Neuroregenerative role of transplanted olfactory ensheathing cells in a model of sciatic nerve crush injury in rats: Histological study

Original Article

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ABSTRACT

Background: Olfactory ensheathing cells (OECs) are highly specialized glial cells that guide olfactory receptor axons from nasal mucosa to olfactory bulb. Olfactory ensheathing cells (OECs) are attractive candidates for transplantation-mediated repair of injured nervous system owing to their neuroregenerative properties.

Aim of the work: To evaluate the neuroregenerative role of OECs in a model of sciatic nerve crush injury in rats.

Materials and Methods: OECs were isolated from the olfactory mucosa of 15 male albino rats (100 gm). Thirty adult female albino rats (200-250 gm) were used and divided into three groups of 10 rats each. Group I (control group) underwent sham operation. Group II underwent crush injury of sciatic nerve. Group III underwent sciatic nerve crush injury then injected with single dose of 3×10^4 OECs at the lesion site. All rats were sacrificed after 3 weeks from the onset of the experiment.

Results: Histological examination of sciatic nerves of group II rats showed discontinuity of nerve fibers with vacuolated axoplasm. OECs transplantation in group III revealed restoration of the normal histological architecture of the sciatic nerves. A significant decrease in the diameter of myelinated nerve fibers and myelin sheath thickness was recorded in group II compared to the control group. However, group III revealed a significant increase in the previous parameters compared to group II.

Conclusions: Olfactory ensheathing cells (OECs) have neuroregenerative properties which might represent a new vision in human cell based therapy.

Key Words: Histology, Olfactory ensheathing cells, rats, sciatic nerve

Revised: 8 July 2017, **Accepted:** 17 August 2017

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ISSN: 1110-0559, September Vol. 40, No. 3

INTRODUCTION

Cellular transplantation strategies using different cellular sources have been established in multiple experimental injuries[1]. Olfactory ensheathing cells (OECs) are a highly specialized glial population which currently considered prime candidate for cell-based therapy to repair the injured CNS[2] due to their unique regenerative potential[1]. Olfactory ensheathing cells are derived from the neural crest and found in both the peripheral and central nervous systems: they ensheath bundles of olfactory axons from their peripheral origin in the olfactory epithelium to their central synaptic targets in the olfactory bulb[3].

Olfactory ensheathing cells (OECs); either residing in olfactory lamina propria or olfactory bulb; are derived from a common progenitor in the olfactory mucosa[4]. They ensheath and promote the growth of olfactory receptor neuron axons as they exit the olfactory epithelium and extend toward the olfactory bulb[5] where they differentiate into olfactory bulb OECs and associate with astrocytes[6].

Bulb- and mucosa-derived OECs differ substantially; for example, with regards to their proliferative ability over time[7]. In contrast to bulb-derived OECs, mucosa-derived OECs are readily accessible and can be biopsied safely without any loss of smell sense[8].

Regenerative potential of OECs is not only attributed to their ability to promote axon growth, remyelinate, and stimulate angiogenesis, but also they migrate into astrocyte-rich areas and do not induce the astrocyte stress response[9].

In rodents, OECs have been successfully used in different models including myocardial infarct[10], spinal cord trauma[11-13], cochlear damage[14], Parkinson's disease[15], and in a mouse model that mimics effects of ischemic/hypoxic injury in the hippocampus[16].

Peripheral nerve injuries (PNI) occur in approximately 3% of all trauma patients[17, 18] causing a debilitating disease with lifelong suffering and disturbance in functions.

 DOI: 10.21608/EJH.2017.4661

Peripheral nerve injury commonly occurs because of accidental trauma, acute compression, or iatrogenic injury leading to neuronal dysfunction which affects quality of life. In clinical practice, surgical repair is usually required for treating nerve transection injuries, while drug administration; such as neurotrophins and vitamins; is a useful therapy for treating nerve crush injuries[19-21].

Sciatic nerve crush injury model in rats is widely used to assess the post-traumatic motor function impairment. Moreover, the sciatic nerve remains the most used and reliable model in nerve regeneration studies due to the several behavioral and functional tests available[22]. Therefore, the aim of the present experiment was to study the histological changes in sciatic nerve crush injury model and to assess the neuroregenerative effects of OECs in this model.

MATERIALS AND METHODS

Animals

This study included 15 male Wistar albino rats of average weight 100 gm (3-4 wks) together with 30 female Wistar adult Albino rats of weight 200-250 gm.

The experiment was performed in the Medical Research Center of Ain Shams University according to the experimental animal ethical committee of Faculty of Medicine and University Hospitals, Ain Shams University.

Experimental Design

Fifteen male Wistar albino rats were sacrificed to obtain olfactory mucosa for isolation and culture of OECs. Thirty female Wistar adult albino rats were kept in standard housing conditions and were given food and tap water ad libitum. Animals were classified into 3 groups; 10 rats each. All rats were sacrificed after 3 week from starting the experiments. The groups were randomly divided as follow:

Group I (control sham group); rats were subjected to sham operation with injection of 0.1ml of phosphate buffer saline (PBS) at the site of the operation.

Group II (Crush injury group); rats were subjected to surgery for crush injury of sciatic nerve.

Group III (OECs treated group); rats were subjected to crush injury of sciatic nerve; as in group II; followed immediately by injection of 3×10^4 OECs in 0.1 ml of PBS distal to the lesion site.

Surgical procedures and OECs transplantation

The rats were anesthetized by intraperitoneal injections of ketamine–xylazine mixture at a dose of 100 mg/kg body weight for ketamine (ketalar 50 mg/ml; Pfizer, New York, USA) and xylazine 10 mg/kg body weight (Xylaject 20 mg/ml; Adwia, Egypt). After hair removal, the skin of right thigh was disinfected with betadine (povidone iodine 10%; El-Nile Company, El Obour City, Cairo, Egypt). The right sciatic nerve was exposed by a gluteal muscle splitting incision. Crushing of sciatic nerve was performed using crusher forceps of 18 cm for 1 minute at the level of sciatic notch. Caution was taken to avoid stretch of the nerve. When the crusher forceps were reopened, the nerve was translucent at the crush site. The site of injury was marked using nonabsorbable black sutures 0.5 cm below and 0.5 cm above the injury site[23]. Immediately after the crush injuries were performed in rats of group III, 3×10^4 OECs suspended in 0.1 ml of PBS was injected just distally to the crush site using[24]. Lastly, the muscle septum and the skin were closed using 50/ polyglactin 910 (Vicryl; Ethicon Inc., Somerville, New Jersey, USA).

