Journal of Chemical Neuroanatomy 88 (2018) 46–54

Contents lists available at ScienceDirect

Journal of Chemical Neuroanatomy

journal homepage: www.elsevier.com/locate/jchemneu

Chitosan-film associated with mesenchymal stem cells enhanced regeneration of peripheral nerves: A rat sciatic nerve model

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ARTICLE INFO

Keywords:
Chitosan
Mesenchymal stem cells
Sciatic nerve
Repair
Rat

ABSTRACT

Objective: Peripheral nerve injuries comprise significant portion of the nervous system injuries. Although peripheral nerves show some capacity of regeneration after injury, but the extent of regeneration is not remarkable. Regeneration might be through the activity of the mesenchymal stem cells (MSCs) which can release growth factors or extracellular matrix components or by the therapeutic effect of some material with the MSCs. The present study aimed to evaluate the regeneration of transected sciatic nerve by a therapeutic value of mesenchymal stem cells (MSCs) associated with chitosan-film (Cs) in rat.

Materials & methods: Male Wistar rats (n = 42, 180-200g) were randomly divided into intact; control; sham; Cs; MSCs; MSCs + Cs groups. Functional recovery was evaluated at 2, 4, 6 and 8 weeks after surgery using sciatic functional index (SFI), hot water paw immersion test, electrophysiological, histological analyses.

Results: The rats in the MSCs + Cs group showed significant decrease in SFI and hot water paw immersion test during the 2nd to 8th weeks after surgery. Electrophysiological findings showed a significant decrease in latency time in the MSCs + Cs group. Amplitude of the nerve impulses also increased. Number of nerve fibers with more than 6 μm diameters increased significantly in MSCs + Cs. The number of nerve fibers with less than 4 μm diameters also increased significantly in MSCs + Cs group.

Conclusion: Taken together, mesenchymal stem cells associated with Cs could improve functional and histomorphological properties of the sciatic nerve after injury which may have some clinical outcomes as well.

1. Introduction

One of the prevalent human disabilities is peripheral nerve damage. The rate of peripheral nerve injuries in developed countries is assessed between 18 per 100,000 persons/year (Jiang et al., 2017). These injuries impose economic burden both to the patients and the society, although these injuries are not life-threatening (Rosberg et al., 2013a). On the other hand, despite the capacity of peripheral nerves to regeneration after injury, the extent of regeneration is not remarkable. The far most experimental model for the study of peripheral nerve regeneration studies is presented by sciatic nerve injury. Various reasons such as easily accessible, large size, representing mixed motor and sensory nerve, and autonomic aspects and huge amount of studies which provide data comparability with previous studies might explain popularity of sciatic nerve injury model among animal models (Geuna, 2015). Several strategies also evaluated for improvement of peripheral nerve regeneration. As a result, much attention has been given by researchers and clinicians to cell-based therapies and use of scaffold materials which are biodegradable, non-toxic/non-inflammatory, mechanically similar to the tissue to be replaced, highly and capable of attaching with other molecules, lasting long enough for axonal regrowth and tissue repair is preferred (Hinderer et al., 2016). Many investigators have focused on mesenchymal stem cells. Yarar and Kuruoglu (2015) concluded that use of treatment with mesenchymal stem cells stem cell improved nerve conduction velocity (Yarar et al., 2015). Guo and Sun (2015) showed that human Wharton's jelly-derived mesenchymal stem cells represent a promising stem cell source for cell-based therapy and MSCs transplantation into the transected sciatic nerve promotes axonal regeneration and functional recovery (Guo et al., 2015). Mesenchymal stem cells are considered a potential

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https://doi.org/10.1016/j.jchemneu.2017.10.003
Received 17 March 2017; Received in revised form 7 October 2017; Accepted 18 October 2017
Available online 26 October 2017
0891-0618/

therapeutic approach in a variety of diseases of the central nervous (CNS) and peripheral nervous system (PNS). Although the mechanisms underlying these benefits remain unclear, a number of experiments have supported that may play a role in supporting peripheral nerve regeneration through axon remyelination, neuronal survival, differentiation and maintenance and protecting sensory, motor and sympathetic neurons as well as promotion of neurite outgrowth. In addition, these cells may enhance nerve regeneration through production of growth factors such or extracellular matrix components and by up-regulation of integrins and membrane proteins that anchor cell matrix (Pereira et al., 2014). Extensive studies have been performed regarding human Wharton's jelly-derived mesenchymal stem cells (MSCs) ability to differentiate into different types of cells such as trilineage mesenchyme cell type; adipocytes, chondrocytes, osteoblasts and neuronal-like cells including astrocytes, oligodendrocytes, microglial, neurons and neuroglial-like cells (Ullah et al., 2015). 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 (Zy et al., 2015). Gonzales-Perez and Cobianchi (2015) showed that chitosan tubes with low and medium degree of acetylation achieved 43% and 57% are promising in effective regeneration and targeted reinnervation, respectively (Gonzalez-Perez et al., 2015). Furthermore, Meyer et al. (2016) results suggested that Cs significantly improved functional and morphological results of nerve regeneration in comparison to simple hollow chitosan nerve guides in 15 mm sciatic nerve defects in adult healthy Wistar rats (Meyer et al., 2016). The present study aimed to evaluate the effects of mesenchymal stem cells on Cs (MSCs + Cs) regeneration of transected sciatic nerve using hot water paw immersion (for evaluation of sensory repair), Sciatic functional index (for evaluation of motor repair), electromyographical (for evaluation of motor unit repair) and morphological assessments in rat model.

2. Materials and methods

2.1. Animals

Forty-two male adult Wistar rats (180–200 g) purchased from Pasture Institute Tehran, Iran (license number: 91.7861) (n = 7/group) were used in these experiments. All animals were housed in cages (four rats per cage) at controlled temperature 23 ± 2 °C and 50% humidity with 12/12 h light/dark and have free access to standard rat chow and tab water. All experiments involving animals and surgical procedures were approved by Ethical Committee of Baqiyatallah University of Medical Sciences (The Baqiyatallah (a.s.) University of Medical Sciences Committee on the use and Care of Animals).

2.2. 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 cm3 and centrifuged at 250*g* for 5 min. Following removal of the supernatant, the precipitate (mesenchymal tissue) was washed with serum-free DMEM (Gibco, USA) and centrifuged at 250*g* for 5 min. Then the precipitate (mesenchymal tissue) was 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. FBS (Hyclone, USA) was then added to the mesenchymal tissue to stop trypsinization. The dissociated mesenchymal cells were further dispersed in 10% FBS-DMEM and counted under a microscope with the aid of a hemocytometer.

