $p75^{NTR}$ in Schwann cells at both the mRNA and protein levels (Taniuchi et al., 1986).

It has been reported that there is a correlation between blood vessels (number and size of blood vessels) and axonal regeneration (Hobson et al., 2000). NGF exerts a direct effect on endothelium and has a pivotal role in neovascularization. Therefore NGF is used as proangiogenic agent (Zhao et al., 2013). In addition, recent investigation has revealed that NGF has various effects on inflammatory conditions (Tanaka et al., 2004). Shahram et al. (2013) reported that after 2 weeks, chitosan membranes were useless and induced an inflammatory response because of being a foreign substance (Shahram et al., 2013). In addition, Ojeda et al. (2011) observed a correlation between an increase in NOS activity in chitosan-treated lesions and an increased number of counted capillaries and that chitosan enhanced angiogenesis and NO release (Ojeda et al., 2011). Thus, the improvement in nerve fiber number might be due to the promotion of angiogenesis, which produces a suitable environment for axon growth and increases nerve regeneration. Among 79 proteins secreted by mesenchmal stem cells of Wharton's jelly, 14 important neurotrophic factors play important roles in neuronal survival, angiogenesis, and enhancement of the neuron regeneration by the regulation of integrin and membrane proteins that anchor cell matrix, Schwann cell viability, and proliferation during the development and regeneration of peripheral nerves. Therefore, it seems that transplantation of MSCs in our study caused the secretion of NGF, BDNF, NT3, and NT4/5, which play important roles in neuronal survival, differentiation, and maintenance. Chitosan-based scaffolds induced adhesion, growth, migration, and orientation of Schwann cells (Guo et al., 2015). Researchers have observed that multipotent mesenchymal stromal cells (MSCs) not only promote tissue repair in response to injury and disease but also induce angiogenesis in impaired tissues by secreting a broad set of archetypal angiogenic factors, such as vascular endothelial growth factor (VEGF), platelet-derived growth factor AA (PDGF-AA), transforming growth factor-β2 (TGF-β2), basic fibroblast growth factor 2 (bFGF-2), and hepatocyte growth factor (HGF). In addition, the paracrine angiogenic effect of the WJ-MSCs is mediated at least in part by netrin-1, which enhances angiogenesis in vivo. Vascular and neuronal development share many guidance factors and receptors that control the angiogenic process (Prieto et al., 2017).

