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Neuro-muscular regeneration using scaffolds with mesenchymal stem cells (MSCs) isolated from human umbilical cord Wharton's jelly: functional and morphological analysis using rat sciatic nerve neurotmesis injury model

Ana Rita Caseiro[a,b,c], Tiago Pereira[a,b], Jorge Ribeiro[a,b,d], Irina Amorim[f,g], Fátima Faria[e], Paulo Jorge Bártolo[h], Paulo Armada[i], Ana Lúcia Luís[a,b,d], Ana Colette Maurício[a,b]*

[a]*Departamento de Clínicas Veterinárias, Instituto de Ciências Biomédicas de Abel Salazar (ICBAS), Universidade do Porto (UP), Portugal.*
[b]*Centro de Estudos de Ciência Animal (CECA), Instituto de Ciências, Tecnologias e Agroambiente da Universidade do Porto (ICETA), Portugal.*
[c]*CDRsp- Centro para o Desenvolvimento Rápido e Sustentado de Produto, Instituto Politécnico de Leiria, Portugal.*
[d]*UPVet, Instituto de Ciências Biomédicas de Abel Salazar, (ICBAS), Universidade do Porto (UP), Portugal.*
[e]*Departmento de Patologia e de Imunologia Molecular, Instituto de Ciências Biomédicas de Abel Salazar (ICBAS), Universidade do Porto (UP), Portugal.*
[f] *Instituto Português de Patologia e Imunologia Molecular da Universidade do Porto (IPATIMUP), Portugal.*
[g] *Escola Superior Agrária do Instituto Politécnico de Viana do Castelo, Ponte de Lima, Portugal.*
[h]*School of Mechanical, Aerospace and Civil Engineering & Manchester Institute of Biotechnology, University of Manchester, Manchester, UK.*
[i]*Centro Interdisciplinar de Estudo de Performance Humana, Faculdade de Motricidade Humana, Universidade de Lisboa, Estrada da Costa, 1499-002 Cruz Quebrada, Dafundo, Portugal.*

Abstract

Peripheral nerves possess the capacity of self-regeneration after traumatic injury but the extent of regeneration is often poor and may benefit from exogenous factors that enhance growth. Neonatal tissues are routinely discarded at parturition so little ethical controversy attends the harvest of the Mesenchymal Stem Cells (MSCs) which may play an important therapeutic role through the secretion of soluble trophic factors which enhance and assist in repair by paracrine activation of surrounding cells. The use of cellular systems is a rational approach for delivering neurotrophic factors at the nerve lesion site, and in our recent research work we have been evaluating the therapeutic value of MSCs isolated from the Wharton jelly (WJ) in nerve repair associated to different tube-guides made of biodegradable and biocompatible biomaterials. The WJ MSCs *in vitro* studies included cell characterization by immunocytochemistry, karyotype analysis, tri-lineage differentiation capacity and flow cytometry and also citocompatibility by measuring the intracellular calcium concentration ($[Ca^{2+}]_i$) in the presence of different tube-guides.

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* Corresponding author. Tel.: +351-919-071-286.
E-mail address: acmauricio@icbas.up.pt; ana.colette@hotmail.com

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Biomaterials like PVA, PVA loaded with MWCNTs (functionalized carbon nanotubes, PVA-CNTs), PVA loaded with polypyrrole (PVA-PPy), and PLC associated to MSCs were tested in terms of cytocompatibility and *in vivo* in the rat sciatic nerve neurotmesis injury model. The regenerated nerves and *tibialis anterior* (TA) muscles were processed for stereological studies after 20 weeks. The functional recovery was assessed serially for gait biomechanical analysis, by EPT, SFI and SSI, and by WRL. Histopathology of lung, liver, kidneys, regional lymph nodes ensured the biomaterials biocompatibility. The karyotype analysis of the MSCs excluded the presence of neoplastic signs, thus supporting the suitability of isolation and expansion protocols. The MSCs were positive for C-kit, Nanog and vimentin, and negative for CD31, following the International Society for Cellular Therapy (ISCT) definition. Results obtained from epifluorescence by measuring the $[Ca^{2+}]_i$ of the MSCs cultured on tube-guides confirmed the ability to support their expansion, adhesion, and differentiation. Our results showed that the use of MSCs enhanced the recovery of sensory and motor function in neurotmesis injuries showing a thicker myelin sheath. MSCs isolated from WJ delivered through biomaterials should be regarded as a potentially valuable tool to improve clinical outcome especially after trauma to sensory nerves. In addition, these cells represent a non controversial source of primitive mesenchymal progenitor cells that can be harvested after birth, cryogenically stored, thawed, and expanded for therapeutic uses.