2.3. Immunocytochemistry of MSCs

MSCs were identified by CD44 and Fibronectin antibodies (Santa Cruz Biotechnology, USA) and CD45 as a marker for hematopoietic stem cell (Santa Cruz Biotechnology, USA). MSCs were washed with PBS, fixed for 30 min in 4% paraformaldehyde (Invitrogen, UK), were washed in PBS, and the primary antibodies (mouse anti-Fibronectin monoclonal antibody diluted at 1:50, mouse anti-CD44 monoclonal antibody diluted at 1:100 and mouse anti-CD45 monoclonal antibody diluted at 1:100 (Santa Cruz Biotechnology, USA)) were incubated overnight at 4° C and washed three times in PBS, incubated with the relevant secondary antibody (anti-mouse FITC conjugated diluted at 1:100 (Chemicon, USA) for 2 h at room temperature, washed in PBS twice, and counterstained with ethidium bromide diluted at 1:10,000 (Sigma, USA) for 1 min to demonstrate the nuclei. The Samples were then washed in PBS and examined using a fluorescence microscope (Axiophot, Zeiss, Germany)). For negative controls, the primary antibodies were omitted and the same staining procedures were similar to positive controls (Pirzad Jahromi et al., 2015).

2.4. Cs preparation

Thin films were prepared with a mixture of 0.25 g of Cs dissolved in 50 ml of 1% acetic acid solution. The mixture was stirred for two 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, dried at 25 °C for 24 h. The films were then dried and cut to patches of 1 cm × 1 cm in size (Emamgholi et al., 2013).

2.5. Animal grouping

Forty-two rats were randomly divided into six groups (n = 7/group) as below: 1) Intact group: healthy rats without any nerve injury; 2) Control group: transected and end-to-end sutured sciatic nerve rats without any intervention; 3) Sham group: transected and end-to-end sutured sciatic nerve rats treated with culture medium on the injured nerve; 4) Cs group: transected and end-to-end sutured sciatic nerve rats treated with a thin Cs around the injured nerve; 5) MSCs group: transected and end-to-end sutured sciatic nerve rats treated with transplantation of 50,000 MSCs around the injured nerve and 6) MSCs + Cs group: transected and end-to-end sutured sciatic nerve rats treated with a thin Cs and transplantation of 50000 MSCs around the injured nerve.

2.6. Surgical procedure

Animals were anesthetized with 80 mg/kg ketamine hydrochloride (alfasan, Netherland) and 5 mg/kg xylazine hydrochloride (alfasan, Netherland) intraperitoneally. For sciatic nerve dissection, after shaving the right hind limbs of rats a longitudinal incision was made on the posterolateral side of the thigh in length of 3 cm to expose the sciatic nerve. The right sciatic nerve was then transected at a midway from by a sharp surgical knife. For end-to-end nerve suture the epineurium was sutured with 7.0 Prolene sutures and the muscle fascia and skin were then sutured with 4.0 nylon sutures (Sadraie et al., 2016) (Fig. 1).

2.7. Sciatic functional index (SFI) assessment

The Sciatic Functional Index (SFI) is a quite useful tool for the evaluation of functional recovery of the sciatic nerve of rats using an apparatus as follows. The SFI apparatus was made from wood with 60 × 7 × 20 cm (L, A, H respectively) dimensions and its floor was covered with white paper. Functional recovery was assessed at 2nd, 4th, 6th and 8th weeks after surgery.

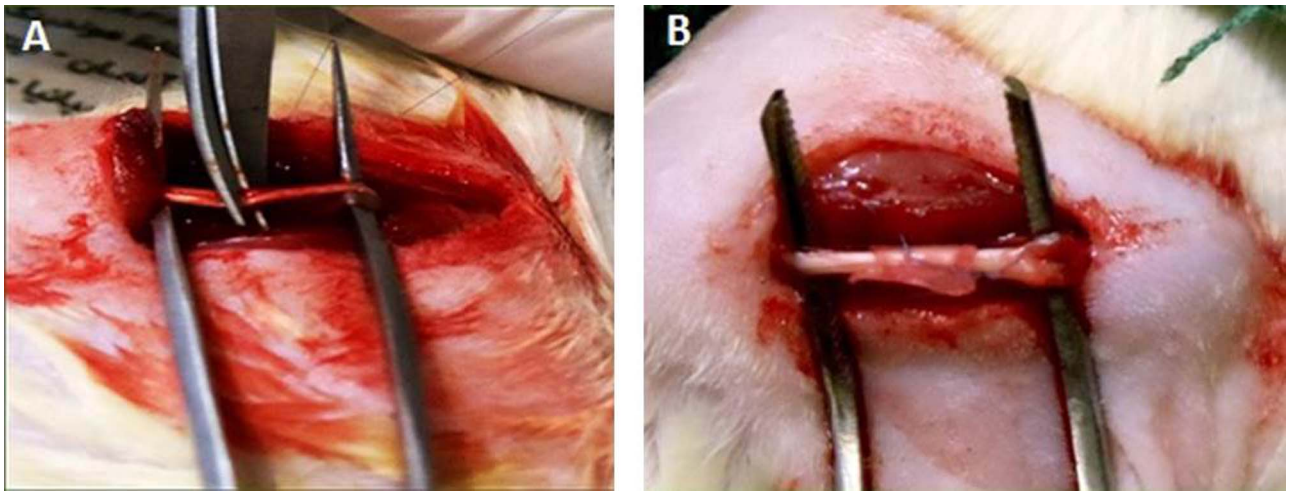

Fig. 1. (A) Operation procedure of the animals. Right sciatic nerves were sectioned under germ free conditions and sutured through the epineurium, (B) Wrapping the MSCs + Cs around the injured sciatic nerve.

After painting of hind paws with water soluble blue ink, rats were permitted to walk through the apparatus and their foot prints were tracked. The lengths of the third toe to it's heel (PL), the second toe to the fourth toe (IT), and the first 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 ranged from 0 (for normal nerve function) till 100 (for total dysfunction). SFI oscillates around 0 (0, +10) indicates normal nerve function, whereas the value is 100 or less indicates that motor activity is completely gone. This method is the best method to measure motor behavior. Since the formula modified by authors, the results are real numbers and the results are reported positive(Sadraie et al., 2016).