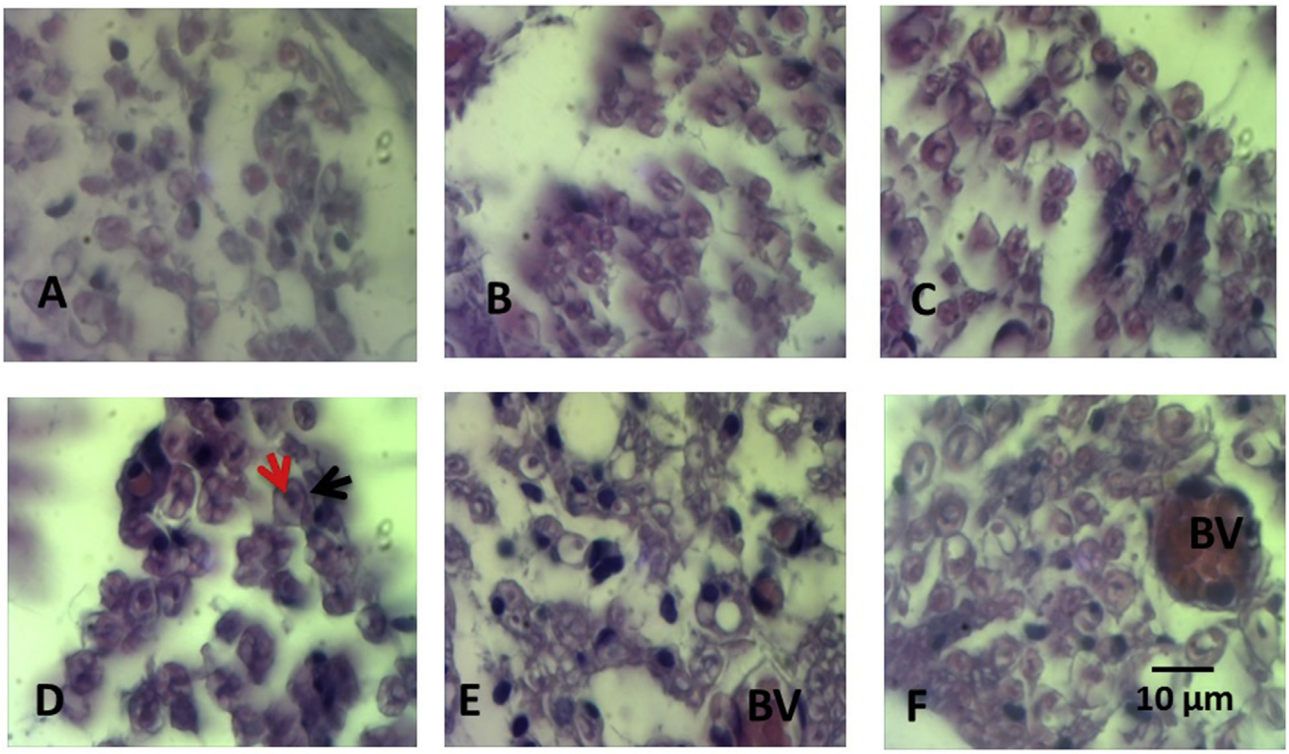

Fig. 9. Photomicrographs of cross-sections through distal part of damaged sciatic nerve at 8 weeks after surgery in different groups. Hematoxylin and eosin staining. Red arrow indicates axon; black arrow indicates nerve fiber. BV, blood vessel. Magnifications × 1000. Groups: (A) control; (B) Mem + Cell; (C) NGF; (D) NGF + Cell; (E) NGF + Mem; and (F) NGF + Mem + Cell. In control group, number of nerve fibers decreased due to nerve transection. Number of nerve fibers increased in NGF + Mem + Cell group compared to other groups, and large vessel was seen.

It has been reported that MSCs do not differentiate at damaged sites; they have low grafting proficiency and act instead by secreting wide-ranging usual growth factors, cytokines, chemokines, and bioactive lipids into the wound microenvironment. Furthermore, netrins are a family of secreted proteins that play important role as guidance cues, axonal path finding and axon migration. Interestingly, among the four common axon guidance family proteins netrins, slits, ephrins, and semaphorins, netrin-1 has the best chemoattractive capacity and characterizes molecules regulating axonal guidance, cell migration, morphogenesis, and angiogenesis. Netrin-1 mRNA and protein are expressed in the adult rat sciatic nerve and Schwann. It was reported that 2 weeks after sciatic nerve transection and direct anastomosis, netrin-1 in Schwann cells of the distal nerve stump was up-regulated (Dun and Parkinson, 2017).

Netrin-1 could guide regenerating axons to the distal nerve stump and enhance axon extension by interacting with DCC, Neogenin, DSCAM, and CD146 on the growth cone of regenerating axons. In addition, netrin-1 enhances Schwann cell and endothelial cell proliferation and migration through the Neogenin receptor and the CD146 receptor, respectively. In addition, netrin-1 prevents endothelial cell apoptosis, capillary density, which enhances the neuronal survival and regeneration. On the other hand, following a peripheral nerve transection, Schwann cell migration plays an important role in effective peripheral nerve regeneration. With Schwann cell guidance, regenerating axons find their correct path to the distal nerve stump and increase their growth speed. For attraction of axons, neurotrophins such as GDNF, NGF, and BDNF have been suggested. These neurotrophins mediate neural connectivity in the peripheral nervous system (PNS). Neurotrophins such as NGF, which are produced by target cells in the periphery, are also implicated in axonal extension and in synapse formation between preganglionic and postganglionic sympathetic neurons (Dun and Parkinson, 2017).

Elevation of cAMP levels in the soma following peripheral nerve injury increases axonal re-growth and requires CREB as a transcription factor. It is reported that NGF triggers cAMP response element binding protein (CREB) translation and retrograde transport (Abe and Cavalli, 2008).