Postoperative care

Animals were housed in cages with free access to food and water. Dressing of the wound was done daily using betadine and amikacin spray. Surgery pain was controlled using diclofenac sodium (Cataflam 75 mg/3 ml ampule, Novartis, Sandoz (Berkeley) 50 mg/kg body weight Intra muscular (IM), and systemic antibiotic ceftriaxone 100 mg/kg body weight (ceftriaxone sodium; Sandoz).

Isolation & culture of OECs

Olfactory ensheathing cells were harvested from olfactory mucosa of male rats. The isolation process was carried out in a laminar flow cabinet under strict sterile conditions. Begin by preparing three 35 mm Petri dishes filled with DMEM/F12 culture medium in a clean culture hood. After the rats had decapitated, the nasal septum was freed by removal of the lower jaw with scissors. Then, the facial muscles on both sides were eliminated with the help of a rongeur. Starting from the back of the incisors, the bone covering the nasal cavity was removed with a rongeur one side at a time. Once the olfactory turbinates came into sight as orange/brown organs located in the back of the nose, the turbinates were delicately discarded with forceps. Using a 26 gauge needle, the olfactory mucosa was isolated by cutting the tissue along three lines: the arc of the perpendicular plate, the cribriform plate, and the ceiling of the nasal cavity. Care was taken to avoid contamination with respiratory epithelium in the anterior edge of olfactory mucosa. Biopsies on both sides were collected and transfered into DMEM/F12 filled Petri dishes. Firstly, the biopsies were washed in DMEM/F12, and then transferred to a 15 ml tube filled with 1 ml of collagenase IA (Serva Company, Germany) for 1 hour at 37°C. The biopsies were dissociated in the tube using a sterile plastic pipette. Then, the tubes were incubated for 10 minutes at 37°C. To terminate the dissociation, the tubes were gently rocked and 9 ml of Ca-free and Mg-free PBS was addded then the tubes were centrifuged at 200 g for 5 minutes

to obtain the cell pellet. The cell pellet was cultured in DMEM/F12 supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 IU/ml penicillin and 100 µg/ml streptomycin. The solutions were purchased from Lonza Company (Basel, Switzerland). The cultured cells were incubated in a humidified incubator at 37°C in 5% CO2 and 95% air. The cultured cells were examined daily with an inverted microscope (Axiovert 100; Carl-Zeiss, Jena, Germany) to follow-up the growth of the cells and to detect any infection. The culture medium was renewed every 2 to 3 days. Five to seven days after, cells began to invade the culture dishes reaching confluency after about two weeks. When confluency was reached, passage and transfer of the cells to culture flasks were done[2].

Immunohistochemistry was carried out to analyze glial fibrillary acidic protein (GFAP) and S100 expression (Dako Cytomation, Glostrup, Denmark). Olfactory ensheathing cells were fixed using freshly prepared precooled (−20°C) mixture of acetone/ methanol at 1:1, for 10 min at room temperature. The fixed adherent OECs were characterized using the streptavidin–biotin immunoperoxidase technique[23].

Histological and immuno-histochemical studies

For light microscopic examination; sciatic nerve specimens were fixed in 10% buffered formalin, dehydrated, cleared, and embedded in paraffin. Serial 5 µm sections of sciatic nerve were prepared and stained with hematoxylin and eosin (H&E) and immuno-histochemical staining for neurofilaments (Dako Cytomation, Glostrup, Denmark)[25].

For transmission electron microscope (TEM) examination; small sciatic nerve specimens (1 mm3) were fixed in 2.5% gluteraldhyde solution, followed by 1% osmium tetroxide then dehydrated and embedded in epoxy resin. Semithin sections of 1 µm were prepared and stained with toluidine blue. Ultrathin sections of 50 nm were collected on copper grids and stained with uranyl acetate and lead citrate[25]. Finally the sections were examined and photographed under a Jeol TEM 1200 Ex in Faculty of Science, Ain Shams University.

PCR detection of male-derived OECs

Genomic DNA was prepared from sciatic nerve tissue homogenate of the rats in each group using QIAamp® DNA Mini and Blood Mini KIT, Germany. The presence or absence of the sex determination region on the Y chromosome male (sry) gene in recipient female rats was assessed by PCR.

Primer sequences for sry gene (forward 5′-CATCGAAGGGTTAAAGTGCCA-3′, reverse 5′-ATAGTGTGTAG-GTTGTTGTCC-3′) were obtained from published sequences (16) and amplified product of 104 bp; purchased from (Sigma-USA). The PCR conditions were as follows; An initial step of denaturation at 94°C for 4 min; 35 cycles (94°C for 50 s, 60°C for 30 s for optimal annealing, and 72°C for 1 min for extension); thereafter, one cycle of final extension at 72°C for 10 min. PCR products were electrophoresed using 2% agarose gel electrophoresis then stained with ethidium bromide. Positive (male white albino rat genomic DNA) and negative (female white albino rat genomic DNA) controls were included in each assay. Y chromosomes marker was expressed as Transilluminated line[26].

Morphometric measurements

Myelinated nerve fiber diameter and myelin sheath thickness; using semithin toluidine blue stained sections; were measured using five specimens from five different rats of each group. Five different captured nonoverlapping fields were taken from each specimen at magnification 400. From every captured photo, five different readings were counted and then the mean was calculated for each specimen. Measurements were taken by an independent observer blinded to the specimens' details for avoiding biased results.

Samples were analyzed using an image Leica Q win V.3 program installed on a computer in the Histology & Cell Biology Department, Faculty of Medicine, Ain Shams University. The computer was connected to a Leica DM2500 microscope with built-in camera (Leica Microsystems GmbH, Ernst-Leitz-StraBe, Wetzlar, Germany).

