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Mast cells improve functional recovery of transected peripheral nerve: A novel preliminary study

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ABSTRACT

Background: Employment of regenerative properties of cells at the service of nerve repair has been initiated during recent decades. Effects of local transplantation of bone marrow-derived mast cells on peripheral nerve regeneration were studied using a rat sciatic nerve transection model.
Materials and methods: A 10-mm sciatic nerve defect was bridged using a conduit chitosan-based hybrid conduit filled with BMMCs in BMMC group. In positive control group (Pos), the conduit was filled with phosphate-buffered saline alone. The regenerated nerve fibers were studied within 12 weeks after surgery. In sham-operated group, the sciatic nerve was only exposed and manipulated. In negative control (Neg) a 10-mm sciatic nerve defect was created and the nerve stumps were sutured to the adjacent muscles. The regenerated nerve fibers were studied functionally, biomechanically, histologically and immunohiscochemically.
Results: Functional and biomechanical studies confirmed faster recovery of regenerated axons in BMMCs transplanted animals compared to Pos group ($p < 0.05$). Morphometric indices of the regenerated fibers showed that the number and diameter of the myelinated fibers were significantly higher in BMMCs transplanted animals than in Pos group ($p < 0.05$). In immunohistochemistry, location of reactions to S-100 in BMMCs transplanted animals was clearly more positive than that in Pos group.
Conclusions: BMMCs transplantation could be considered as a readily accessible source of cells that could improve functional recovery of transected sciatic nerve.

Introduction

A commonly encountered clinical problem in regenerative medicine is peripheral nerve injury that often ends up long-term functional deficits [1]. Widespread research is continuing toward the development of methods to improve regeneration following nerve injury especially where a transection injury is the case [1].

Employment of regenerative properties of the cells at the service of nerve repair has been initiated during recent decades [2–4]. Mast cells are fascinating, multifunctional, bone marrow-derived, tissue dwelling cells. They can be activated to degranulate in minutes, not only by IgE and antigen signaling *via* the high affinity receptor for IgE, but also by a diverse group of stimuli. These cells can release a wide variety of immune mediators, including an expanding list of cytokines, chemokines, and growth factors [5]. Mast cells have an armamentarium of inflammatory mediators interleukins such as IL-6 and IL-8, and growth factors, such as vascular endothelial growth factor, platelet derived growth factor and proteases that are released in degranulation [6]. As a result of extra cellular matrix degradation and changes in the microenvironment following initial mast cell secretion, the mast cell populations may change significantly in number, phenotype and function. There is, moreover, strong evidence that mast cells significantly influence angiogenesis [7,8].

These characteristics of the mast cells has encouraged us to conduct a study to assess local mast cell therapy in site of transection of sciatic nerve to observe whether the cells could be of benefit in peripheral nerve regeneration. The aim of the present preliminary study was a single local transplantation of bone marrow-derived mast cells after sciatic nerve transection and entubulation using chitosan-based hybrid conduit in rat.

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Materials and methods

Animals and surgery

Sixty adult male Sprague Dawley rats ~300 g were randomized into four groups (n = 15). Positive control group (PC). Animals were housed five animals per cage in a temperature and humidity controlled room with 12:12 h light/dark cycles, and were allowed normal cage activities under standard laboratory conditions. The animals were fed with standard chow and water *ad libitum*. Adequate measures were taken to minimize pain and discomfort taking into account human endpoints for animal suffering and distress. Animals were housed for 2 weeks before entering the experiment. All procedures were performed with the approval of the Ethical Committee of Urmia University of Medical Sciences. The surgical procedures were described in our previous work in detail [5]. A 10-mm sciatic nerve defect was bridged using a conduit chitosan-based hybrid conduit filled with 20 μL BMMCs (1×10^6 cells/20 μL) in BMMC group. In positive control group (Pos), the conduit was filled with the same volume of phosphate-buffered saline alone. The regenerated nerve fibers were studied 4 weeks, 8 weeks, and 12 weeks after surgery. In Sham-surgery group, the sciatic nerve was only exposed and manipulated. In negative control (Neg) a 10-mm sciatic nerve defect was created and the nerve stumps were sutured to the adjacent muscles.

