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ORIGINAL ARTICLE BASIC SCIENCE/EXPERIMENTAL

A Reliable Stem Cell Carrier: An Experimental Study in Wistar Rats

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Abstract

Introduction Treatments based on cell biology need reliable and precise carriers for reaching the desired targets. For that reason, a PDO-based cell carrier was idealized, with the purpose of carrying stem cells to distant sites at room temperature.

Materials and Methods Three modalities of the same carrier were evaluated: one containing undifferentiated human dental pulp stem cells (DPSCs); one loaded with stem cells induced to neurogenic differentiation (DPSCNs); and one without cells (Blank). The carriers were implanted in sciatic nerve gaps in 48 Wistar rats that were divided in three groups. Two other rats were included in a SHAM control group. Immunohistochemical, histological and clinical analyses were performed in two, four, six and eight weeks of time.

Results Efficacy of human stem cell transportation at room temperature to rats was attested. Moreover, it was possible to confirm that those cells show tropism for inflamed environments and are also prone to induction of neurogenesis in the first two weeks, vanishing after that period.

Conclusion Clinical evaluation of the animals' gait recovery shows a promising perspective of success with the inclusion of stem cell-loaded PDO tubes in nerve gaps, which may be positively compared to previously published studies.

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Keywords Stem cells · PDO carrier · Nerve regeneration · Nerve guide · Tropism for inflammation

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Introduction

The development of treatments based on cell biology, genetic engineering or even intralesional therapies needs carriers that must allow for the exact placement of therapeutic agents to achieve their desired action.

Transfer of either stem cells or differentiated cells carries a great perspective of use, since the standardization of their harvest, described by Rodbell [1]. There is a scenario of several therapeutic applications [2–8] for carriers that

may accomplish such purposes, so that their usage is highly likely to occur. In the near future, these carriers loaded with adipocytes will be able to be used as fillers in aesthetic procedures.

Repair of damaged peripheral or facial nerves, which is warranted in multiple situations, has been achieved with the current level of expertise, with the use of nerve grafts, frequently aided by microsurgery. However, results are not always the most adequate, leading to deficits in the donor areas, development of neuromas and truncated recovery [9, 10]. For years, there has been an effort to supply such demands with the utilization of stem cells, differentiation-induced stem cells or even differentiated cells [11–20].

Stem cell banks are reality, allowing for the development of mesenchymal tissues, autogenous or not, according to necessity, and, sometimes, presenting low immunogenicity.

Plastic surgeons have started to take an important share in this market, since it was observed that adipose tissue shows an exuberant presence of stem cells [21–24]. Based on that information, there has been a range of therapies that have derived from the capacity of fat to induce inflammation, which has led to a great development of regenerative lipografting [25, 26], with research showing amplification of the potential of integration of stem cell-enriched grafts [27], as well as their regenerative action on damaged tissues, such as nerves.

Considering the possibility of on-demand nerve repair, the authors aware that such procedures will need adequate tools dependent on cell biology and have sought easy alternatives to meet such requests: using tubes made from PDO—an absorbable synthetic material widely known in surgery—for a user-friendly, dependable and precise execution.

The main goal of this study was to evaluate whether stem cells maintained viability when transferred with a carrier from one culture medium to a laboratory animal, at room temperature; and to determine whether undifferentiated mesenchymal stem cells and mesenchymal stem cells induced to neurogenic differentiation showed any differences in nerve regeneration.

Materials and Methods

The study was approved by the Animal Ethics Committee at State University, under protocol 4051–1.

A population of 48 male Wistar rats (HanUnib: WH) was studied, with an average weight of 407 g (ranging from 322 to 664 g), born between March 14 and April 15, 2016, raised at the State University vivarium, and randomly distributed into three groups (16 animals) with two subgroups (8 animals) in each group; a SHAM control group with two animals was also studied.

Harvesting of surgical specimens was performed at 2 weeks, 4 weeks, 6 weeks and 8 weeks postoperatively. One rat from the SHAM group was killed at 4 weeks, and the other one at 12 weeks.