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Keywords: nerve regeneration; neurogenic muscle atrophy; mesenchymal stem cells; umbilical cord; Wharton´s jelly; functional assessment; histomorphometry.

Nomenclature

MSCs	Mesenchymal stem cells
SCs	Schwann cells
TA	*Tibialis anterior* muscle
ISCT	International Society for Cellular Therapy
UC	Umbilical cord
WJ	Wharton jelly
UCT	Umbilical cord tissue
MHC	Major Histocompatibility Complex
PVA	Poly(vinyl alcohol)
CNTs	Carbon nanotubes
MWCNTs	COOH-functionalized multiwall carbon nanotubes
PPy	Polypyrrole
PLC	Poly(DL-lactide-ε-caprolactone)
$[Ca^{2+}]_I$	Intracellular calcium concentration
EPT	Extensor Postural Thrust
WRL	Withdrawal Reflex Latency
IC, OT, HR, TO	Initial Contact, Opposite Toe-off, Heel Rise, Toe-off

1. Introduction

Tissue engineering focusing on the *in vitro* fabrication of autologous, living tissues with the potential of regeneration is a promising scientific and clinical field. Peripheral nerve regeneration studies should be included in a multidisciplinary team able to develop biomaterials, to develop cell therapies, to elaborate *in vitro* analysis and pre-clinical trials concerning animal welfare and the most appropriate animal model before the clinical trials and clinical application approval [1]. As a matter of fact, the peripheral nerve injury has a high regenerative potential, but functional recovery rarely occurs when total nerve transection occurs. A full understanding of nerve regeneration, especially complete functional achievement and organ re-innervation after nerve injury, still remains an important goal of regenerative medicine because nerve regeneration is a complex biological phenomenon. In the peripheral nervous system, nerves can spontaneously regenerate without any treatment if nerve continuity is maintained (axonotmesis) whereas more severe type of injuries must be surgically treated by direct end-to-end surgical reconnection of the damaged nerve ends [2,3,4]. Cell transplantation has been proposed as a method of improving peripheral nerve regeneration, enhancing axon outgrowth and survival both *in vitro* and *in vivo* [5,6]. Mesenchymal stem cells (MSCs) are one of the most interesting cell products that can be harvested from different sources, including extra-fetal tissues without any religious or ethical concern [7]. Tissue engineering of peripheral nerves associates biomaterials, some of them, previously studied by our group [1,4,8] to cellular systems, able to differentiate into neuroglial-like cells or even by modulating the inflammatory process, which might improve nerve regeneration, in terms of motor and sensory recovery, and also, by shortening the healing period avoiding regional muscular atrophy [5,6]. The MSCs are capable of forming a variety of mesenchymal tissues in response to tissue damage including bone, cartilage, fat, muscle, tendons and ligaments, amongst others. The MSCs are characterized by several important and distinct properties: a) being plastic adherent; b) specific surface protein expression, staining positively for markers of the mesenchymal lineage like CD10, CD13, CD29, CD44, CD90, and CD105, and negatively for markers of the hematopoietic lineage; and c) tri-lineage differentiation capacity of the isolated MSCs. These are the criteria that were defined by the International Society for Cellular Therapy (ISCT) [9]. The high plasticity and low immunogenicity of these cells turns them into a desirable form of autologous and allogenic cell therapy for the injured nervous system [1,7]. A reported potential alternative tissue source of MSCs is the connective tissue (Wharton's Jelly, WJ) of human umbilical cord tissue (UCT) [1,7]. Interestingly, these cells, which are major histocompatibility complex (MHC) class II negative, not only express both an immuno-privileged and immuno-modulatory phenotype, but their MHC class I expression levels can also be manipulated [9], making them a potential cell source for MSC-based therapies. In addition, these cells represent a non-controversial source of primitive mesenchymal progenitor cells that can be harvested after birth, cryogenically stored, thawed, and expanded for therapeutic uses [1,7]. Concerning the peripheral nerve regeneration, it is important to identify an appropriate vehicle to the local application of the MSCs in the injury site. This vehicle must be biocompatible and may be a hydrogel or a membrane / tube-guide that support the adhesion, expansion and survival of the cellular system [1,7]. The resorption of a biomaterial should be adjusted to the regeneration process, which depends on its molecular weight, composition, crystal structure and thermal history. When associating a biomaterial to a cellular system it is also important to determine properties such as hydrophobicity, surface charge and surface rugosity to establish its ability to support adhesion and cell growth [3]. Among several biomaterials, our research group focused its attention in some biodegradable ones like those made of poly (DL-lactide-e-caprolactone) copolyester (Vivosorb®, PLC) [5,6,10] and more recently, the poly(vinyl alcohol) (PVA) loaded with electrical conductive materials, such as carbon nanotubes (CNTs) and polypyrrole (PPy) to produce a conductive biomaterial with higher electrical conductivity than the polymer matrix. Entubulation offers advantages compared to graft implantation in neurotmesis injuries where an end-to-end suture is not possible without tension, including the potential to manipulate and to improve the regeneration environment within the tube, by adding to the tube-guide lumen, growth factors and/or cellular systems. Consequently, guidance of regenerating axons is not only achieved by a mechanical effect but also by cellular growth factors, a chemical and electrical cues [1,5,6,7,11,12].