Preparation of the conduit

The chitosan-based hybrid conduit was prepared based on a methods described by others [9]. Chitosan conduit was made by 80 gentle injection of the solution into a home-made mold [10]. The prepared conduit was 2 mmin external diameter, 1.8 mm in internal diameter, and 10 mm in length. This internal diameter complied with optimal function in rat models.

Pokeweed mitogen-stimulated spleen cell conditioned medium (PWM-SCM)

Spleen cells from a donor rat were cultured at a density of 2×10^6cells/ml in RPMI 1640 medium containing 4 mM L-glutamine, 5×10^{-5} M 2-mercaptoethanol, 1 mM sodium pyruvate, 100 U/ml penicillin, 100 mg/ml streptomycin, and 0.1 mM nonessential amino acids (complete RPMI1640) containing lectin (8 mg/ml) and placed in 75-cm^2 tissue culture flasks. The cells were incubated at 37–8 °C in a 5% CO2 humidified atmosphere. After 5–7 days, medium was collected, centrifuged for 15 min at 3200*g*, filtered through a 0.22 μm Millipore filter and used as PWM-SCM.

Preparation of the bone marrow derived mast cells (BMMCs)

Bone marrow of a donor male rat was used to generate mast cells based on a method described by others [11]. Briefly, the animal was anesthetized, euthanized (see above) and intact femurs were removed. Sterile endotoxin-free medium was repeatedly flushed through the bone shaft using a needle and syringe. The suspension of bone marrow cells was centrifuged at 320*g* for 10 min, and cultured at a concentration of 0.5×10^6 nucleated cells/ml in RPMI 1640 with 10% FCS, 100 units/ml penicillin, 100 mg/ml streptomycin (Life technology), 10 mg/ml gentamycin, 2 mM L-glutamine and 0.1 mM nonessential amino acids (referred to as enriched medium). PWM-SCM 20% (v/v) was added to the enriched medium. Flasks were then incubated at 37 °C in a 5% CO2 humidified atmosphere. Non-adherent cells were transferred to fresh medium at least once a week. After 3–4 weeks, mast cell purity of >90% was achieved as assessed by toluidine blue staining and flow cytometery.

Staining of the mast cells

The granularity of the mast cells was determined by Toluidine blue, Alcian blue and Gimsa stainings. In brief, the cells were cytospun, fixed with Carnoy's fluid, and in Toluidine blue staining specimens stained by either 2 min with acid toluidine blue (pH = 2.7). Cells were examined by light microscopy. Staining procedure was the same for Alcian blue staining on cytospun. Briefly, slides were incubated in 3% acetic acid, 3 min alcian blue solution microwave: Hi power, 30 s and Washed in running water for 2 min, rinsed in distilled water and counterstained in nuclear fast red solution for 5 min, dehydrated, cleared and coversliped [12].

Characterization of mast cells

Mast cells were harvested, and after washing with cold PBS, the cell-surface Fc receptors were blocked with 2.4G2 (PharMingen, San Diego, CA, USA) before staining. We used a PE-conjugated anti-mouse *c-kit* (PharMingen, USA) to stain *c-kit,* and mouse FcεRI was stained with an FITC-conjugated anti-mouse FcεRI antibody (PharMingen, USA) and compared with matched isotype control antibodies. The cells were incubated with antibodies in 50 μL of PBS for 1 h at 4 °C, washed with PBS, and analyzed on BD FACSCanto II flow cytometer (Becton Dickinson, San Jose, CA, USA). Dead cells were gated out when performing the analysis [12].

Walking track analysis

The animals were tested in a restricted footpath with a dark chamber at the end. A white paper was placed on the floor of the footpath and the paws of the hind limb of the rats were pressed down on to a ink-soaked sponge, and they were then allowed to walk down the path leaving its hind paw prints on the paper [13]. The walking tracks were obtained during the healing period of 12 weeks on a weekly basis.