PDO tubes were implanted in gaps that resulted from resection of the sciatic nerve, as described below:

Group 1 16 animals, divided into two subgroups of 8:
Subgroup *A*: Right side, empty carrier (Blank). Left side, carrier loaded with undifferentiated dental pulp stem cells (DPSCs).
Subgroup *B*: Right side, carrier loaded with DPSCs. Left side, empty carrier.
Group 2 16 animals, divided into two subgroups of 8:
Subgroup *A*: Right side, empty carrier. Left side, carrier loaded with stem cells induced to neurogenic differentiation (DPSCNs).
Subgroup *B*: Right side, carrier loaded with DPSCNs. Left side, empty carrier.
Group 3 16 animals, divided into two subgroups of 8:
Subgroup *A*: Right side, carrier loaded with DPSCs. Left side, carrier loaded with DPSCNs.
Subgroup *B*: Right side, carrier loaded with DPSCNs. Left side, carrier loaded with DPSCs.

SHAM Group

Adequate alignment of the nerve stumps was done in the right side of SHAM 01, after careful section of the sciatic nerve with microsurgical scissors; the PDO carrier was used as an immobilization splint. In the left side, a gap was made in the same way as with the other animals, with carrier interposition, in order to reproduce the elements that were present in the proposed procedures, except for the inclusion of stem cells.

In SHAM 02, bilateral section of the sciatic nerve and alignment of the nerve stumps were performed, followed by placement of the carrier as an immobilization splint on both sides. This animal was taken as a reference [28], having undergone a longer postoperative follow-up.

Cell Preparation

Stem cells were prepared at a high-level sterilization laboratory (Stem Cell Laboratory), where human dental pulp stem cells were chosen due to their easy obtainment and low immunogenicity [29]. The cells were cultivated and expanded, then marked with Invitrogen™ Molecular Probes™ CellTracker™ CM-Dil dye (Thermo Fisher Scientific, Waltham, MA) and distributed into two groups: DPSCs, cultivated for 3 days; and DPSCNs, cultivated for

7 days. Afterward, the multiperforated, tubular PDO carriers (Fig. 1) were loaded using a technique of competition between the carrier and the culture plate, which had been treated to prevent cell adhesion (Fig. 2).

Both cell groups were placed in a liquid medium to guarantee cell viability, and each carrier was cut into 6-cm-long pieces and kept inside a syringe, which was then placed in a sterile package. Each carrier received one million cells, possessing a capacity of storing 750,000 cells on its inner floor, with an estimated density of 125,000 cells per linear centimeter.

These cell-loaded carriers were collected at 7 am by the senior author at the cell biology laboratory and then taken in a 125-km car trip to the experimental surgery laboratory at State University, where the carriers were utilized according to the Stem Cell Laboratory protocols. At the end of the same day, the remnants contained in each syringe were kept and taken back to the Stem Cell Laboratory, for cell viability control.

Surgical Procedure

Each animal was weighed and then anesthetized through inhalation of 100% Isoflurane (Isoforine®, Cristália, Itapira, Brazil). Subsequently, the animals were prepared for surgery with aseptic technique and immobilized to a board with elastic bands. Shaving of the surgical site was performed with an electric shaver, followed by disinfection with a 1% povidone-iodine aqueous solution (S.C. Johnson, Rio de Janeiro, Brazil) and preparation with a 1% povidone-iodine tincture (S. C. Johnson, Rio de Janeiro, Brazil).

The surgeon, who is also the senior author, used a customized 420-mm 3 × binocular loupe (Microdont, Sao Paulo, Brazil) during all procedures.