Statistical analysis:

The measured parameters in each group were collected, revised, and compared with each other using one-way analysis of variance performed by SPSS.21 program (IBM Inc.,Chicago, Illinois,USA). Differences were considered significant when the p value was ≤0.05. Summary of the data was expressed as mean±standard deviation (SD).

RESULTS

Primary culture of OECs results:

Examination of the primary culture of OECs using an inverted light microscope showed that most of the cells on day 5 were attached with multiple cytoplasmic processes. Some cells were star-shaped while others were spindle-shaped with granular cytoplasm and vesicular nuclei (Fig. 1A). On day 14, the attached cells appeared forming dense homogenous fibroblast like cell colonies with granular cytoplasm and multiple interdigitating process (Fig. 1B). Using streptavidin–biotin immunoperoxidase technique for S100, the cultured OECs revealed positive brownish cytoplasmic reaction in most of the attached cells on day 14 of the

culture (Fig.1C); however weak cytoplasmic reaction was noticed for GFAP in the cultured OECs (Fig. 1D).

Light microscope examination:

Histological examination of H&E stained sections of the sciatic nerve in the control rats (group I) showed that, the sciatic nerve fibers appeared parallel and packed with Schwann cells nuclei appeared in between the nerve fibers. Perineurium was seen surrounding the nerve fascicles (Fig. 2A). Some sections of the sciatic nerve of group II (crush nerve injury group) showed discontinuity of most of the nerve fibers, vacuolated axoplasm, and numerous deeply stained pyknotic nuclei (Fig. 2B). However, other sections of the sciatic nerve of group II revealed disorganization of the nerve fascicles as the axons sprouts advanced in between the proliferated Schwann cells. The nerve fibers appeared degenerated (Fig. 2C). However, sciatic nerve sections of group III showed nearly the same histological results as the control group (Fig. 2D).

Immunohistochemical analysis for neurofilaments revealed positive brownish immune reaction in the axoplasm of most of the nerve fibers in group I (Fig. 3A). However, different sections of the sciatic nerve from different rats in group II showed weak brownish immune reaction in the axoplasm (Fig. 3B, 3C) as compared to the control group. The sections of group III showed strong brownish immune reaction in the axoplasm of many nerve fibers (Fig. 3D).

Toluidine-blue stained semithin sections of the sciatic nerve of group I showed uniform thick myelin sheath (Fig. 4A). Nevertheless, sciatic nerve sections of group II revealed severely degenerated axons and multiple myelin globules (Fig. 4B). In contrast, sciatic nerve sections of group III showed regaining of the normal appearance of myelin sheath as it appeared thick, regular, and uniform (Fig. 4C).

Electron microscopic examination:

Transmission electron microscopy (TEM) provided further evidence for Toluidine-blue stained semithin sections. Ultrastructural examination of transverse section of the sciatic nerve of group I (control group) revealed that sciatic nerve was formed of multiple axons; some appeared myelinated with thick regular myelin sheath while others were unmyelinated as they appeared encompassed within the cytoplasm of Schwann cells. The nerve fibers were seen surrounded by collagen fibers of the endoneurium (Fig. 5A). In group II, myelin sheath had different pictures as it either appeared fragmented forming ellipsoids, or had a whorled appearance. Few fibers revealed thin disorganised myelin sheath. The nerve fibers appeared severly degenerated and lost their organelles and cytoskeleton (Fig. 5B). However, group III showed restoration of the structural integrity of the sciatic nerve fibers as the Schwann cells appeared surrounding the axons with thick, compact, and regular myelin sheath. The axons appeared healthy as their sarcoplasm restored the organelles. However, group of the regenerated unmyelinated nerve fibers ensheathed with Schwann cell cytoplasm was noticed (Fig. 5C). In higher magnification, myelin sheath appeared as a well defined electron dense structure. The axon displayed an abundance of neurofilaments which had been cross-sectioned; giving the axon a stippled appearance. The Schwann cells appeared with normal cytoplasm density, uniformly distributed chromatin, and intact basal lamina. Collagen fibers constituted the fibrillar component of the endoneurium (Fig. 5D).

PCR detection of male-derived OECs:

The SRY gene which was used as the Y chromosome marker was detected in male rats from which OECs were isolated. It was also expressed in female rats that were injected by male derived OECs in group III. Y chromosome marker was not detecting in the control group and group II (Fig. 4D).

Morphometric analysis:

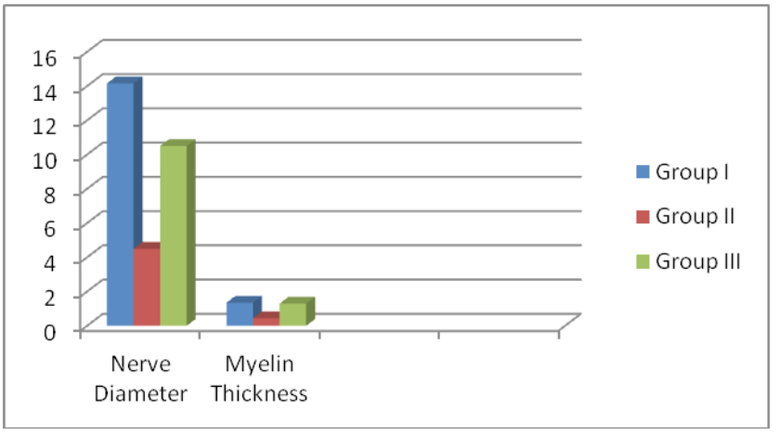
Analysis of the morphometric data from Toluidine-blue stained semithin transverse sections revealed a significant decrease in the diameter of myelinated nerve fibers and myelin sheath thickness in group II as compared to group I. However, OECs transplantation in group III resulted in a significant increase in the previous parameters as compared to group II with no significant difference was recorded in comparison to the control group (Table 1, Histogram 1).

Table 1: Mean±SD of myelinated nerve fiber diameter and myelin sheath thickness in different groups:

	Group I	Group II	Group III
Mylinated nerve fiber diameter(μm)	14.16± 1.57■	4.5±0.89*▲	10.58±0.73■
Myelin sheath thickness (μm)	1.35 ± 0.11■	0.44±0.07*▲	1.31±0.05■

* Significant difference from group-I.
■ Significant difference from group-II.
▲Significant difference from group-III.