Distance from the heel to the third toe known as the print length (PL), distance from the first to the fifth toe known as the toe spread (TS) and distance from the second to the fourth toe known as the intermediary toe spread (ITS) were obtained. All three measurements were taken from the experimental (E) and normal (N) sides. The Sciatic function index (SFI) in each animal was calculated using the following formula:

$$SFI = -38.3 \times (EPL-NPL)/NPL + 109.5 \times (ETS-NTS)/NTS + 13.3 \times (EIT-NIT)/NIT-8.8$$

An SFI of 0 and −100 indicated normal and total impairment, respectively.

Biomechanical testing

The nerve samples were harvested and fixed between frozen fixtures in a mechanical apparatus. The TA.XTPlus Texture Analyzer mechanical test device was used for the assessment (Stable Micro Systems, Surrey GU7 1YL, UK). After 5 min, the frozen fixtures were tightened to ensure that no slippage occurred during testing. The initial length was set to 10 mm. Each sample was stretched at a constant rate of 1 mm/min. The load and displacement were sampled 5 times per second. Each sample was stretched to complete tensile failure. Samples were kept wet moist during testing using a drop of normal saline solution to the nerve segments.

Histological assessments

The regenerated nerves from all groups were isolated and post fixed in OsO4 (2%, 2 h), dehydrated through an ethanol series and

embedded in Epon. Semi thin transverse (5 μm) sections were next stained with toluidine blue. An image analyzing software (Image-Pro Express, version 6.0.0.319, Media Cybernetics, Silver Springs, MD, USA) was used to perform morphometric analysis.

Immunohistochemical assessments

For myelin sheath determination anti-S-100 (1:200, DAKO, USA) was used as a marker based on a method described in a previous study [9]. In brief, samples were post fixed with 4% paraformaldehyde for 2 h and embedded in paraffin. Before immunreaction the samples were dewaxed and rehydrated in PBS (pH 7.4). The samples were then incubated with 0.6% hydrogen peroxide for 30 min. After blocking of non-specific immuno-reactions, sections were then incubated in S-100 protein antibody solution for 1 h at room temperature. Following washing with PBS and incubating in biotinylated anti-mouse rabbit IgG solution for 1 h, horseradish peroxidase-labelled secondary antibody was applied for 1 h. The samples were then incubated with chromogene substrate solution (DAB, DAKO, USA) for 10 min. The results of immunohistochemistry were examined under a light microscope and assessed qualitatively.

Statistical analysis

The Results were analyzed using repeated measures and a factorial ANOVA with two between-subjects factors and the Bonferroni test was used to examine the effect of time and treatments. Experimental results were expressed as means ± SD. Statistical analyses were performed using PASW 18.0 (SPSS Inc., Chicago, IL, USA) and the significant difference was set at $P < 0.05$.

Results

Findings of mast cell staining

Bone marrow stromal cells of the mice were successfully harvested and cultured. In the first culture of the cells both adherent and confluent cells were observed that were appeared as heterogeneous cells. Within the first week the adherent cells were observed as confluent cells. In contrary to other common culture media, the confluent cells could live longer. In the second passage, because of limited space in the smaller flasks (T25), the confluent cells were appeared densely and on days 18 and 19 the first culture cells were appeared more homogenous. A few dividing cells were also observed. Following 3 to 4 passages and change of the culture media on day 21, the cells were homogenous enough to be harvested. The harvested cells were counted and their viability was assessed using trypan blue with Neubauer method. From each flask 12,000,000 cells with viability rate of 90% were harvested. After centrifugation, the supernatant was discarded and the pellet was resuspended in a 1 mL culture media and spread on slides. The slides were air dried at room temperature. They were fixed using carnoy and stained using toluidine blue, alcin blue and gimsa stains.The granules of mast cells were purple to red where stained with toluidine blue. These cells were matachromatic. The granules were blue and the nuclei were red where stained with alcin blue and violet where stained with gimsa (Fig. 1).

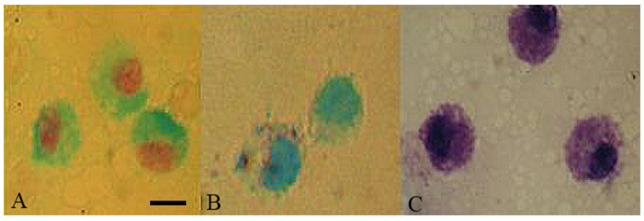

Fig 1. Bone marrow mast cells from rat were cultured in the medium during the third week of culturing bone marrow cells. (A) Alcian blue, (B) Toluidine blue and (C) Gimsa staining. Scale bar: 10 μm.