The surgeon made skin incisions with a No. 15 scalpel blade, extending for about 1.8 cm, in the femoral regions,

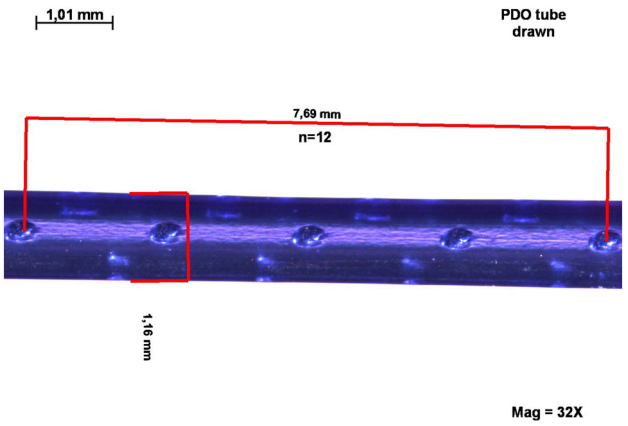

Fig. 1 Characteristics of the PDO carrier

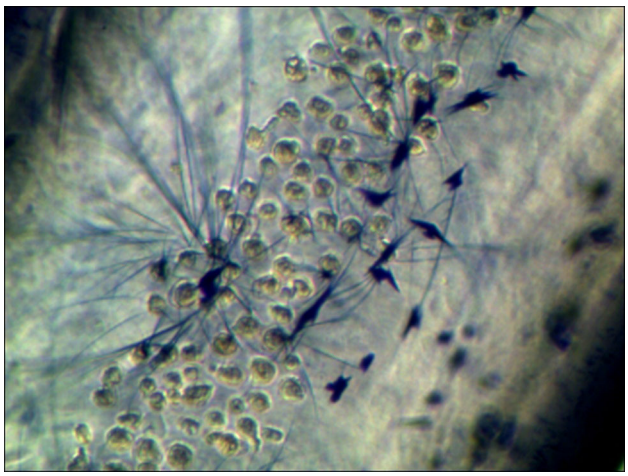

Fig. 2 Close of the carrier loaded with cells

followed by divulsion and exposure of the sciatic nerve. Subsequently, the carriers (which had been previously cut into 0.8-cm-long pieces) were randomly placed, according to the groups' distribution.

A tension-free suture was performed at the limits of the carrier, encompassing both carrier and nerve, with a 7–0 polypropylene monofilament thread (Premilene®, Aesculap/B. Braun, Tuttlingen, Germany). The PDO carrier was fixed parallel to the nerve. Afterward, the surgeon used microsurgical scissors to resect a segment of about a third of the visible nerve, approximately 0.6 cm (Fig. 3). That step was also performed at the left side of animal 01 from the SHAM group. In animal 02 from this group, the surgeon only did a complete section of both nerves, after fixing the carriers. Finally, the retracted muscles were reapproximated, followed by skin closure with interrupted 4–0 Nylon monofilament sutures (B. Braun, Melsungen, Germany).

Animals were kept warm until total recovery from anesthesia was achieved. Subsequently, they were transferred to large cages (each measuring 41 × 34 × 16 cm) that were placed in a specific unit, designed for

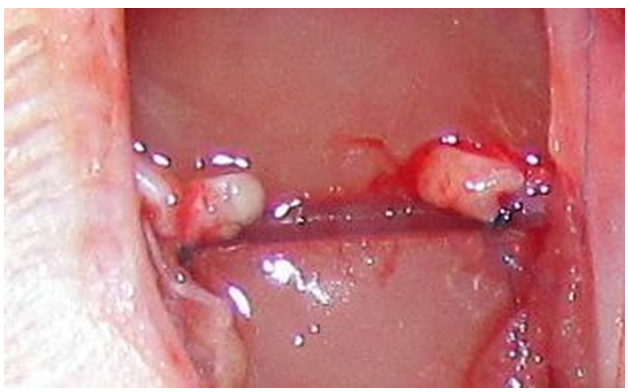

Fig. 3 Proximal and distal section of the sciatic nerve

postoperative care at the State University vivarium, where they were offered water and feed ad libitum. Analgesia was provided for three days with piroxicam, at a single daily dose of 3 mg/kg orally. Following the observation periods of 2, 4, 6 and 8 weeks postoperatively, all animals from the three groups were killed by deepening of anesthesia with intraperitoneal injection of xylazine, as recommended by Flecnell P (Laboratory Animal Anaesthesia, 2nd edition; Academic Press, 1986), with attention being paid to the schedule, so that all subgroups and dates could be considered. Animal 01 from the SHAM group was killed after 4 weeks, while animal 02 was killed after 12 weeks.