2.8. Hot water paw immersion test

Hot water paw immersion test was performed using the hot water bath (DID SABZ Co. Iran). The water temperature was set on 50 ± 1 °C. Measurement of reaction time in hot water paw immersion test was done at 2nd, 4th, 6th and 8th weeks after surgery. Paw immersion procedure was as follow: each rat was gently arrested by the experimenter and one of its feet (i.e. intact or experimental) immersed into the water till its paw. The passed time in which the rat withdraws its paw from the water was recorded. Each test was performed three times, with interval of 10 min. Then results were expressed as the reaction time(Sadraie et al., 2016).

2.9. EMG testing

At 8 weeks after surgery, the sciatic nerves were exposed in the anesthetized rats. The sciatic nerve in Control, Sham, Cs, MSCs and Cs + MSCs groups; transected and end-to-end nerve sutured. The therapeutic procedure were performed in each group. In addition, electrical stimulation was done by two monopolar needles as cathode and anode stimulate and the rate of 3–5 mA located next to the sciatic nerve. The compound action muscle protential (CAMP) were recorded by insertion of surface electrodes in active formed on the gastrocnemius muscle and the reference electrode inserted to the knee. Device was set as follows: 1 millisecond per division and sensivity was set 1 ms per division. Amplitude and latency of the impulses were recorded as the factors of nerve conductivity from the gastrocnemius muscle and a reference cap electrode inserted on the surface of knee joint. Filter settings for the recording motor wave and distal latency including the time interval between stimulation to the start of motor wave was employed to indicate that regeneration or remyelination process which creating connections between axons. The cAMP also indicates the number of active axons. In other hands, the amplitude when decreased showed axonal damage and when increased revealed axonal restoration. Amplitude and latency of the impulses were recorded as the factors of nerve conductivity from the gastrocnemius muscle and a reference cap electrode inserted on the surface of knee joint. Another stainless steel needle was used as the ground electrode, which inserted into the tail skin

2.10. Histological study

For histological assessment, 8th week after surgery, the sciatic nerves were surgically taken out one millimeter before and one millimeter after the anastomosis and fixed in10% formalin, After 72 h of fixation the specimen processing, embedded in paraffin. Five μm transverse sections from distal portion of sciatic nerves were prepared and stained by hematoxylin and eosin using standard techniques. The number of nerve fibers in different categories based on the diameter (including > 6 μm, 4–6 μm and < 4 μm diameters) were counted using MOTIC software (Nikon, Japan, 2001) under light microscopy from at least 5 randomly selected at × 1000 magnification (Sadraie et al., 2016).

2.11. Statistical analysis

All data were expressed as mean ± standard error of mean (SEM). Results were analyzed using Two-Way Repeated Measurements analysis of variance (ANOVA) [for hot water paw immersion test and SFI] with training Weeks (Week) as within-subjects factor and experimental group (Group) as between-subjects factor or One-Way analysis of variance (ANOVA) [electrophysiological and morphological assessments] followed by least significant different (LSD) Post Hoc test (SPSS 22.0 software package, SPSS Inc., Chicago, IL). Difference less than 0.05 ($P < 0.05$) level was considered as statistically significant.

3. Results

3.1. Immunocytochemistry of MSCs

As in our previous study was shown, Immunocytochemical results showed the cells had positive expression of CD90 and fibronectin as

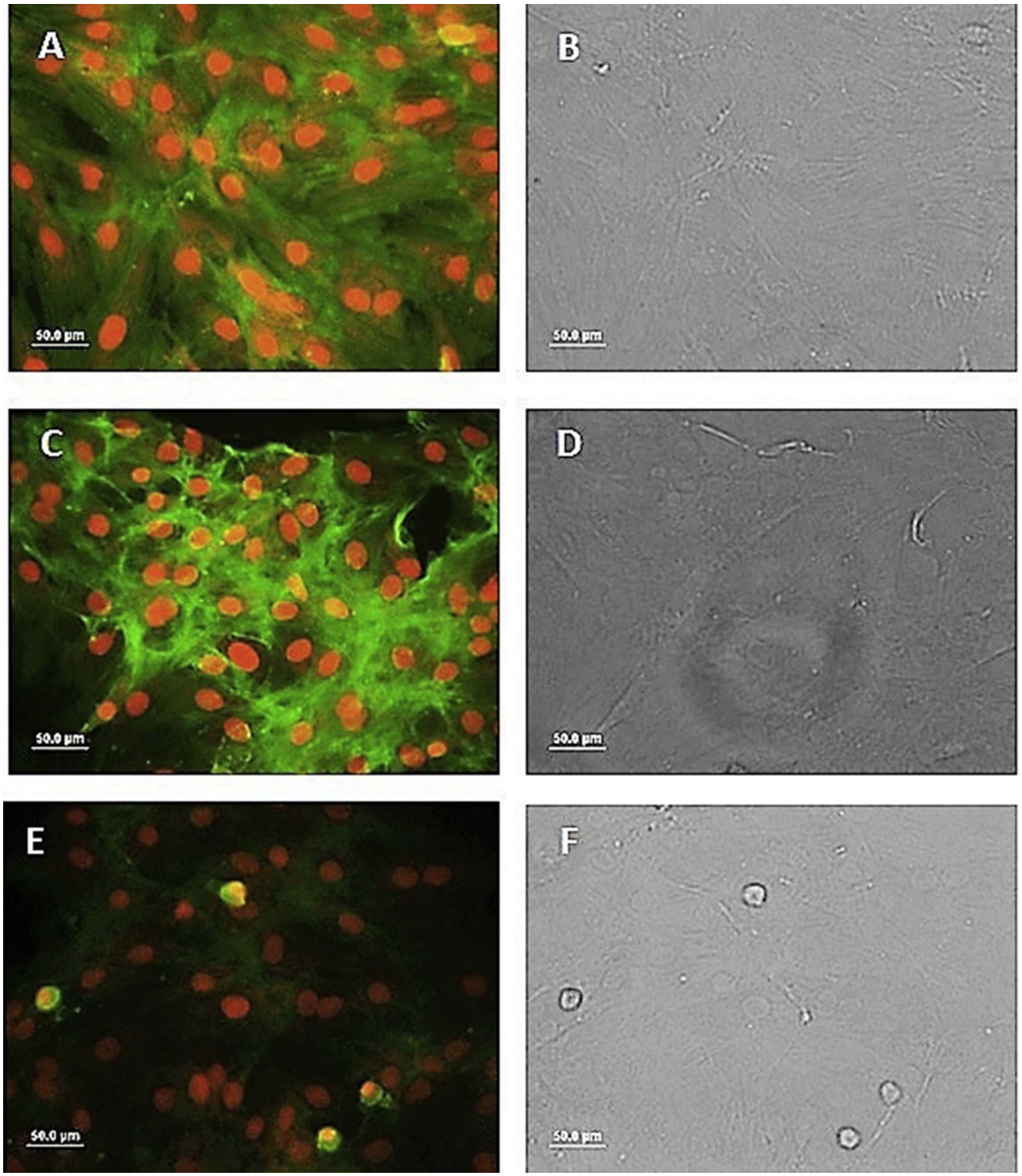

Fig. 2. Immunocytochemical photomicrographs of the mesenchymal stem cells of Wharton's jelly. At 3rd passage mesenchymal stem cells were incubated with (A) anti-CD44 (B) anti-fibronectin, and (C) anti-CD45 as primary antibodies, followed by the secondary antibody conjugated with FITC. Nuclei were stained with ethidium bromide. Magnifications × 1000. Scale bar: 50 µm.

mesenchymal stem cells markers but did not express CD45, a hematopoietic stem cell marker(Fig. 2).