Transplantation of mesenchymal stem cells that produce netrin-1 in a rat model of crushed injury, increased the level of NGF and enhanced the function of sciatic nerve (Ke et al., 2015). Indeed, in addition to NGF promoting local axonal growth as well as long-range retrograde transcriptional regulation and survival, it also controls synaptogenesis from a distance (Sharma et al., 2010).

5. Conclusion

Nowadays, one serious health problem is peripheral nerve injury. Our study results suggest that use of NGF in conjunction with biodegradable membrane and mesenchymal stem cells improved electromyographical and histological analysis. These findings provide greater support for the use NGF plus membrane plus mesenchymal stromal cells for the clinical repair of peripheral nerve damage.

Acknowledgements

This study is extracted from a Ph.D. thesis at Kharazmi University by Mehrnaz Moattari. We express gratitude from Neuroscience Research Center, Baqiyatallah University of Medical Sciences for supporting this article. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The authors report no conflicts of interest.

References

Abe N, Cavalli V: Nerve injury signaling. Curr Opin Neurobiol 18: 276–283, 2008

Asplund M, Nilsson M, Jacobsson A, von Holst H: Incidence of traumatic peripheral nerve injuries and amputations in Sweden between 1998 and 2006. Neuroepidemiology 32: 217–228, 2008

Bain J, Mackinnon S, Hunter D: Functional evaluation of complete sciatic, peroneal, and posterior tibial nerve lesions in the rat. Plast Reconstr Surg 83: 129–136, 1989

Barmpitsioti A, Konofaos P, Ignatiadis I, Papalois A, Zoubos AB, Soucacos PN: Nerve growth factor combined with an epineural conduit for bridging a short nerve gap (10 mm). A study in rabbits. Microsurgery 31: 545–550, 2011

Bhang SH, Jeon O, Choi CY, Kwon YHK, Kim BS: Controlled release of nerve growth factor from fibrin gel. J Biomed Mater Res 80: 998–1002, 2007

Chen Y, Wang D, Wang Z, Weng Z, Deng Z: Effect of adenovirus expressing NGF on sciatic nerve injury in rats. Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi 23: 947–953, 2009

Chen ZW, Wang MS: Effects of nerve growth factor on crushed sciatic nerve regeneration in rats. Microsurgery 16: 547–551, 1995

Cheng H, Qiu L, Ma J, Zhang H, Cheng M, Li W, et al: Replicative senescence of human bone marrow and umbilical cord derived mesenchymal stem cells and their differentiation to adipocytes and osteoblasts. Mol Biol Rep 38: 5161–5168, 2011

Deuse T, Stubbendorff M, Tang-Quan K, Phillips N, Kay MA, Eiermann T, et al: Immunogenicity and immunomodulatory properties of umbilical cord lining mesenchymal stem cells. Cell Transplant 20: 655–667, 2011

Dubový P, Raška O, Klusáková I, Stejskal L, Čelakovský P, Haninec P: Ciliary neurotrophic factor promotes motor reinnervation of the musculocutaneous nerve in an experimental model of end-to-side neurorrhaphy. BMC Neurosci 12: 58, 2011

Dun X-P, Parkinson DB: Role of netrin-1 signaling in nerve regeneration. Int J Mol Sci 18: 491, 2017

Guo Z-Y, Sun X, Xu X-L, Zhao Q, Peng J, Wang Y: Human umbilical cord mesenchymal stem cells promote peripheral nerve repair via paracrine mechanisms. Neural Regen Res 10: 651, 2015

Hobson MI, Green CJ, Terenghi G: VEGF enhances intraneural angiogenesis and improves nerve regeneration after axotomy. J Anat 197: 591–605, 2000

Hsu S-H, Chan S-H, Weng C-T, Yang S-H, Jiang C-F: Long-term regeneration and functional recovery of a 15 mm critical nerve gap bridged by tremella fuciformis polysaccharide-immobilized polylactide conduits. Evid Based Complement Altern Med 2013, 2013

Ke X, Li Q, Xu L, Zhang Y, Li D, Ma J, et al: Netrin-1 overexpression in bone marrow mesenchymal stem cells promotes functional recovery in a rat model of peripheral nerve injury. J Biomed Res 29: 380, 2015