2. Material and Methods

2.1. Cell culture of MSCs from the Wharton's jelly

Our research group has been testing MSCs from the Promocell GmbH (C-12971, lot-number: 8082606.7) that are cultured and expanded in a humidified atmosphere with 5% CO2 at 37°C in Mesenchymal Stem Cell Medium, PromoCell (C-28010) [5,6,7]. To ensure that MSCs were not modified by the culture conditions, their phenotype is always confirmed by our research group immediately before *in vivo* testing by flow cytometry with the following antibodies and their respective isotypes (all from BioLegend unless stated otherwise): PE anti-human CD105 (eBioScience); APC anti-human CD73; PE anti-human CD90; PerCP/Cy5.5 anti-human CD45: FITC anti-human CD34; PerCP/Cy5.5 anti-human CD14; Pacific Blue anti-human CD19 and pacific-blue anti-human HLA-DR. Also cytogenetic analysis has been carried out before *in vivo* application between P4-P5 as previously described [5,6,7]. Intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) by using dual wavelength spectrofluorometry as previously described

[5,6,11,13], was measured in Fura-2-loaded MSCs cells. Results obtained from epifluorescence technique are referred to measurements from MSCs which correspond to $[Ca^{2+}]_i$ from cells that did not begin the apoptosis process (data not shown) cultured without the presence of any biomaterial, or sub-cultured over different biomaterial discs of 10 mm diameter. Immediately previously to *in vivo* application, a MSCs suspension is prepared in 1 ml syringes. Each syringe contained MSCs at a concentration of 10^6/µl for posterior intra-operatively nerve injection. To induce tri-lineage differentiation including adipogenic, chondrogenic and osteogenic differentiation, MSCs were cultured and histologically prepared as previously described by the authors [10].

2.2. MSCs immunocytochemistry

At P3, MSCs were trypsinized, washed and re-suspended in Shandon™ Cytoblock™ Cell Block Preparation System (Thermo Scientific, USA) at a concentration of $1x10^5$cells/ml and processed for immunocytochemistry. Antigen retrieval was performed on dewaxed sections by immersion in citrate buffer (10 mM, pH 6.0) in a pressure cooker for 3 min. The Novolink™ Max-Polymer detection system (Novocastra, UK) was used for visualization according to the manufacturer's instructions and the following monoclonal antisera were employed: vimentin (clone V9, Dako) diluted 1:500; CD117 (c-Kit) (A4502; DakoCytomation) diluted 1:450; CD31 (clone JC70A, Dako) diluted 1:50 and NANOG-1 (clone MAB, ABGent) diluted 1:50.