3.2. Sciatic functional index (SFI) evaluations

SFI track analysis was performed at 2, 4, 6 and 8 weeks after surgery. The SFI values after surgery in all surgical groups significantly increased. At 8 weeks after surgery, SFI decreased significantly and improvement was found in MSCs + Cs group compared to control and sham group [Two-Way repeated measurement ANOVA; Week effect: $F(1, 3) = 39.144$, $P < 0.001$; Cs effect $F(1, 5) = 233.668$, $P < 0.001$; MSCs effect: $F(1,5) = 233.668$ $P < 0.001$] (Fig. 3).

3.3. Hot water paw immersion test

At 8 weeks after surgery, the reaction time in hot water paw

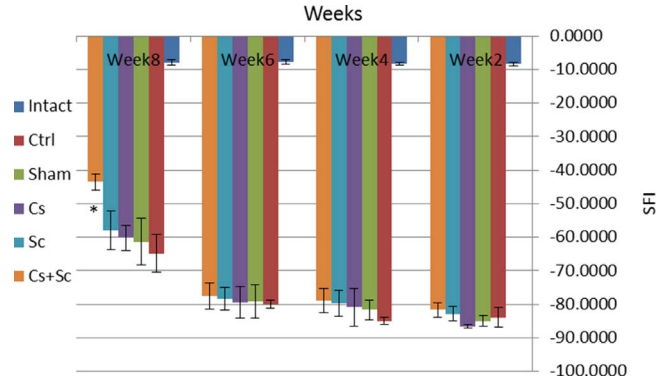

Fig. 3. Comparison of the mean functional recovery of SFI (mean ± SEM). At 8 weeks after surgery, SFI decreased significantly and improvement was found in MSCs + Cs group compared to control and sham group ($p < 0.05$).

immersion test significantly decreased in therapeutic groups especially in the MSCs + Cs group compared with control, sham, Cs and MSCs groups [Two-Way repeated measurement ANOVA; Week effect: $F(1,3) = 32.48$, $P < 0.0001$; Cs effect $F(1,5) = 230.223$, $P < 0.0001$; MSCs effect: $F(1,5) = 230.223$, $P < 0.0001$] (Fig. 4).

3.4. EMG results

Our data showed the mean of latency in MSCs + Cs group were significantly decreased when compared to control and sham. Mean amplitude (mV) decreased significantly in surgical groups compared to intact group at 8 weeks after surgery. In addition, at 8 weeks after surgery, mean amplitude were significantly increased in MSCs group compared to control and sham groups [Two-Way ANOVA; Cs effect: $F(2,35) = 2.42$, $P > 0.05$, MSCs effect: $F(2,35) = 2.42$, $P > 0.05$, MSCs × Cs effects: $F(1,42) = 5.83$, $P < 0.05$](Figs. 5 and 6).

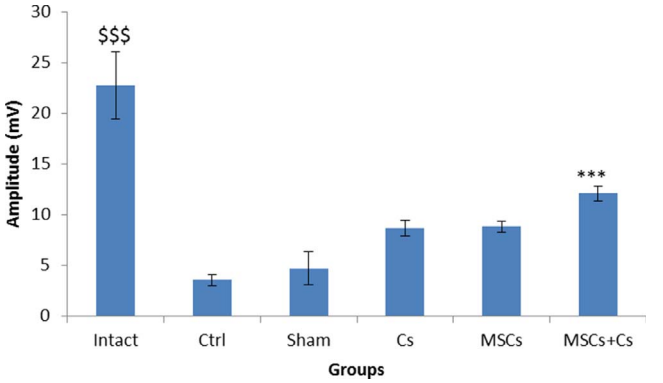

Fig. 5. Comparison of mean amplitude (mV) analysis in all experimental groups (mean ± SEM). $$$ shows that there are significant differences among intact group and other groups. *** shows that amplitude was increased significantly in MSCs + Cs group compared to other groups at 8 weeks after surgery ($p < 0.05$).

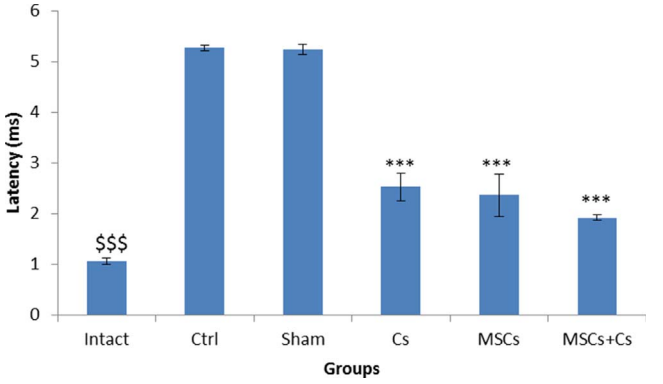

Fig. 6. Comparison of mean latency (ms) analysis in all experimental groups (mean ± SEM). $$$ shows that there are significant differences among intact group and other groups. *** shows that Latency decreased significantly in Cs, Mem and MSCs + Cs group compared to other groups at 8 weeks after surgery ($p < 0.001$).