Kemp SW, Webb AA, Dhaliwal S, Syed S, Walsh SK, Midha R: Dose and duration of nerve growth factor (NGF) administration determine the extent of behavioral recovery following peripheral nerve injury in the rat. Exp Neurol 229: 460–470, 2011

Ma J, Liu J, Yu H, Wang Q, Chen Y, Xiang L: Curcumin promotes nerve regeneration and functional recovery in rat model of nerve crush injury. Neurosci Lett 547: 26–31, 2013a

Ma S, Peng C, Wu S, Wu D, Gao C: Sciatic nerve regeneration using a nerve growth factor-containing fibrin glue membrane. Neural Regen Res 8: 3416, 2013b

Margossian T, Reppel L, Makdissy N, Stoltz J-F, Bensoussan D, Huselstein C: Mesenchymal stem cells derived from Wharton's jelly: comparative phenotype analysis between tissue and in vitro expansion. Biomed Mater Eng 22: 243–254, 2012

Nunes e Silva D, Silva ACMBA, Aydos RD, Viterbo F, Pontes ERJC, Odashiro DN, et al: Nerve growth factor with fibrin glue in end-to-side nerve repair in rats. Acta Cir Bras 27: 325–332, 2012

Ojeda JE, Cardenas G, Klassen R, von Plessing C, Grez P, Sepulveda J: Nitric oxide synthase activity and angiogenesis measured by expression of CD34 in burns treated with chitosan films. Compend Contin Educ Dent 23: 135–143, 2011

Prieto C, Villanueva A, Carrillo D, Lattus J, Aedo S, Lois P, et al: Netrin-1 acts as a non-canonical angiogenic factor produced by human Wharton's jelly mesenchymal stem cells. Placenta 51: 104, 2017

Sadraie SH, Parivar K, Arabi F, Moattari M, Kaka G, Mansouri K: Study of transected sciatic nerve repair by amniotic membrane with betamethasone in adult albino Wistar rats. Arch Iran Med 19, 2016

Santos P, Winterowd J, Allen G, Bothwell M, Rubel E: Nerve growth factor: increased angiogenesis without improved nerve regeneration. Otolaryngol Head Neck Surg 105: 12–25, 1991

Shahram E, Sadraie SH, Kaka G, Khoshmohabat H, Hosseinalipour M, Panahi F, et al: Evaluation of chitosan–gelatin films for use as postoperative adhesion barrier in rat cecum model. Int J Surg 11: 1097–1102, 2013

Sharma N, Deppmann CD, Harrington AW, Hillaire CS, Chen Z-Y, Lee FS, et al: Long-distance control of synapse assembly by target-derived NGF. Neuron 67: 422–434, 2010

Sun W, Sun C, Zhao H, Lin H, Han Q, Wang J, et al: Improvement of sciatic nerve regeneration using laminin-binding human NGF-β. PLoS One 4: e6180, 2009

Tanaka A, Wakita U, Kambe N, Iwasaki T, Matsuda H: An autocrine function of nerve growth factor for cell cycle regulation of vascular endothelial cells. Biochem Biophys Res Commun 313: 1009–1014, 2004

Taniuchi M, Clark HB, Johnson EM: Induction of nerve growth factor receptor in Schwann cells after axotomy. Proc Natl Acad Sci 83: 4094–4098, 1986

Truzzi F, Marconi A, Lotti R, Dallaglio K, French LE, Hempstead BL, et al: Neurotrophins and their receptors stimulate melanoma cell proliferation and migration. J Invest Dermatol 128: 2031–2040, 2008

Wang H, Zhao Q, Zhao W, Liu Q, Gu X, Yang Y: Repairing rat sciatic nerve injury by a nerve-growth-factor-loaded, chitosan-based nerve conduit. Biotechnol Appl Biochem 59: 388–394, 2012

Yu H, Peng J, Guo Q, Zhang L, Li Z, Zhao B, et al: Improvement of peripheral nerve regeneration in acellular nerve grafts with local release of nerve growth factor. Microsurgery 29: 330–336, 2009

Yu H, Peng J, Sun H, Xu F, Zhang L, Zhao B, et al: [Effect of controlled release nerve growth factor on repairing peripheral nerve defect by acellular nerve graft]. Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi 22: 1373–1377, 2008