2.3. Biomaterial production for tube-guide construction

Synthetic biodegradable tubes of PVA (Aldrich, Mowiol 10-98), PVA loaded with COOH-functionalized multiwall carbon nanotubes MWCNTs (Nanothinx, NTX5, MWCNTs 97% -COOH) (PVA-CNTs tube-guides), and PVA loaded with PPy (Aldrich, 10-40 S/cm of conductivity) (PVA-PPy tube-guides) were prepared using a casting technique to a silicone mould. A 15% (%w/v) aqueous solution of PVA was prepared. Then the solution of PVA was mixed with 0.05% of COOH-functionalized MWCNTs and 0.05% of PPy. The tube-guides were produced by freezing/thawing process. The treatment consisted in three cycles of freezer (-30ºC)/ incubator (25ºC), and an annealing treatment started with a stage of 14 h on an incubator (25ºC) followed by a ramp rate of 0.1ºC/min until 80ºC, and then a stage of 20h at 80 ºC. The tube-guides were sterilized by gama-radiation and hydrated in a sterile saline solution during 2h before microsurgical application in the rat neurotmesis injuries. Poly(DL-lactide-ε-caprolactone) (PLC) membranes (Vivosorb®) were purchased from Polyganics BV, Groningen, Netherlands (FS01-006/20 Lot: FSA2009092311). Vivosorb® is a flexible bioresorbable polymer film, made of poly(DL-lactide-ε-caprolactone) copolymer which presents retention of mechanical strength for up to 10 weeks throughout the critical healing period.

2.4. Rat sciatic nerve model – surgery procedure

The rats Sasco Sprague Dawley (Charles River Laboratories, Barcelona, Spain) weighted approximately 300 g, were divided in experimental groups of 6 animals. Immediately before the surgery procedure, the rats were anesthetized with ketamine 9 mg/100 g and xylazine 1.25 mg/100 g, (body weight), by intra-peritoneal (IP) administration. The right sciatic nerve was exposed unilaterally and a neurotmesis injury above the terminal nerve ramification was performed. The proximal and distal nerve stumps were inserted 3 mm into tube-guides, maintaining a nerve gap of 10 mm, with two epineural sutures using 7/0 monofilament polypropylene suture. In one group, the PVA loaded with 0.05% (%w/v) of COOH-functionalized MWCNTs tube-guide was filled with 100µm of MSCs at a concentration of 10^6cells/µl and locally infiltrated. To test the PLC membrane associated to MSCs, immediate cooptation with 7/0 monofilament nylon epineural sutures of the 2 transected nerve endings was performed (End-to-End) or the sciatic nerve was bisected immediately above the terminal nerve ramification and at a10mm distal point. The resulting nerve graft, with a length of 10mm, was inverted 180º and sutured with 7/0 monofilament nylon. No immunosuppressive treatment was given to any of the experimental animals during the entire study.

2.5. Functional assessment

All animals were tested pre-operatively (week 0), and every week until week 12 and every 2 weeks until the end of follow-up time (20 weeks). Motor performance and nociceptive function were evaluated by measuring extensor postural thrust (EPT) and withdrawal reflex latency (WRL), respectively and described elsewhere [5,6,10,11]. Ankle kinematics was carried out before nerve injury (week 0), and at week 20. A Perspex track with length, width (adjustable) and height of 120, 12, and 15 cm, respectively was used to record the rat locomotion in a straight line. The animals' gait was video recorded at a rate of 300 Hz images per second (Casio Exilim PRO EX-F1, Japan) by a camera with a visualization field of 14 cm wide. The software APAS® (Ariel Performance Analysis System, Ariel Dynamics, San Diego, USA) was used for a 2D biomechanical analysis (sagittal plan) applying a two-segment model of the ankle joint, adopted from the model firstly developed by [14,15]. The rats' ankle angle was determined using the scalar product between a vector representing the foot and a vector representing the lower leg. With this model, positive and negative values of position of the ankle joint (Θ^{o}) indicate dorsiflexion and plantarflexion, respectively. For each step cycle the following time points were identified: initial contact (IC), Opposite Toe off (OT), and Heel Rise (HR) and toe-off (TO) [14,15] and were time normalized for 100% of step cycle. Stance phases lasting between 150 and 400 ms were considered for analysis, since this corresponds to the normal walking velocity of the rat (20–60 cm/s) [8,14,15].