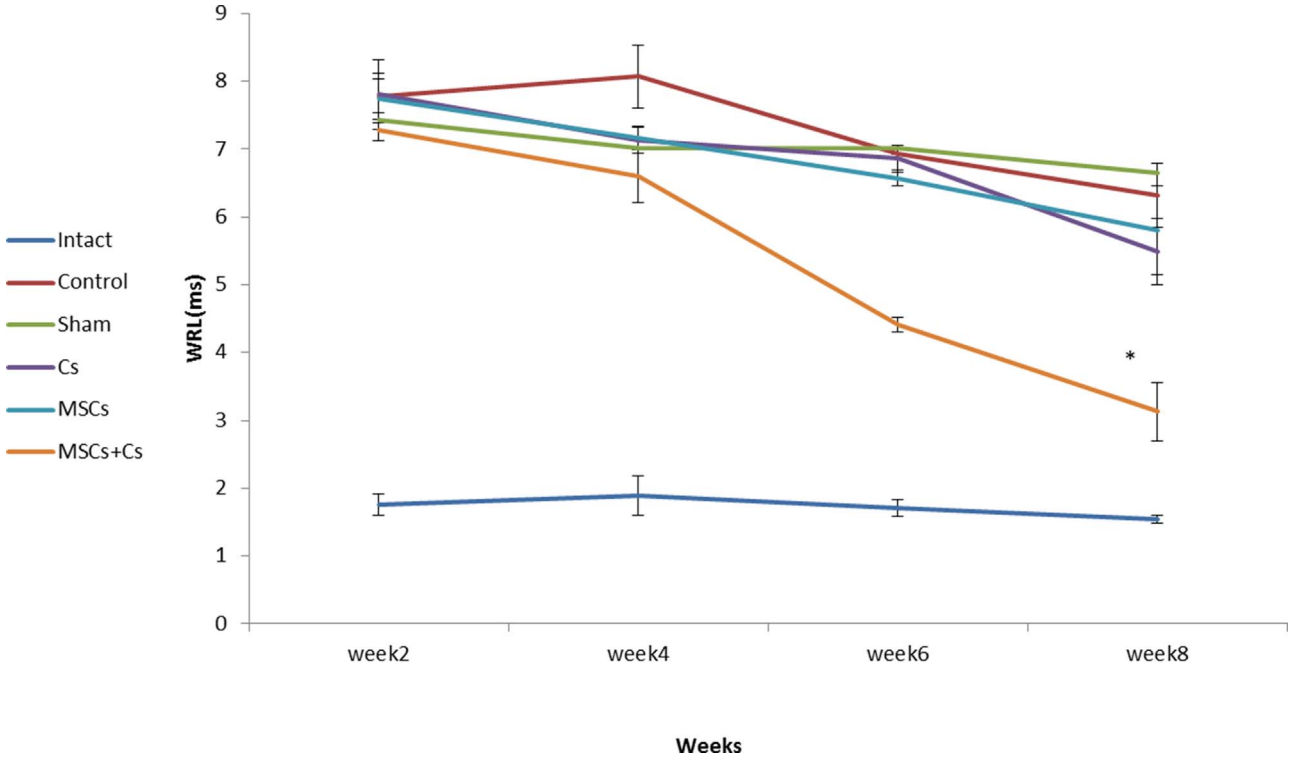

Fig. 4. Hot water paw immersion test (mean ± SEM). At 8 weeks after surgery, the reaction time in hot plate test decreased significantly in MSCs + Cs ($P < 0.05$).

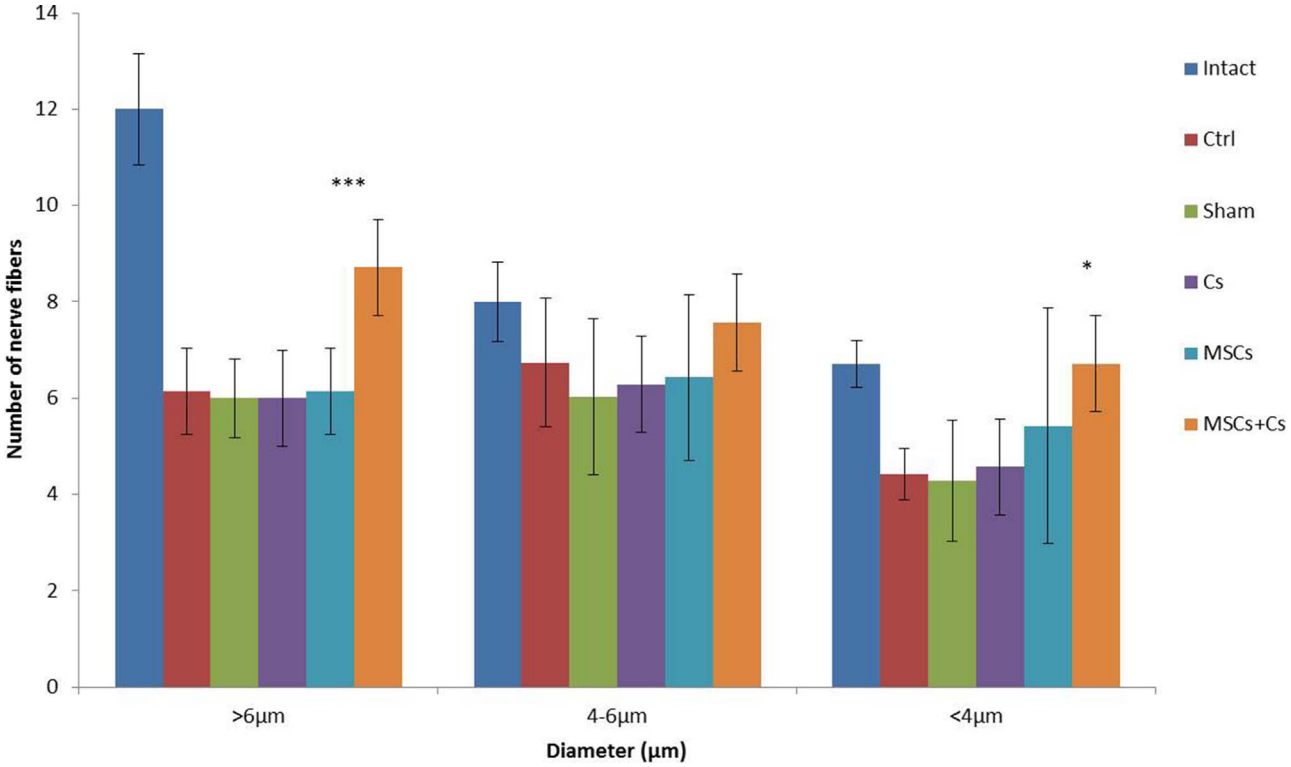

Fig. 7. Photomicrographs of cross sections through distal part of damaged sciatic nerve at 8 weeks after surgery in different groups. Diameter is measured. (A) Intact group, (B)Control group, (C) Sham group, (D) Cs group, (E) MSCs group and (F) MSCs-Cs group. Hematoxylin and eosin staining., magnifications × 1000.