Histogram 1: Showing myelinated nerve fiber diameter and myelin sheath thickness in the different groups:



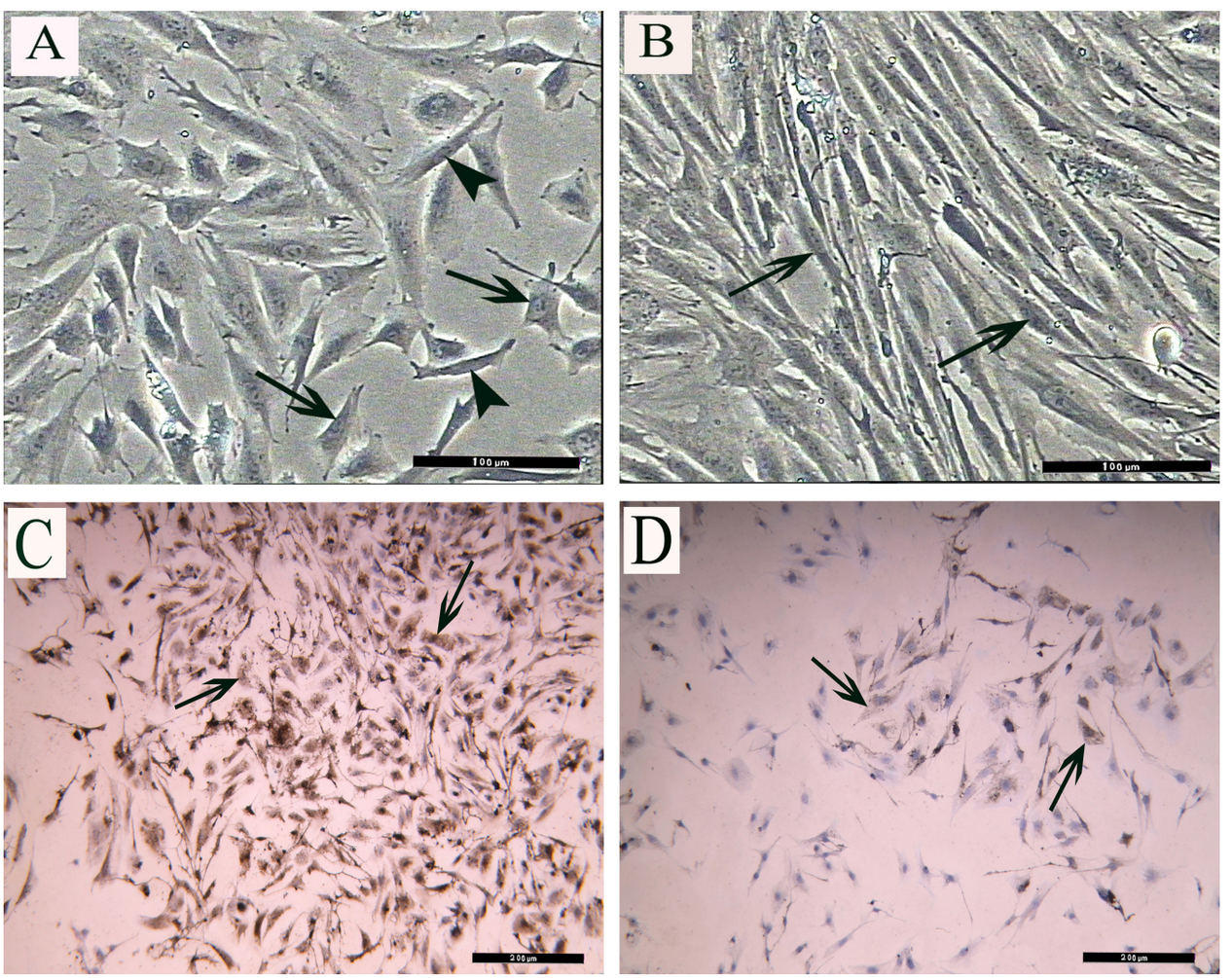


Fig. 1: (A) Culture on day five, showing most of the cells appears attached with multiple cytoplasmic processes. Some cells appear star-shaped (↑) while others are spindle-shaped (▲). They have granular cytoplasm and vesicular nuclei (Inverted microscope X200). (B) Culture on day fourteen, showing attached homogenous fibroblast like cells (↑) which form colonies. The cells show granular cytoplasm and multiple interdigitating process (Inverted microscope X200). (C) Primary culture of OECs on day fourteen showing most of the cultured cells exhibit positive brownish cytoplasmic reaction for S100 (↑) (Streptavidin-biotin peroxidase S100 X100). (D) Primary culture of OECs on day fourteen showing few cells only exhibit positive brownish cytoplasmic reaction for GFAP (↑) (Streptavidin-biotin peroxidase GFAP X100).