2.6. Nerve and muscle histomorphometry

For histomorphometric analysis, nerve samples obtained from the 10-mm-long sciatic nerve segments distal to the neurotmesis site and from un-operated controls [16-19]. The histological nerve preparation followed the previously reported protocol [5,6,10,19,20] and a systematic random sampling and D-disector were adopted [5,6,10,19,20]. At the end of the healing period tested (week 20), *tibialis anterior* (TA) muscles of the experimental with PVA, PVA-CNTs, PVA-PPy, Graft, End-to-End, and PVA-CNTs-MSCs, and Control groups were collected, and the tissue samples were fixed in 10% buffered formalin, routinely processed, dehydrated and embedded in paraffin wax. Consecutive 3 μm transverse sections from the mid-belly of each muscle were cut, stained with haematoxylin and eosin (HE) and used for morphometry evaluation and determination of the degree of atrophy. For the morphometric analysis, an unbiased sampling procedure was applied and the following measures were calculated: area, perimeter, "Feret's angle" and "minimal Feret's diameter" (which is the minimum distance of parallel tangents at opposing borders of the muscle fiber) and described elsewhere [6,20].

2.7. Scanning electron microscopy (SEM)

Prior to scanning electron microscopy (SEM) analysis, the MSCs cultured on PLC and PVA, PVA-CNTs and PVA-PPy discs were first fixed with 1.5% glutaraldehyde in 0.14 M sodium cacodylate buffer (pH 7.3) for 2h at 4°C. Afterwards, the samples were dehydrated using graded ethanol solutions from 60 % to 100%, 5 minutes each, and subjected to critical point drying. Finally, the samples were mounted on aluminum stubs using double-side adhesive tape and sputter coated with gold/palladium thin film, using the SPI Module Sputter Coater equipment for 100 seconds and with a 15 mA current. The SEM / EDS exam was performed using a High resolution (Schottky) Environmental Scanning Electron Microscope with X-Ray Microanalysis and Electron Backscattered Diffraction analysis: Quanta 400 FEG ESEM / EDAX Genesis X4M [5,6].

2.8. Statistical analysis

Data was analyzed using two-way mixed factorial ANOVA (General Linear Model). Mauchly's test was used to assert sphericity and, if necessary, degrees of freedom correction was introduced using the Greenhouse-Geiser's epsilon. Pairwise comparisons between groups were carried out by the HSD Tukey's test. All statistical procedures were performed with the statistical package SPSS (version 14.0, SPSS, Inc). Data is presented as mean ± standard deviation of the mean (SD).

3. Results and discussion

MSCs are defined by the International Society for Cellular Therapy (ISCT) which includes the following characteristics: a) their capacity to adhere to plastic surfaces during cell culture; b) expression of the specific surface markers, CD73, CD90, and CD105, and no expression of CD14, CD19, CD34, CD45 and HLA-DR; and c) are able to undergo tri-lineage differentiation into adipocytes, chondrocytes and osteoblasts [9]. MSCs isolated from the UC Wharton jelly were expanded and the culture appeared homogeneous and cells presented their typical fusiform, fibroblast-like, morphology (Figure 1A).

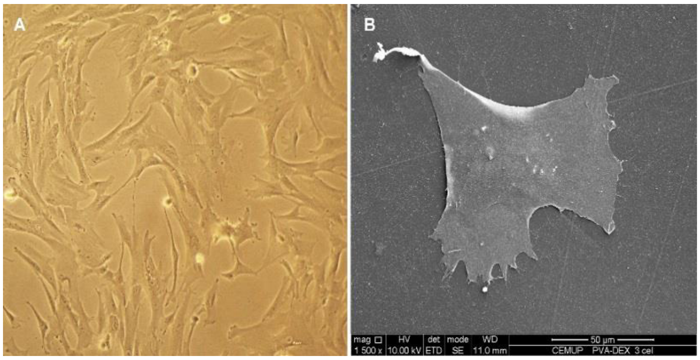

Fig.1. Monoculture of MSCs (P3) exhibiting a mesenchymal-like shape with a flat polygonal morphology in standard adherent culture conditions (Magnification: 100x) (A). SEM image of MSCs cultured over a PVA disc (Magnification: 1000x) (B).