3.5. Histomorphometric results

At 8 weeks after surgery, the numbers of nerve fibers in different categories based on diameter were significantly decreased in surgical groups compared to intact group. Mean number of nerve fibers with more than 6 µm diameter increased significantly in MSCs + Cs group compared to control, sham, Cs and MSCs group[Two-Way ANOVA; Cs effect: $F(2,35) = 9.54$, $P < 0.001$, MSCs effect: $F(2,35) = 9.54$, $P < 0.001$, MSCs × Cs effects: $F(1,42) = 2.59$, $P > 0.05$]. There was no significant differences in therapeutic groups compared to control and sham groups based on the number of nerve with 4–6 µm diameter [Two-Way ANOVA; Cs effect: $F(2,35) = 20.382$, $P < 0.001$, MSCs effect: $F(2,35) = 20.382$, $P < 0.001$, MSCs × Cs effects: $F(1,42) = 21.518$, $P < 0.05$]. The number of nerve fibers with less than 4 µm increased significantly in MSCs + Cs group compared to control and sham group [Two-Way ANOVA; Cs effect: $F(2,35) = 14.115$, $P < 0.001$, MSCs effect: $F(2,35) = 14.115$, $P < 0.001$, MSCs × Cs effects: $F(1,42) = 2.974$, $P > 0.05$]. [Two-Way ANOVA; Cs effect: $F(1,48) = 2.11$, $P < 0.01$, MSCs effect: $F(2, 48) = 1.24$, $P < 0.05$, MSCs × Cs effects: $F(7, 48) = 3.22$, $P < 0.01$] (Figs. 7 and 8).

4. Discussion

The present study aimed to evaluate the effects of human Wharton's jelly-derived mesenchymal stem cells on chitosan/polyethylene oxide membrane (MSCs + Cs) for repairing of transected sciatic nerve in a rat model. In this regard, functional (sciatic functional index, hot water paw immersion test and electrophysiological evaluation) and histomorphometric assessments was performed on transected sciatic nerve during 8 weeks after surgery.

According to our previous study immunocytochemical results of this investigation showed the cells had positive expression of CD44 and fibronectin as mesenchymal stem cells markers but did not express CD45, a hematopoietic stem cell marker at the P3. The immunoreactivity of the MSCs to fibronectin and CD44 antibodies confirmed by successful isolation of MSCs. In addition, appropriate amount of MSCs from bone marrow were harvested and obtained at passage 3.

Previous studies reported that MSCs was useful in sciatic nerve regeneration (Pereira et al., 2014; Rosberg et al., 2013b). This issue makes them valid candidates for cell-based therapy and tissue engineering in peripheral nervous system. Our results showed that use of MSCs in combination with Cs membrane could improve transected sciatic nerve regeneration. This improvement was pretty impressive 8 weeks after surgery. Although it seems that considering a longer recovery time for example for 12 weeks or more probably enhance the probability of observing a greater improvement.

The selection of a proper cell candidate for replacement therapy is very important. In this regard, MSCs derived from the Wharton's jelly of the umbilical cord (MSCs) exhibit unique features (e.g. primitive nature, multilineage potential immunomodulatory ability, ease of isolation, extensive proliferation) that may make them more valuable therapeutic tools for promoting anatomical and functional recovery upon transplantation in rodent models of central and peripheral nerve dysfunction.

The motor function recovery was assessed by the walking track. Application of sciatic functional index (SFI) is a reliable quantitative method for the evaluation of the recovery of motor performance because of low cost and easy to apply compared to other methods proposed for the assessment of functional condition. In this regard, our data demonstrated that at 8 weeks after surgery, SFI results significantly improved in MSCs + Cs group. Our findings are in disagreement with the results of Shalaby et al. (2017) who reported that SFI decreased significantly in combination group (conduit and stem cells derived from adipose tissue) at 12 weeks after surgery(Shalaby et al., 2017). Liu et al. (2014) also reported conserved dopamine neurotrophic factor-transduced mesenchymal stem cells promoted functional recovery (SFI) 8 weeks after surgery (Liu et al., 2014). Nociceptive function was assessed by hot water paw immersion test. At 8 weeks after surgery, hot water paw immersion test results of this study showed significant improvement in MSCs + Cs group. This results are