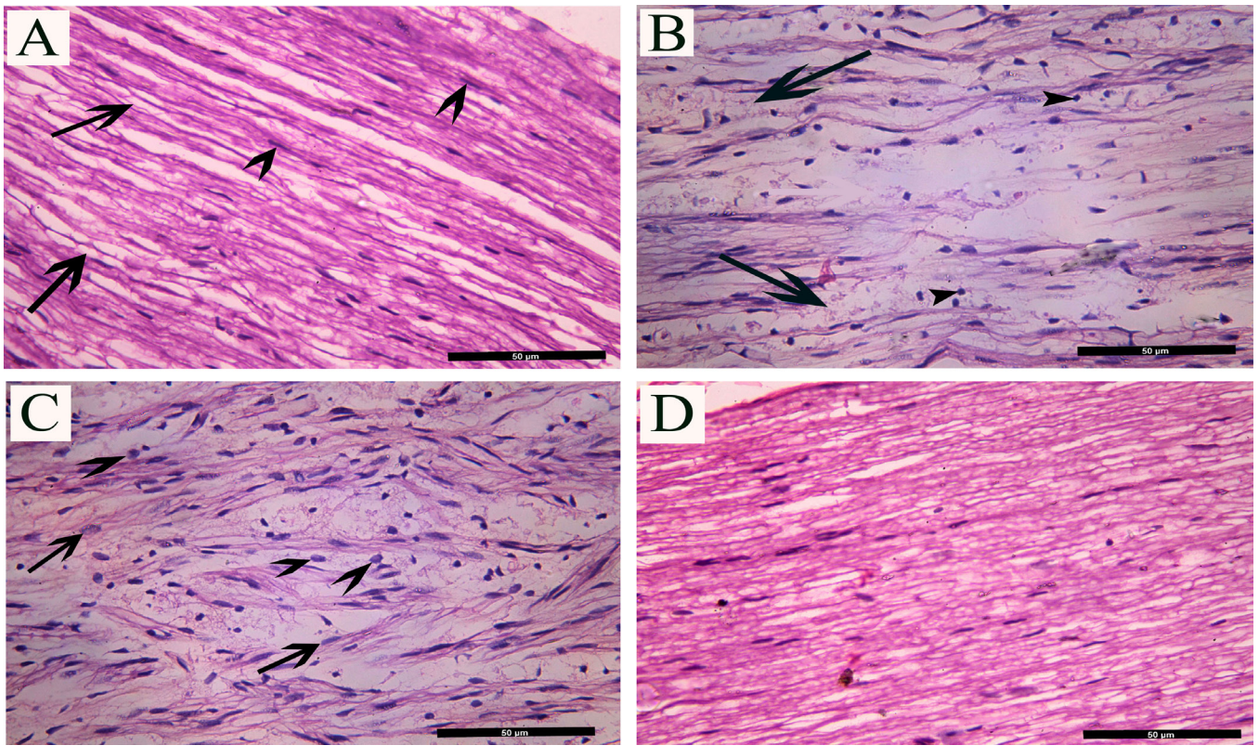


Fig. 2: (A) Show the sciatic nerve fibers appear packed, parallel with thick dissolved myelin sheath (↑). Schwann cells nuclei (ʌ) appear in-between the nerve fibers (Control, H&E X400). (B) Show discontinuity of most of nerve fibers which appear with vacuolated axoplasm(↑), degraded myelin sheaths, and numerous deeply stained pyknotic nuclei (▲). (Group II, X400). (C) Show disorganization of nerve fascicles as the axons sprouts advance in between Schwann cells (↑). Nerve fibers appear degenerated with inflammatory cell infiltrate (ʌ) (Group II, X400). (D) Show nearly the same histological picture as the control group (Group III, X400).

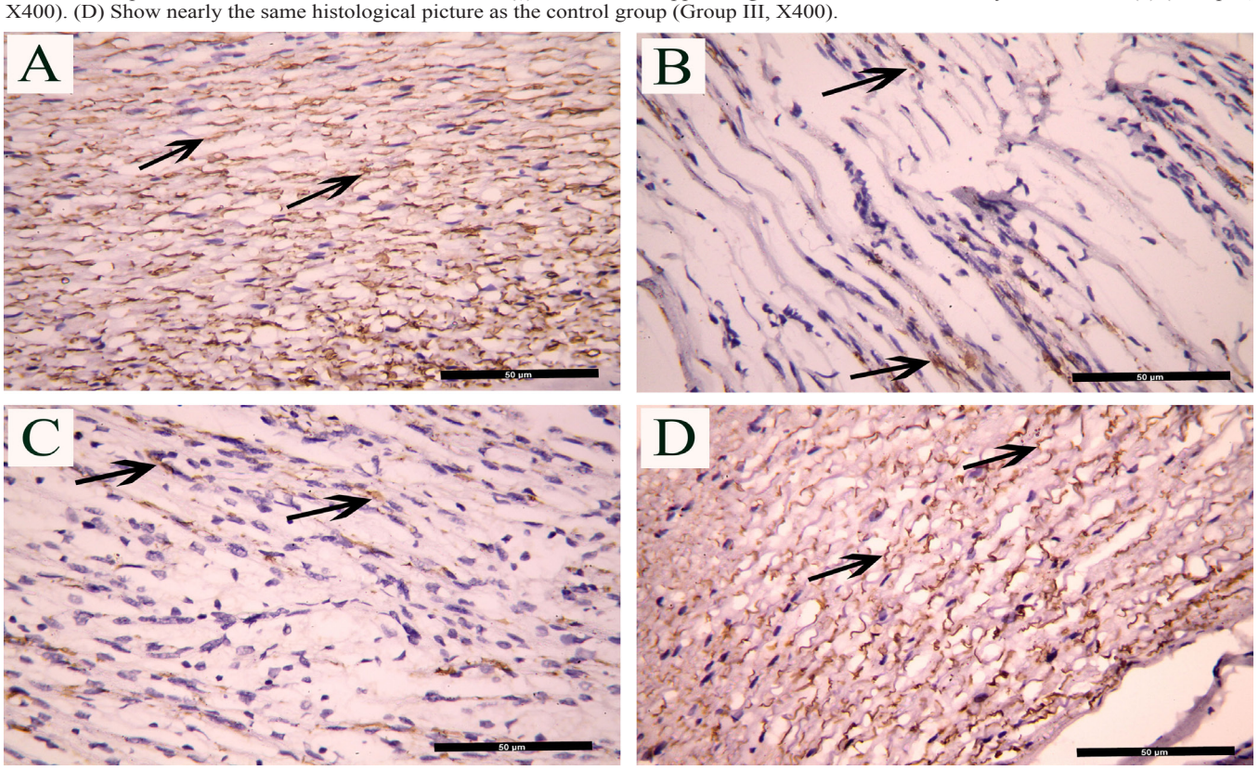


Fig. 3: (A) Show positive brownish immune reaction for the neurofilaments (↑) in the axoplasm of most of nerve fibers (Control, Neurofilament X400). (B) Show weak brownish immune reaction for neurofilaments (↑) in the axoplasm of the degenerated discontinued nerve fibers (Group II, Neurofilament X400). (C) Show weak brownish immune reaction for neurofilaments (↑) in the axoplasm of the disorganized nerve fibers (Group II, Neurofilament X400). (D) Show strong brownish immune reaction for neurofilaments (↑) in the axoplasm of the nerve fibers (Group III, Neurofilament X400).