Findings of characterization of mast cells

To characterize the harvested cells, 100000 cells were also assessed based on their surface markers. In the present study, for the harvested cells from differentiated bone marrow stromal cells specific markers, CD117 (c-kit) and FCεRI, were used using flow cytometers. The results showed that the cells were positive for Mast cell-related antigens for each of CD117 and FCεRI 95% and 93%, respectively, and for both of the markers 90% (Fig. 2). This result was consistent with successful differentiation of the cells.

Analysis of the walking tracks

The analysis of the walking tracks showed that the MMCs treated animals were improved in movement significantly better than other non-treated animals (P = 0.001) (Fig. 3).

Findings of biomechanical testing

Maximum pull force (F_{max}) of normal sciatic nerve was found to be 5.40 ± 0.33. Biomechanical parameters including F_{max}, tensile strength, ultimate strain and toughness of the nerve samples in experimental groups are shown in Fig. 4. The biomechanical

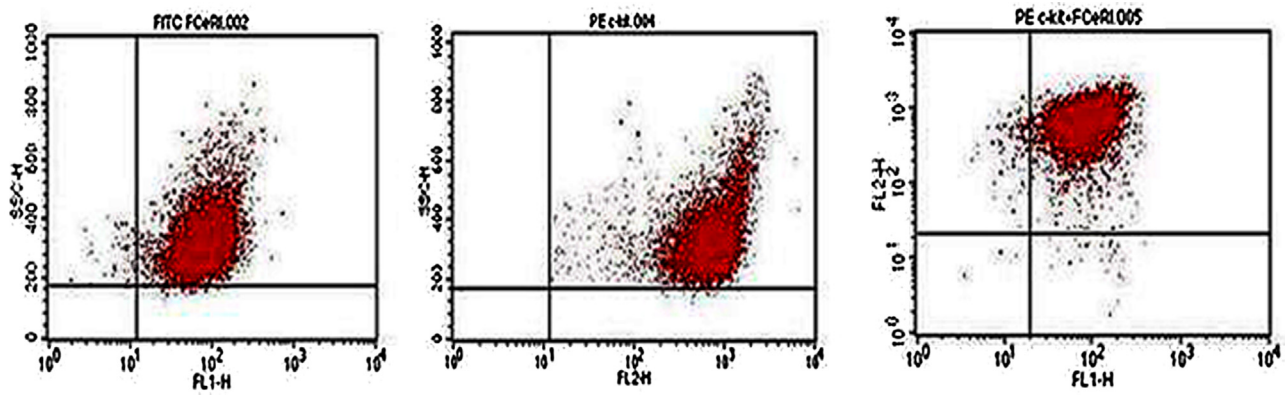

Fig. 2. Characterization of bone marrow derived mast cells (BMMC) after 3 weeks. Flow cytometric analyses of cell surface markers showed that the cells were positive for BMMC-related antigens of FCεRI (93%), CD117 (c-kit) (95%) and for BMMC-related double positive cells (90%).