Flow cytometry analysis performed previously to *in vivo* application to expanded MSCs showed that over 95% of the cells in the population were consistently positive for the cell surface markers CD44, CD73, CD90 and CD105 and less than 2% positive for CD14, CD19, CD31, CD34, CD45 and HLA-DR (data not shown). Also, the phenotype of MSCs was assessed by PromoCell by flow cytometry analysis for an additional comprehensive panel of markers: PECAM (CD31), HCAM (CD44), CD45, and Endoglin (CD105). Overall, these results are the ones expected for MSC-type stem cells according to ISCT [9]. Giemsa-stained cells of MSCs at P5 were analyzed for cytogenetic characterization. The karyotype of MSCs was determined previously to *in vivo* application to ensure that no neoplastic cells were used proving the cell therapy safety. The karyotype analysis demonstrated that no structural alterations were found, as well as chromosomal stability in terms of number and structure of the somatic and sexual chromosomes, to the cell culture procedures. Results obtained from epifluorescence technique confirmed that MSCs did not begin the apoptosis process, showing that the *in vivo* applied MSCs were viable even in the presence of the tube-guides biomaterials (PLC, PVA, PVA-CNTs and PVA-PPy). The MSCs cultured and expanded in the presence of the tested biomaterials reached confluence and exhibited a normal star-like shape with a flat morphology in culture, also evident with SEM analysis (Figure 1B). According to these results, it is reasonable to conclude that the biomaterials (PLC, PVA, PVA-CNTs, and PVA-PPy) are viable substrate for MSCs culture and survival and may be used in the pre-clinical trials. MSCs expanded to P5-P6 were incubated with specific differentiation media as described in the methods section. Results showed that MSCs have the capacity for tri-lineage differentiation into adipocytes, chondrocytes and osteoblasts.

Also, the isolated cell populations presented mainly fibroblastic spindle shape morphology and the great majority expressed MSCs markers such as vimentin, c-kit and NANOG-1 (Figure 1A, Figure 2A-C). The isolated cell populations were negative for the vascular market CD31 (Figure 2D). The MSCs cultured over the PLC and PVA membranes also presented normal shape and could easily expand, evidenced in SEM images (Figure 1B).

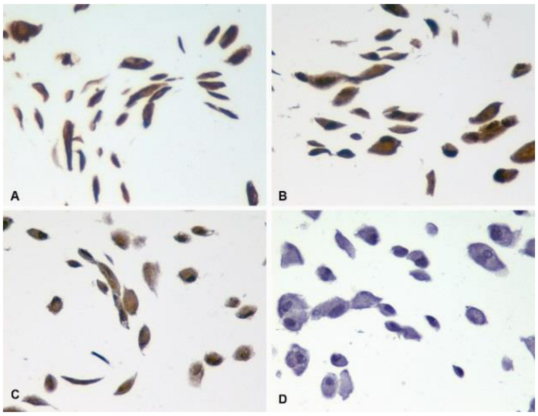

Fig.2. MSCs immunocytochemistry staining with Vimentin (A); Nanog (B); c-kit (C) and CD-31 (D). Magnification: 400x.