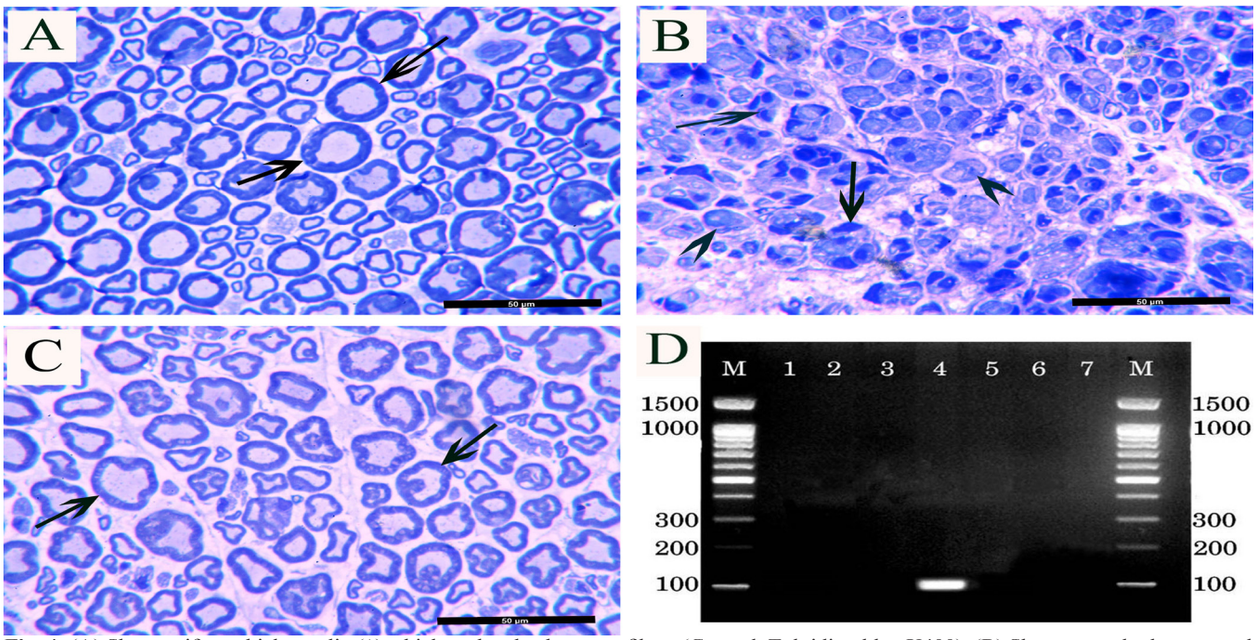


Fig. 4: (A) Show uniform thick myelin (↑) which ensheaths the nerve fibers (Control, Toluidine-blue X400). (B) Show severely degenerated axons (ʌ) and multiple myelin globules (↑) (Group II, Toluidine-blue X400). (C) Show restoration of the normal appearance of myelin sheaths (↑) as compared with the control group (Group III, Toluidine-blue X400). (D) Show the presence of Y chromosomes in female rats which were treated with OECs in group III (lane 4). Y chromosome marker is not detected in female rats of the control group I (lane 3) and group II (lane 5). M: PCR marker (U.V. trans-illuminated agarose gel of PCR products of SRY gene).