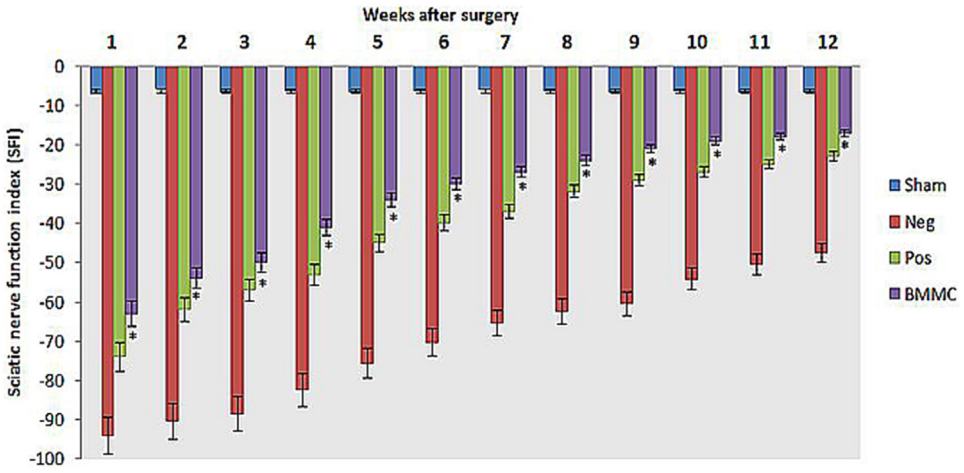

Fig. 3. Bar graph indicating static functional index (SFI) values in each experimental group during the study period. Local administration of BMMCs gave better results in functional recovery of the sciatic nerve than in POS group. Data are presented as mean ± SD. * $P < 0.05$ *vs* Pos group.

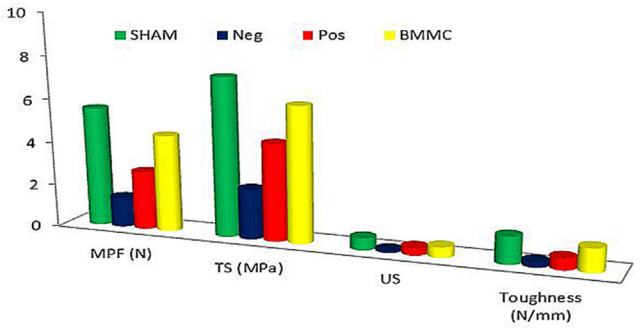

Fig. 4. The graph indicating biomechanical analyses of regenerated nerves for each of the experimental groups. MPF: Maximum Pull Force. TS: Tensile Strength. US: Ultimate Strain.

findings indicated that the parameters were significantly improved in the BMMCs treated animals than non-treated ones (P = 0.032)

Histological studies

The animals in BMMC group demonstrated significantly greater nerve fiber, axon diameter, and myelin sheath thickness during the study period compared to non-treated animals (Table 1) ($P < 0.05$).

Immunohistochemistry

The qualitative analysis of immunohistochemistry of regenerated nerve fibers showed extensive immunoreactivity to S-100 protein in BMMC group. The expression of S-100 protein signal was located mainly in the myelin sheath. The axon also showed a slight expression showing that there was Schwann cell-like phenotype around the myelinated axons (Fig. 5).

Discussion

The findings of the present preliminary study indicated that the local transplantation of BMMCs improved functional recovery, biomechanical and histomorphometric indices in animals with transected sciatic nerves.

Analysis of the walking track in the present study showed that local treatment of the animals with BMMCs ended up significant improvement in locomotion. Walking track analysis has been reported to be reliable in evaluation of repair process in sciatic nerves mainly because in this test sensory input, motor response and cortical integration are involved [14].

In the present study local administration of BMMCs resulted in the enhanced biomechanical properties. Peripheral nerves are remarkable tissues that not only conduct electrical impulses, but also must bend and stretch to accommodate the movement of limbs. In order to achieve this they have a complex structure consisting of bundles of neurons packed into fascicles and surrounded by connective tissue layers, the perineurium and epineurium. Both neural and connective tissue elements are tethered proximally at the spinal cord and have numerous branch points allowing neurons from a single nerve trunk to synapse with various target organs. The strongest connective tissue layers in peripheral nerves are the perineurium and, to a lesser extent, the epineurium. Changes in the epineurium and perineurium extracellular matrix composition are likely to have significant effects on the biomechanical properties of acellular nerve [15]. The connective tissue from the epineurium forms a layer of fiber membrane at the 3rd day postoperatively and then forms collagen at the 8th day. The key point influencing functional recovery is the number of axons throughout the suture that enhances the anti-tension capacity of the nerve [16]. Identifying the maximum tension which nerves can withstand and understanding the origin of their mechanical resilience is of great importance to improve the

Table 1
Morphometric analyses of sciatic nerve in each of the experimental groups: Values are given as mean ± SD.