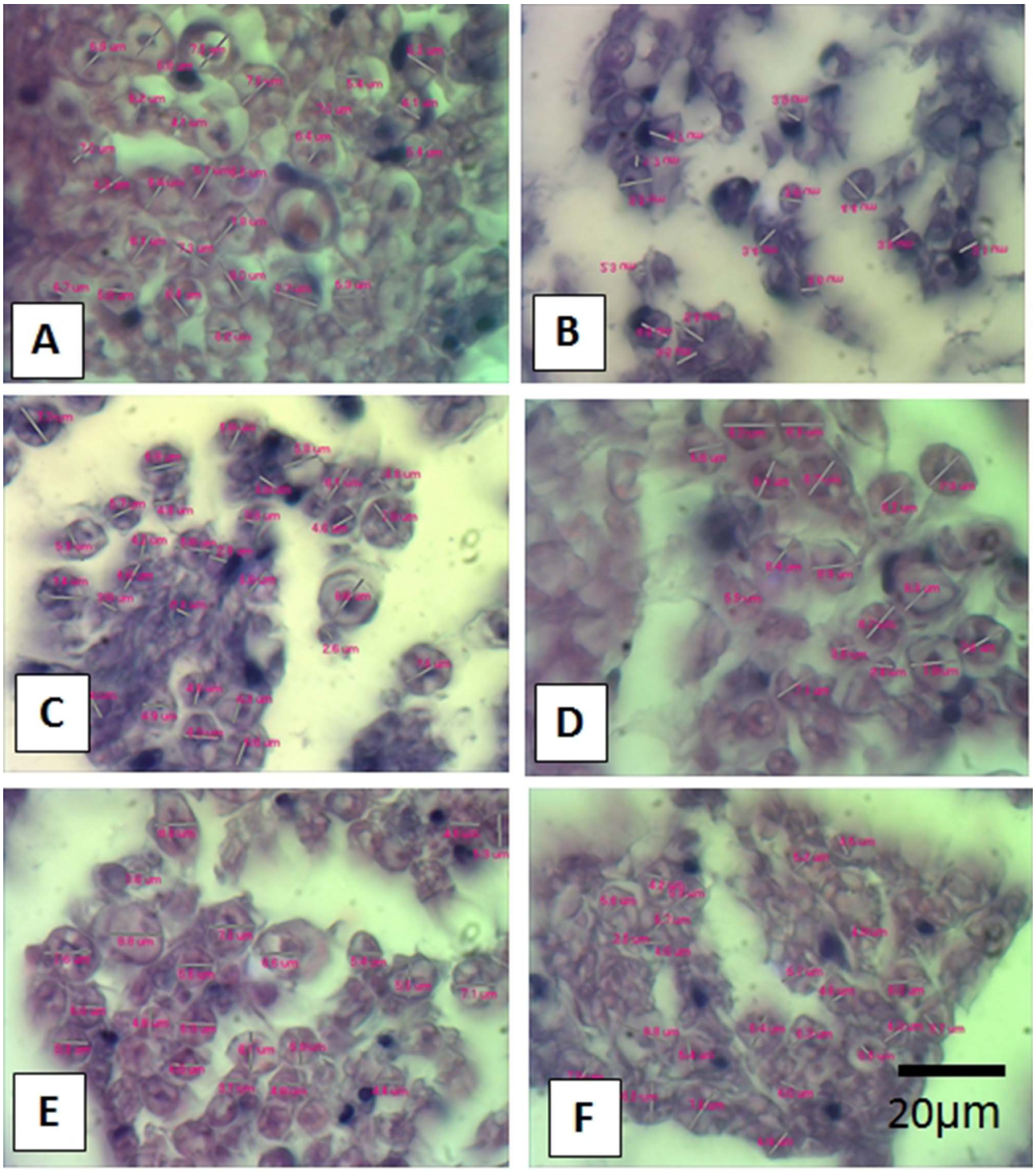

Fig. 8. Number of nerve fibers is shown in different categories based on diameter (mean ± SEM). *,*** shows that the number of nerve fibers with less than 4 μm and more than 6 μm increased significantly in MSCs + Cs group compared to control and sham group at 8 weeks after surgery ($p < 0.05$ and $p < 0.001$).

in agreement with the findings of Shirosaki et al. (2014) demonstrated that the reaction time in hot water paw immersion test improved at 12 weeks after surgery(Shirosaki et al., 2014). In addition, our results are in agreement with study by Pereira et al. (2014) showed better recovery in end-to-end groups containing poly (DL-lactide-ε-caprolactone) membrane and mesenchymal stem cells from the Wharton's jelly compared to groups receiving a graft on the nociception function 20 weeks after surgery (Pereira et al., 2014). Our electrophysiological data showed that at 8 weeks after surgery, latency and amplitude of CAMP improved significantly in Cs, MSCs and MSCs + Cs group compared to control and sham groups. This shows the functionality of nerve conduction. The amplitude indicates the nerve conduction strength. A higher amplitude shows that more axons regenerated and arrived at the measured muscles in the paws. However it should be mentioned that latency is decreased in the Cs + MSCs group compared to other groups. This means that there are more neural connections with the target muscle. In other words, shorter latency results from better nerve myelination (Pan et al., 2017). Of course, our data showed the amount of Cs + MSCs, in separate treatment groups have less time after stimulation. These results are in contradiction with the findings of Pan et al. (2017) reported

improvement of electrophysiological tests in cell therapy groups (Pan et al., 2017).

Histomorphometric findings of our study showed the number of nerve fiber in different categories increased in MSCs + Cs group at 8 weeks after surgery. Our results are in agreement with Shirosaki et al. (2014) who reported total number of nerve fibers were significantly higher in chitosan and cell therapy group during the healing period of 12 weeks (Shirosaki et al., 2014). In addition, Goulart et al. (2016) reported that the poly (L-lactic acid) PLA conduit improved the regenerative process of the sciatic nerve in mice by increasing the number of myelinated fibers after 8 weeks of regeneration (Goulart et al., 2016). Several possible mechanisms are described to explain the effects of mesenchymal stem cells in peripheral nerve repair. In fact, overall effects of mesenchymal stem cells in improvement of nerve regeneration including cell replacement, the production of growth factors, extracellular matrix molecule synthesis and deposition, releasing anti-inflammatory, anti-apoptic molecules and immune modulators to establish adequate microenvironment for neural regeneration (Zy et al., 2015). Additionally, mesenchymal stem cells of umbilical cord participate in regeneration of peripheral nerves by expressing neurotrophins. The effect of bioactive molecules secreted by mesenchymal stem cells can be either directly or indirectly or even both: Direct effects causing by intracellular signaling and indirect effects by forcing other cells to release the active agent operating in that area. This indirect activity is called trophic. Among 79 proteins secreted by mesenchmal stem cells of Wharton jelly, 14 important neurotrophic factors play important role in neuronal survival, angiogenesis, enhancement of the neuron regeneration by up-regulation of integrin and membrane proteins that anchor cell matrix, schwann cell viability and proliferation during development and regeneration of peripheral nerves (Zy et al., 2015). Therefore, it seems transplantation of MSCs in our study caused secretion of NGF, BDNF, NT3 and NT4/5 that play important role in neuronal survival, differentiation and maintenance (Zy et al., 2015). To promote peripheral nerve injuries, tissue engineering has produced many biomaterials the objective of repairing wounds in a timely and cost effective manner. Chitosan is a derivation of chitin which is found in the cell wall of crustacean and is obtained by the *N*-deacetylation of chitin. It is reported that chitosan accelerate wound healing by promoting inflammatory reaction. As above-mentioned, chitosan has several advantages which has found in bone tissue engineering, skin tissue engineering and neural tissue regeneration. Chitin based associated with other materials show an affinity for nerve cells. It has been reported that chitosan based nerve chambers can be useful for nerve regeneration. Chitosan based scaffolds induce adhesion, growth, migration and orientation of Schwann cells. Schwann cells promote nerve tissue regeneration by releasing neurotrotropic factors, expressing neuron-specific ligands and guiding neurite outgrowth. Schwann cells also secrete and deposit ECM. In addition, chitosan has roles in survival and differentiation of neuronal cells and the prevention of painful neuromas (Raisi et al., 2012). It is declared that in wound healing, the inflammatory cells produce NO in the early phase. Ojeda et al. (2011) reported that there is a correlation between the increase of NOS activity in chitosan-treated lesions and the increased number of counted capillaries chitosan enhances angiogenesis and NO release. Improvement of the nerve fiber number might be due to the promotion of angiogenesis which produces a suitable environment for axon growth and increases nerve regeneration (Ojeda et al., 2011). Their results showed that chitosan was biocompatible and had positive effects on the survival and orientation of Schwann cells, as well as the survival and differentiation of neuronal cells and the prevention of painful neuromas (Ojeda et al., 2011).