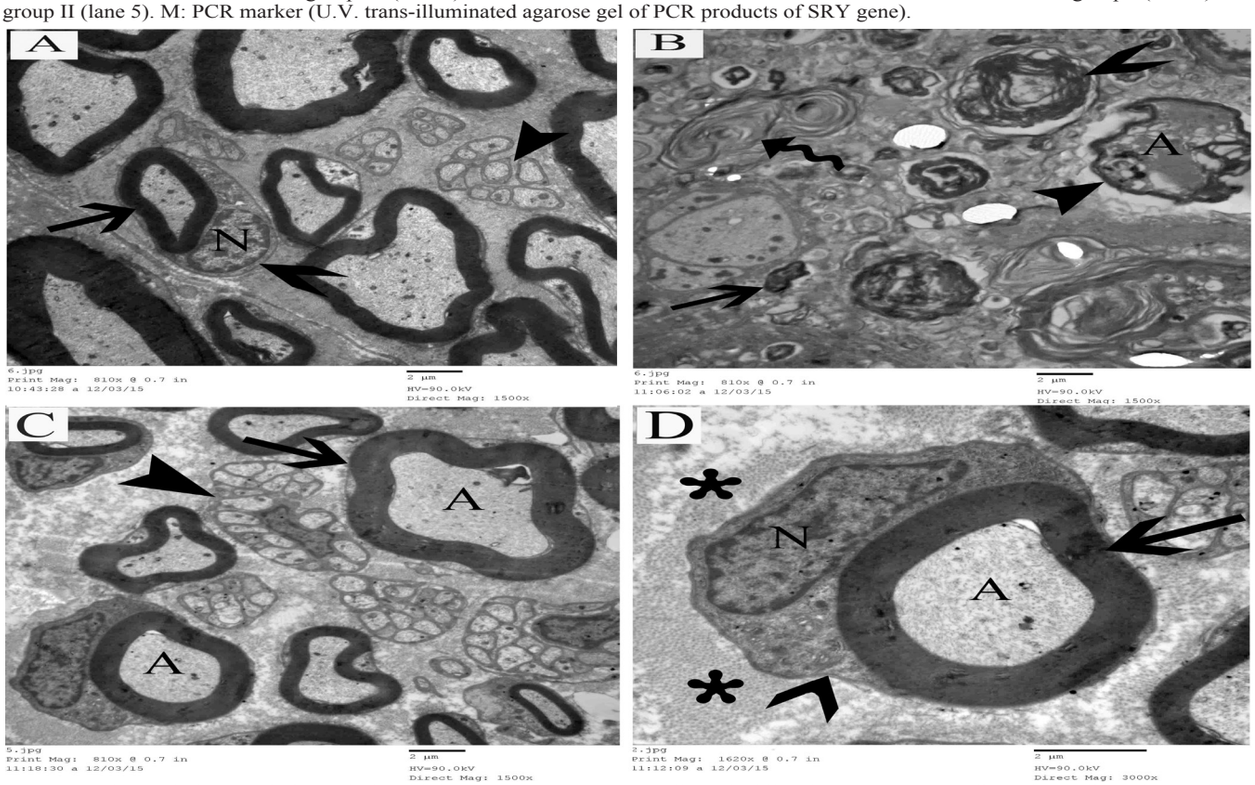


Fig. 5: (A) Show sciatic nerve is formed of multiple axons; some appear myelinated while others appear unmyelinated. Myelinated axons have thick regular myelin sheath (↑). Some unmyelinated axons (▲) are seen surrounded within Schwann cell cytoplasm. Notice the presence of Schwann cell nuclei (N) (Control, TEM X1500). (B) Show myelin sheaths either appear fragmented forming ellipsoids (↑), or have a whorlly appearance (zigzag arrow). Few fibers revealed thin disorganized myelin sheath (▲). The axons (A) appear severely degenerated losing their organelles and cytoskeleton (Group II, TEM X1500). (C) Show restoration of the structural integrity of the sciatic nerve as Schwann cells appear surrounding the axons with thick, compact, and regular myelin sheath (↑). The axons (A) appear healthy with restoration of cytoplasmic organelles. Group of regenerated unmyelinated nerve fibers (▲) can be seen inside Schwann cell cytoplasm (Group III, TEM X1500). (D) Show higher magnification of the previous image which reveals a well defined electron dense myelin sheath (↑). The axon (A) displays an abundance of neurofilaments which have been cross-sectioned; giving the axon a stippled appearance. The Schwann cell appears with normal cytoplasm density, uniformly distributed chromatin in the nucleus (N), and with intact basal lamina (ʌ). Collagen fibers (*) could be seen in the endoneurium (Group III, TEM X3000).

DISCUSSION

Nerve regeneration is a complex process and needs a combination of different modalities such as surface architecture, appropriate physical and electrical properties, biological cues, and contact guidance[27]. After peripheral nerve injury, axon regeneration does not always provide adequate functional recovery, and consequently patients do not recover a normal motor function[28]. Therefore, in the clinical practice, surgical repair is usually recommended especially in long distance defect injuries[29]. In the present study, we aimed to elucidate the neuroregenerative effects of OECs on a sciatic nerve crush injury model, hoping to achieve a new therapeutic strategy that can regenerate the crushed peripheral nerves.

In the current study, crush nerve injury produced severe structural changes in the sciatic nerves of rats in group II, including discontinuity of most of the nerve fibers with vacuolated axoplasm and numerous deeply stained pyknotic nuclei. Myelin sheaths were irregular, splitted, and disrupted. These results were in accordance with what were mentioned by Xavier *et al* 2012[30].

In the recent years, OECs have attracted attention as a promising cell transplantation candidates due to their dual nature as astroglial cells (regarding anatomical location and GFAP expression) and Schwann cells (regarding axon ensheathment and myelination and neurotrophics expression)[27]. OECs are responsible for the successful regeneration of the olfactory axons throughout the life in adult mammals[31] as they ensheath large fascicles of unmyelinated primary olfactory axons and maintain their continuous regeneration into the central nervous system[27].

For clinical application, obtaining OECs from the olfactory bulb of a patient needs an invasive and risky brain operation with subsequent risks of anosmia, stroke, meningitis, or even death[32]. Therefore, biopsies of olfactory mucosa are considered as a safer alternative and that what we did in the current experiment.

Olfactory ensheathing cells population in the present study was formed of adherent cells with heterogeneous morphologies, but mostly displayed spindle shape and this was supported by[33]. Multiple markers are used in OECs identification[24]. Generally, OECs have strong reactivity to S100[32] which is a calcium binding protein and considered to be a marker for Schwann cells (SCs), oligodendrocytes, and astrocytes[24]. Nevertheless, GFAP; an intermediate size cytoskeletal protein and considered a marker for astrocytes[34]; showed weak reactivity in the cultured OECs which is considered a clear difference in the phenotype of OECs and SCs[32]. These results were comparative to our findings regarding OECs immunohistochemical analysis of S100 and GFAP.

To exclude possibility of SCs contamination in OECs culture[35] explained this point in their experiment. Schwann cells from the peripheral nerve bundles in the olfactory mucosa and the surrounding arterioles may be considered as a source of SCs contamination in OECs cultures. Although p75 nerve factor receptor (75 NGFR) and S100 staining can differentiate between OECs and other cell types in the olfactory bulb, it cannot fully distinguish between OECs and SCs. While we cannot exclude the possibility of minor SCs contamination in OECs cultures, the relative degree of myelination after transplantation of equal numbers of SCs and OECs was nearly similar. This result suggests that a small contaminant of SCs in the OECs culture did not account for the remyelination after OECs transplantation[36].

Moreover, OECs have been reported to have S-type (Schwann cell-like) considered as a OECs-subtype that can form peripheral myelin and have A-type (fibroblast-like) which can form a cellular channel through which axons can grow[35, 37].

In the present study, H&E stained sections of the sciatic nerve from rats of group III showed nearly the same histological picture as the control group as the nerve fibers appeared packed and parallel with Schwann cells nuclei in-between them. This can be explained by wide range of the therapeutic effects which were induced by OECs transplantation as they can enhance nerve regeneration and functional recovery through reducing inflammation and lesion cavitation, enhancing angiogenesis, promoting neuronal survival and plasticity, enhancing remyelination of spared axons, and promoting regeneration of damaged axon by bridging the lesion with a growth-permissive substrate[32].

Additionally, the regenerative properties of OECs were attributed to their ability to express Notch signaling pathway which needs Jag1 ligand that is expressed in the sciatic nerve. Binding of ligand proteins to the extracellular domain of Notch receptor induces proteolytic cleavage and release of the intracellular domain of the receptor, which enters the cell nucleus triggering the target gene expression[3]. Notch signaling pathway participates in a variety of cellular processes such as cell fate specification, differentiation, proliferation, apoptosis, adhesion, epithelial-mesenchymal transition, migration, and angiogenesis[38]. Furthermore, Notch signaling pathway appears to have an instructive role in gliogenesis as it is directly promoting the differentiation of many glial subtypes[39].