Groups	Axon counts fb/mm^2	Axon diameter (μm)	Myelin sheath thickness (μm)
Sham	28674 ± 1982	11.35 ± 0.15	2.60 ± 0.03
Neg	5123 ± 1746	3.34 ± 0.12	1.03 ± 0.04
Pos	18293 ± 2005	6.27 ± 0.16	1.34 ± 0.02
BMMC	22980 ± 2076*	7.72 ± 0.15*	1.38 ± 0.03

* The mean difference is significant at the 0.05 level *vs.* Pos group.

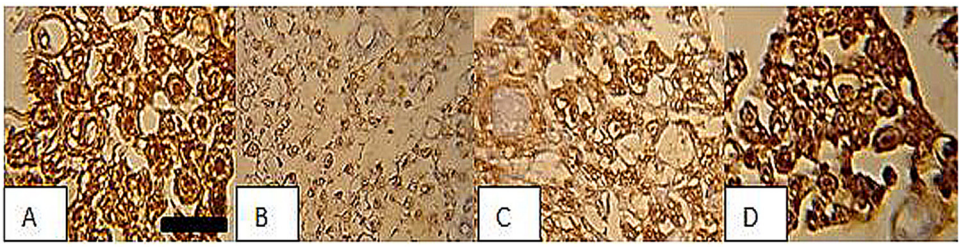

Fig. 5. Immunohistochemical analysis of the regenerated nerves 12 weeks after surgery from middle cable (A) Sham, (B) Neg, (C) Pos and (D) BMMC. There is clearly more positive staining of the myelin sheath-associated protein S- 100 (arrow) within the periphery of nerve, indicating well organized structural nerve reconstruction in BMMSc treated nerve. Scale bar: 20 μm.

outcome of surgical nerve repairs. Their behaviour under loading is viscoelastic and is likely to be dependent upon a number of factors such as the internal fluid pressure maintained by the impermeable perineurium, the outer-inner layer integrity, the number and arrangement of fascicles and the molecular structural elements of the extracellular matrix such as collagen and elastin [16].

Morphometric analysis of the repaired nerve fibers indicated that there was significant difference between BMMC and Pos animals. Regarding better functional and morphometric indices in BMMC group, it could be stated that cell therapy both accelerated and improved the process of nerve regeneration.

In immunohistochemistry the expression of axon and myelin sheath special proteins was apparent in cell treated animals demonstrating the normal structure in histology. The response to S-100 in BMMCs treated animals was evidently more positive than in Pos group. This further implied that both repaired axon and Schwann cell-like cells were present and accompanied by the process of myelination and the structural recovery of repaired nerve fibers.

Depending on the mast cell phenotype and stimulus, mast cells initiate the transcription, translation and secretion of a varied array of cytokines including PDGF, VEGF. It has already been shown that PDGF, VEGF bear beneficial effects on peripheral nerve regeneration [17–20].

Mast cells have been proposed as angiogenesis promoters and the mast cell count appears to be a reliable prognostic marker in some tumors [21,22]. Mast cells cause neovascularization by producing angiogenic factors, such as VEGF, or substances with angiogenic properties, such as tryptase, FGF, TNF, interleukin (IL)-8, histamine and heparin.

Angiogenesis is a complex process governed by many different variables. Growth factors, including VEGF, platelet derived growth factor (PDGF) and fibroblast growth factor (FGF), play important roles. Consequently their generation within nerve conduits is vital to achieving positive clinical outcomes [23].

The regulatory influence of the mast cells is also demonstrated by the nerve repair phenomena characteristic of the proliferation stage [24]. The release of specific mediators (vasoactive amines, tryptase, IL-4 and NGF) of mast cell origin is essential to initiate regeneration of damaged nerve fibres, and lead to temporary hyperinnervation of the scar at this stage [21–29].

Mast cell- derived cytokines, including TNF, and growth factors, such as NGF, lower the threshold for activation of local neurons and promote nerve fiber growth. There is anatomical evidence for mast cell associations with peripheral myelinated and unmyelinated nerves [30].

The mast cells were introduced in the site of injury in the present preliminary study regarding this fact that changing the mast cell microenvironment alters significant changes in phenotype of the mast cells and they may act as growth factors packages that only degranulate *in situ* and do not induce inflammatory responses [4].