The present experimental work includes a variety of independent evaluation tools considering the morphologic and functional recovery, in order to understand and estimate the potential therapeutic benefit of a nerve repair strategy [1,15,19,20]. MSCs associated to PLC or locally infiltrated enhanced EPT recovery in animals graft and end-to-end nerve suture, showing values of residual functional impairment. The MSCs helped in minimizing the negative consequences of grafting in functional outcome. Also, the nociception function was almost completely recovery to values near the 4 seconds at week-20 [1,5,6,19,20]. When the neurotmesis injury can be surgically reconstructed with an epineural end-to-end suture or by grafting, the addition of a PLC and MSCs seems to bring significant advantage, especially concerning the motor function recovery. Considering the PVA, PVA-CNTs, PVA-CNTs-MSCs, and PVA-PPy experimental groups, in the weeks following sciatic nerve neurotmesis there was a large increase in the time latency to withdraw the paw in response to the thermal stimulus, with most animals reaching the cutoff time of 12 seconds. A mild improvement in latency times occurred over the weeks of recovery at a rate that was similar between the experimental groups so that no significant interaction effect existed [$F(55,341) = 5.727$; $P < 0.503$]. According to ANOVA results, differences between treated groups (PVA, PVA-CNTs, PVA-PPy, PVA-CNTs-MSCs, Graft and End-to-End) in the severity of changes in WRL times existed [$F(5,31) = 2.942$; $P < 0.05$], but these differences were not large enough to be detected by the pairwise comparisons. The use of biomechanical techniques and rat's gait kinematic evaluation was a progress in documenting functional recovery, largely published by our research group [1,14,15,19,20]. Indeed, the use of biomechanical parameters has given valuable insight into the effects of the sciatic denervation/reinnervation, and thus represents an integration of the neural control acting on the ankle and foot muscles [1,8,14,15,19,20]. For that reason, ankle joint angle values were similar across the experimental groups at the times of IC, OT and HR. However, less acute ankle joint angles observed in PVA-CNTs-MSCs group, compared with PVA-PPy and PVA-CNTs groups might suggest improved ankle muscles function during the push off phase of the rat's gait cycle. The morphometry and histological analysis of TA muscles collected from rats where the neurotmesis injury was reconstructed with PVA-CNTs-MSCs tube-guides is in fact somehow surprising since in the treatment groups presented in a previous published study [10], no adverse effect was noticed when MSCs isolated from UCT were used. The TA muscles from PVA-CNTs-MSCs group presented the worst results in terms of muscular atrophy after denervation, results not correlated with functional analysis. The decreased fiber size observed in these TA muscles was coincident with the smaller sized muscles that were collected at 20 weeks, macroscopically evaluated during the collection for histological evaluation. Histologically it was also possible to detect a considerable amount of necrosis with delayed muscle regeneration in this group's TA muscles. Some toxic metabolite resulting from the interaction between the vehicle where the MSCs are suspended (Phosphate Buffered Solution, PBS) which has Ca^{2+} and Mg^{2+}, and PVA-CNTs tube-guide, could explain the histological results. These results may occur, not because there is a deleterious effect of the cellular system, but because the vehicle is not appropriate to the cells microenvironment. As a matter of fact these MSCs were previously tested with Floseal® and some local nerve necrosis could be observed by histological analysis [10]. On the other hand, the myelin sheath of the MSCs-treated regenerated nerves was thicker, suggesting that MSCs might exert their positive effects on SCs, the key element in Wallerian degeneration and consequent axonal regeneration [10]. From the data obtained from the stereology analysis of PVA, PVA-CNTs, PVA-PPy, Graft, End-to-End and PVA-CNTs-MSCs groups, it was also demonstrated a synergistic positive effect on regenerated nerve fibers resulting from combined use of MSCs with the PVA-CNTs tube-guides, where PVA-CNTs-MSCs presented higher myelin thickness (M), ratio myelin thickness/axon diameter (M/d) and ratio axon diameter/fiber diameter (d/D; g-ratio) when compared to PVA, PVA-CNTs, PVA-PPy groups. Also, these three stereologic parameters (M, M/d, and g-ratio) measured in PVA-CNTs-MSCs regenerated nerves, were not significantly different from the values obtained from End-to-End and Graft groups. As it was previously reported [5,6,10], in the MSCs-based cell therapy applied to neurotmesis nerve injuries, it is observed a significantly higher myelin thickness, and similar to Graft and End-to-End regenerated nerves, which are considered the Gold Standards in nerve neurosurgery. These results clearly are consisted with functional data and kinematic gait analysis. The PVA-CNTs and PVA-PPy groups presented significantly higher axon diameters (d) and fiber diameters (D), suggesting also the positive effect of these electric conductive materials on axon regeneration and reestablishment of the neuro-muscular junction in agreement with the TA muscle morphometry analysis.

4. Conclusions

The results revealed that treatment with MSCs associated to *PVA-CNTs* tube-guides induced an increased number of regenerated fibers and thickening of the myelin sheet. Functional and kinematics analysis has revealed positive synergistic effects brought by MSCs and PVA-CNTs. The PVA-CNTs and PVA-PPy are promising scaffolds with electric conductive properties, bio- and cytocompatible that might prevent the secondary neurogenic muscular atrophy by improving the reestablishment of the neuro-muscular junction. When the neurotmesis injury can be surgically reconstructed with an epineural end-to-end suture without tension or by grafting, the addition of a PLC membrane and MSCs seem to bring significant advantage, especially concerning the motor function recovery, basically by the secretion of local growth factors and cytokines secretion.

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