Moreover[34] correlated between the regenerative properties of OECs and their migratory capacity[34]. Actually, it is assumed that OECs migrate ahead of the growing axons and through secreting neurotrophic factors such as glial-derived neurotrophic factor[40] or with matrix metalloproteinase activity[41] they are aiding and stimulating axons elongation.

Additionally, OECs can promote glial migration from the nerve stump and control both the direction of migration of endogenous cells and the newly sprouted axons through providing contact guidance cues of the aligned fibers[27].

Regarding neurofilaments expression in the axons, it is well correlated with the axonal regeneration and accordingly restoration of the nerve fibers integrity. Although neurofilaments immunopositive nerve fibers were detectable in all groups, however their expression was more visibly prominant in group III as compared with group II. These immunohistochemical results were in accordance with[27] findings who pointed out that OECs transplantation promoted better nerve regeneration as evidenced by better axonal regeneration and myelination in the group received cell transplantation versus that with no cells were incorporated.

Electron microscopy of sciatic nerve fibers in group III revealed restoration of the structural integrity as Schwann cells appeared surrounding the axons with thick, compact, and regular myelin sheath. These results were analyzed by[42] who stated that the transplanted OECs integrated into the crushed peripheral nerve and formed peripheral-like myelin on the regenerated peripheral nerve fibers. Noteworthy, while endogenous Schwann cells can perform this function, additional transplantation of glia cells; such as OECs into the injured nerves; may facilitate the repair process and this could explain the significant increase in myelinated nerve fibers diameter and myelin sheath thickness in group III which were received OECs as compared to group II with no transplanted cells.

EM examination of sections from group III revealed intact basal laminae of Schwann cells and abundant longitudinally arranged collagen fibers; forming the fibrillar component of endoneurium; which might play an important role in guiding of the axons sprouts during the regeneration process.

Moreover[42] explained the accelerated neuroregenerative properties of OECs in comparison to Schwann cells. They proposed that, while endogenous SCs are intrinsic facilitators of peripheral nerve injury repair, they need several days to mobilize after nerve injury as they retract from injured axons with subsequent expression of the low affinity p75 nerve growth factor receptor, nerve growth factor, and other molecules which promoting axonal regeneration. Nevertheless, cultured OECs are "primed" as they express these factors at the time of transplantation.

Our results provide new insights toward the regenerative properties of OECs and therefore, the possibility of their transplantation as a human cell based therapy in the peripheral nerve injuries.

CONCLUSION

Because of the unique neuroregenerative properties of OECs, transplantation of OECs in rats with crush injured sciatic nerve led to restoration of sciatic nerve structural integrity.

Though enhancement of axonal regeneration and remyelination following OECs transplantation into the injured peripheral nervous system appear to be a fact, yet many questions are to be addressed regarding the best source of OECs, the threshold number of transplanted cells, and the optimal culture conditions to be used prior to transplantation.

RECOMMENDATIONS

Long-term experiments are recommended to allow an extensive follow-up of the engrafted cells to define their proper role and fate within the sciatic nerve before future transplantation approaches can be successfully applied in the clinical application.

CONFLICT OF INTEREST

The authors have no conflicting financial interest.

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الملخص العربى

الدور العصبى التجديدى للخلايا المغلفة الشمية المزروعة فى نموذوج لإصابة العصب الوركي فى الجرذان دراسة هيستولوجية

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المقدمة: تعتبر الخلايا المغلفة الشمية واحدة من الخلايا الدبقية عالية التخصص والتى بدورها تقود المحور العصبيّ للمستقبلات الشمية من الغشاء المخاطي للأنف حتى البصلة الشمية. وتصنف الخلايا المغلفة الشمية واحدة من الخلايا الجذابة فى مجال علاج إصابة الجهاز العصبي بوساطة الزرع وذلك يعود لخصائصها العصبية التجديدية.

الهدف من البحث: تقييم الدور العصبى التجديدى للخلايا المغلفة الشمية فى نموذوج لإصابة العصب الوركي فى الجرذان.

المواد وطرق البحث: تم فصل الخلايا المغلفة الشمية من خمسة عشر جرذ من ذكورالجرذان البيضاء التي تزن حوالي ١٠٠ جم. وقد تم ايضا استخدام ثلاثين من أنثى الجرذان البيضاء والتى تزن ٢٠٠-٢٥٠ جم حيث صنفوا الى ثلاث مجموعات فى كل مجموعة عشر جرذان. المجموعة الأولى (المجموعة الضابطة) خضعوا لعمليات صورية. اما جرذان المجموعة الثانية فقد خضعوا لسحق العصب الوركي. وفى المجموعة الثالثة خضعت الجرذان لسحق العصب الوركي ثم تم حقن كل جرذ بجرعة واحدة من الخلايا المغلفة الشمية $٣x^{٤}١٠ ٣ x ١٠^{٤}$ وذلك في مكان الإصابة. تم التضحية بالجرذان فى كل المجموعات بعد ثلاث أسابيع من بداية التجربة.

النتائج: كشف الفحص الهيستولوجى للعصب الوركي من المجموعة الثانية عن انقطاع فى الألياف العصبية وظهر السيتوبلازم فجوي وبزرع الخلايا المغلفة الشمية فى المجموعة الثالثة فقد تم استعادة التركيب النسيجي الطبيعي للعصب الوركي. وقد لوحظ انخفاض ملحوظ في قطر الألياف العصبية الميالينية وسمك الغمد الميالييني وذلك فى المجموعة الثانية مقارنة بالمجموعة الضابطة. امالمجموعة الثالثة فقد كشفت عن زيادة ملحوظة في القياسات السابقة مقارنة مع المجموعة الثانية.

الاستنتاج: تمتلك الخلايا المغلفة الشمية خصائص عصبية تجديدية و التى قد تمثل رؤية جديدة في العلاج القائم على الخلايا البشرية.