As the title indicates this preliminary study was conducted to assess effects of *in situ* transplantation BMMCs at the site of peripheral nerve injury and motor testing modalities like electrophysiology and/or electromyography are needed in the future experiments to support other results including functional assessments. Since mast cells bear armamentarium of inflammatory mediators, the authors aimed to assess whether the BMMCs could positively affect the nerve repair process. We aimed to use the cells as package of mediators and growth factors and we expected autolysis of the cells *in situ* and release of the agents. Whether the release of these factors only is responsible for nerve regeneration of the transected sciatic nerve is not abundantly clear and further study on proliferation and differentiation of the cells remain to be conducted in the future.

Future experiments could possibly use the same chitosan hybrid conduit but coated with growth factors in addition to mast cells to examine if this accelerates regeneration.

The major limitation of the present study was comparison of the cells with extracellular matrix, microtubules, fibroblasts, and Schwann cells and other nerve segment constituents and conduits with giving the histological and molecular evidences for neuroprotective action of BMMCs. This would be considered for further studies. Therefore, the authors stress that the current investigation was conducted to evaluate a single local dose and clinical treatment potential of BMMCs on nerve repair and precise mechanisms of neuroprotective action of BMMCs in transection models remain to be investigated.

In conclusion, this alteration in the behavior of mast cells could be favorable in cell therapy where readily accessible and instant source of cells in large quantities are required and could be taken into consideration in the emerging field of regenerative medicine and surgery. It could be considered clinically as a translatable route towards new methods to enhance peripheral nerve repair in clinical applications.

Conflicts of interest

There are no conflicts of interests to declare.

Author contributions

Behrooz Ilkhanizadeh: Analysis of data and writing of the manuscript.
Leila Zarei: Study Design and Analysis of data.
Negin Farhad: Collection of Data.
Mehran Bahrami-Bukani: Collection of Data.
Rahim Mohammadi: Surgical Procedures.