5. Conclusion

Nowadays, one of the serious health problems is peripheral nerve injury. Our results suggested that use of mesenchymal stem cells and Cs (MSCs + Cs) improved functional, electrophysiological and histomorphometric recovery of transected sciatic nerves in rat model which may be useful for the clinical outcomes improvement that is often unsatisfactory and there is rarely a complete return of function.

Ethical statement

It is also declared that the paper has not been submitted for consideration elsewhere. All experiments involving animals and surgical procedures were approved by Ethical Committee of Baqiyatallah University of Medical Sciences (The Baqiyatallah (a.s.) University of Medical Sciences Committee on the use and Care of Animals).

Acknowledgements

We express gratitude from Neuroscience Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran for supporting this article.

References

Emamgholi, A., Kaka, G.R., Sadri, M., Sadraee, S.H., Hosseini, S.H., 2013. The synthesis of chitosan/polyethylene nanofibersoxide on chitosan membrane: an applicable model in tissue engineering. Ann. Mil. Health Sci. Res. 10, 300–305.

Geuna, S., 2015. The sciatic nerve injury model in pre-clinical research. J. Neurosci. Methods 243, 39–46.

Gonzalez-Perez, F., Cobianchi, S., Geuna, S., Barwig, C., Freier, T., Udina, E., Navarro, X., 2015. Tubulization with chitosan guides for the repair of long gap peripheral nerve injury in the rat. Microsurgery 35, 300–308.

Goulart, C.O., Lopes, F.R.P., Monte, Z.O., Dantas, S.V., Souto, A., Oliveira, J.T., Almeida, F.M., Tonda-Turo, C., Pereira, C.C., Borges, C.P., 2016. Evaluation of biodegradable polymer conduits–poly (l-lactic acid)–for guiding sciatic nerve regeneration in mice. Methods 99, 28–36.

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

Hinderer, S., Layland, S.L., Schenke-Layland, K., 2016. ECM and ECM-like materials—biomaterials for applications in regenerative medicine and cancer therapy. Adv. Drug Deliv. Rev. 97, 260–269.

Jiang, L., Jones, S., Jia, X., 2017. Stem cell transplantation for peripheral nerve regeneration: current options and opportunities. Int. J. Mol. Sci. 18, 94.

Liu, Y., Nie, L., Zhao, H., Zhang, W., Zhang, Y.-Q., Wang, S.-S., Cheng, L., 2014. Conserved dopamine neurotrophic factor-transduced mesenchymal stem cells promote axon regeneration and functional recovery of injured sciatic nerve. PLoS One 9, e110993.

Meyer, C., Stenberg, L., Gonzalez-Perez, F., Wrobel, S., Ronchi, G., Udina, E., Suganuma, S., Geuna, S., Navarro, X., Dahlin, L.B., 2016. Chitosan-film enhanced chitosan nerve guides for long-distance regeneration of peripheral nerves. Biomaterials 76, 33–51.

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

Pan, M., Wang, X., Chen, Y., Cao, S., Wen, J., Wu, G., Li, Y., Li, L., Qian, C., Qin, Z., 2017. Tissue engineering with peripheral blood-derived mesenchymal stem cells promotes the regeneration of injured peripheral nerves. Exp. Neurol. 292, 92–101.

Pereira, T., Gärtner, A., Amorim, I., Almeida, A., Caseiro, A., Armada-da-Silva, P.A., Amado, S., Fregnan, F., Varejão, A., Santos, J., 2014. Promoting nerve regeneration in a neurotmesis rat model using poly (DL-lactide-Îµ-caprolactone) membranes and mesenchymal stem cells from the Wharton's jelly: in vitro and in vivo analysis. BioMed. Res. Int. 302659.

Pirzad Jahromi, G., Shabanzadeh, A.P., Sadr, S.S., Kaka, G., Jafari, M., Seidi, S., Charish, J., 2015. Multipotent Bone Marrow Stromal Cell Therapy Promotes Endogenous Cell Proliferation Following Ischemic Stroke. Clinical and Experimental Pharmacology and Physiology.

Raisi, A., Azizi, S., Delirezh, N., Heshmatian, B., Amini, K., 2012. Use of chitosan conduit for bridging small-gap peripheral nerve defect in sciatic nerve transection model of rat. Iran. J. Vet. Surg. 5, 89–100.

Rosberg, H.-E., Carlsson, K.S., Cederlund, R.I., Ramel, E., Dahlin, L.B., 2013a. Costs and outcome for serious hand and arm injuries during the first year after trauma–a prospective study. BMC Public Health 13, 1.

Rosberg, H.-E., Carlsson, K.S., Cederlund, R.I., Ramel, E., Dahlin, L.B., 2013b. Costs and outcome for serious hand and arm injuries during the first year after trauma –a prospective study. BMC Public Health 13, 501.

Sadraie, S.H., Parivar, K., Arabi, F., Moattari, M., Kaka, G., Mansouri, K., 2016. Study of transected sciatic nerve repair by amniotic membrane with betamethasone in adult albino wistar rats. Arch. Iran. Med. 19, 612–617.

Shalaby, S.M., Amal, S., Ahmed, F.E., Shaban, S.F., Wahdan, R.A., Kandel, W.A., Senger, M.S., 2017. Combined Wharton's jelly derived mesenchymal stem cells and nerve guidance conduit: a potential promising therapy for peripheral nerve injuries. Int. J. Biochem. Cell Biol. 86, 67–76.

Shirosaki, Y., Hayakawa, S., Osaka, A., Lopes, M.A., Santos, J.D., Geuna, S., Mauricio, A.C., 2014. Challenges for nerve repair using chitosan-siloxane hybrid porous scaffolds. BioMed. Res. Int. 153808.
Ullah, I., Subbarao, R.B., Rho, G.J., 2015. Human mesenchymal stem cells-current trends and future prospective. Biosci. Rep. 35, e00191.
Yarar, E., Kuruoglu, E., Kocabıcak, E., Altun, A., Genc, E., Ozyurek, H., Kefeli, M., Marangoz, A.H., Aydın, K., Cokluk, C., 2015. Electrophysiological and histopathological effects of mesenchymal stem cells in treatment of experimental rat model of sciatic nerve injury. Int. J. Clin. Exp. Med. 8, 8776.
Zy, G., Sun, X., Xl, X., Peng, J., Wang, Y., 2015. Human umbilical cord mesenchymal stem cells promote peripheral nerve repair via paracrine mechanisms. Neural Regener. Res. 10, 651.