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References

[1] Lin H, Wang H, Chen D, Gu Y. A dose-effect relationship of ginkgo biloba extract to nerve regeneration in a rat model. Microsurgery 2007;27:673–7.
[2] Haghighat A, Mohammadi R, Amini K. Transplantation of undifferentiated bone-marrow stromal cells into a vein graft accelerates sciatic nerve regeneration in streptozotocin induced diabetic rats. Curr Neurovasc Res 2014;11:230–41.
[3] Mohammadi R, Mahmoodzadeh S. Combination of local transplantation of in vitro bone-marrow stromal cells and pulsed electromagnetic fields accelerate functional recovery of transected sciatic nerve regeneration: a novel approach in transected nerve repair. Curr Neurovasc Res 2015;12:222–31.
[4] Mohammadi R, Asadollahi A, Amini K. Uncultured undifferentiated adipose-derived nucleated cell fractions combined with inside-out artery graft accelerate sciatic nerve regeneration and functional recovery. Int J Oral Maxillofac Surg 2014;43:1161–8.
[5] Krishnaswamy G, Chi DS. Methods in molecular biology. In: Frangogioannis NG, Entman ML, editors. Mast Cells: Methods and Protocols. Totowa, NJ: Humana Press; 2006. p. 91–113.
[6] Gaber MA, Seliet IA, Ehsan NA, Megahed MA. Mast cells and angiogenesis in wound healing. Anal Quant Cytopathol Histopathol 2014;36:32–40.
[7] Norrby K. Mast cells and angiogenesis. APMIS 2002;110:355–71.
[8] Mokarizadeh A, Mehrshad A, Mohammadi R. Local polyethylene glycol in combination with chitosan based hybrid nanofiber conduit accelerates transected peripheral nerve regeneration. J Invest Surg 2016;29:167–74.
[9] Bhattaraia N, Edmondson D, Veiseh O, Matsen FA, Zhang M. Electrospun chitosan-based nanofibers and their cellular compatibility. Biomaterials 2005;26:6176–84.
[10] Ojagh SM, Rezaei MA, Razavi SH, Hosseini SMH. Development and evaluation of a novel biodegradable film made from chitosan and cinnamon essential oil with low affinity toward water. Food Chem 2010;122:161–6.
[11] Mortaz E, Redegeld FA, Nijkamp FP, Engels F. Dual effects of acetylsalicylic acid on mast cell degranulation, expression of cyclooxygenase-2 and release of pro-inflammatory cytokines. Biochem Pharmacol 2005;69:1049–57.
[12] Givi ME, Blokhuis BR, Da Silva CA, Adcock I, Garssen J, Folkerts G, et al. Cigarette smoke suppresses the surface expression of c-kit and FcεRI on mast cells. Mediators Inflamm 2013;2013:813091.
[13] Varejão AS, Melo-Pinto P, Meek MF, Filipe VM, Bulas-Cruz J. Methods for the experimental functional assessment of rat sciatic nerve regeneration. Neurol Res 2004;26:186–94.
[14] Castaneda F, Kinne RK. Omental graft improves functional recovery of transected peripheral nerve. Muscle Nerve 2002;26:527–32.
[15] Ma XL, Sun XL, Yang Z, Li XL, Ma JX, Zhang Y, et al. Biomechanical properties of peripheral nerve after acellular treatment. Chin Med J (Engl) 2011;124:3925–9.
[16] Jiang B, Zhang P, Yan J, Zhang H. Dynamic observation of biomechanic properties of sciatic nerve at the suture site in rats following repairing. Artif Cells Blood Substit Immobil Biotechnol 2008;36:45–50.
[17] Jippo T, Lee YM, Ge Y, Kim DK, Okabe M, Kitamura Y, et al. Tissue-dependent alteration of protease expression phenotype in murine peritoneal mast cells that were genetically labeled with green fluorescent protein. Am J Pathol 2001;158:1695–701.
[18] Welker P, Grabbe J, Zuberbier T, Grützkau A, Henz BM. GM-CSF downregulates c-kit, Fc(epsiolon) RI(alpha) and GM-CSF receptor expression as well as histamine and tryptase levels in cultured human mast cells. Arch Dermatol Res 2001;293:249–58.
[19] Mohammadi R, Ahsan S, Masoumi M, Amini K. Vascular endothelial growth factor promotes peripheralnerve regeneration after sciatic nerve transection in rat. Chin J Traumatol 2013;16:323–9.
[20] Golzadeh A, Mohammadi R. Effect of local administration of platelet-derived growth factor B on functional recovery of peripheral nerve regeneration: a sciatic nerve transection model. Dent Res J 2016;13:225–32.
[21] Elpek GO, Gelen T, Aksoy NH, Erdoğan A, Dertsiz L, Demircan A, et al. The prognostic relevance of angiogenesis and mast cells in squamous cell carcinoma of the oesophagus. J Clin Pathol 2001;54:940–4.
[22] Noli C, Miolo A. The mast cell in wound healing. Vet Dermatol 2001;12:303–13.
[23] Carmeliet P, Jain RK. Molecular mechanisms and clinical applications of angiogenesis. Nature 2011;473:298–307.
[24] Shih B, Garside E, McGrouther DA, Bayat A. Molecular dissection of abnormal wound healing processes resulting in keloid disease. Wound Repair Regen 2010;18:139–53.
[25] Kennelly R, Conneely JB, Bouchier-Hayes D, Winter DC. Mast cells in tissue healing: from skin to the gastrointestinal tract. Curr Pharm Biotechnol 2011;17:3772–5.
[26] Ng MF. The role of mast cells in wound healing. Int Wound J 2010;7:55–61.
[27] Shiota N, Nishikori Y, Kakizoe E, Himoura K, Niibayashi T, Shimbori C, et al. Pathophysiological role of skin mast cells in wound healing after scald injury: study with mast cell-deficient W/W (V) mice. Int Arch Allergy Immunol 2010;151:80–8.
[28] El-Agamy DS. Targeting c-kit in the therapy of mast cell disorders: current update. Eur J Pharmacol 2012;690:1–3.
[29] Krishnaswamy G, Ajitawi O, Chi DS. The human mast cell: an overview. Methods Mol Biol 2006;315:13–34.
[30] Forsythe P, Bienenstock J. The mast cell- nerve functional unit: a key component of physiologic and pathophysiologic responses. In: Bienenstock J, editor. Allergy and the Nervous System. Chem Immunol Allergy. Basel: Karger; 2012. p. 196–221.