Surgical specimens were resected en bloc, containing the nerve and the adjacent and underlying muscles. Specimens were attached to textured kraft paper and then placed in vials containing 10% formalin solution, which were individually labeled. Vials were sent to the State University Department of Pathology, where they were processed into 100 paraffin blocks, from which sections were made and used for histological and immunohistochemical analyses.

The following criteria were chosen to evaluate results:

First Criterion

To verify the presence of human cells in the Wistar rats, sections underwent immunohistochemical analysis by a specialized pathologist, using the MAB1281 Anti-Nuclei Antibody, clone 235-1 (Lot No. 3065575, MilliporeSigma, Burlington, MA) at a dilution of 1:50. The analysis required the use of the PT Link pre-treatment system (Agilent, Santa Clara, CA), the EnVision FLEX Target Retrieval Solution, Low pH (Agilent, Santa Clara, CA) for antigenic recovery, also the FLEX + Mouse 2 × 5 DAB visualization system (Agilent, Santa Clara, CA) and the FLEX + Mouse (LINKER) amplifier (Agilent, Santa Clara, CA).

Second Criterion

Specimens were evaluated by two senior histologists/pathologists and their assistants through optical microscopy, observing the occurrence of nerve regeneration (presence of differentiated cells), with counting of cell blocks composed of neoformed nerves, focusing only on the nerves who were clearly identifiable.

Evaluated topics:

1. *Percentage of the circumferential perimeter of the carrier covered with neoformed nerves: 1: from 33 to 66% of the perimeter; 2: from 67 to 100% of the perimeter.* Objective: to evaluate the carrier as a repair guide.
2. *Presence of A point in the circumferential perimeter of the carrier that showed radial neoformation of nerve tissue with a minimum of 0.5 mm: yes* or *no*. Objective: analysis of the consistency of a potential regeneration.
3. *Nerve tissue in the carrier lumen: yes:* presence of nerve tissue in the lumen, either with usual morphology (as in a normal peripheral nerve fiber) or with aberrant morphology. *No:* absence of nerve tissue in the lumen. Objective: to determine whether cells that had remained in the lumen originated nerve regeneration, as well as the morphology of this new tissue.
4. *The emergence of neoformed, consistent nerve sprouts* around the carrier; they were counted in each case. Such reference was identified as Grimaldi Index, which was considered suggestive of nerve regeneration.

Third Criterion

Clinical evaluation of the rats' gait, randomly carried out in 30 animals through 33 videos that encompassed all different nerve reconstructions, and followed the criteria shown in Table 1.

That evaluation was executed by the senior author, together with laboratory staffs (a biologist and two technicians) who were familiar with laboratory animal care, besides a second surgeon.

Statistical analysis

For statistical analysis, the following programs were used: IBM® SPSS Statistics 20, Minitab® 16 and Microsoft® Excel 2010. As statistical methods, the Kruskal–Wallis, Mann–Whitney and Chi-squared tests were used, a 95% confidence interval for a mean and a p value.

Results

All animals survived surgery and underwent good postoperative recovery during all the proposed period of analysis. The timetable for obtainment of specimens was strictly followed.

Table 1 Rat gait evaluation

Gait evaluation	
Score	Gait manifestation
0	Animal walks on the knee
1	Weak support in paw
2	Stronger support in paw
3	Totally supported in paw

Mean operative time: right side—14.37 min; left side—15.52 min (8% longer).

The immunohistochemical evaluation was focused on the *presence* or *absence* of human cells in the groups (Blank, DPSCs and DPSCNs) and in the different periods (two, four, six and eight weeks).

The presence of human cells was attested *in all animal groups* in the second week, including the Blank sides, which only received empty carriers (Fig. 4). Regarding the Blank cases, the cells were restricted to the implantation area of the tube, not being observed in more distant locations. Vanishing of these cells after two weeks [30] was not unexpected.

As foreseen, no human cells were detected in either animal from the SHAM group (Fig. 5).

Results from the immunohistochemical analysis are shown in Table 2.

Histology demonstrated that there was some extent of nerve regeneration (presence of differentiated cells) *in all specimens* with cell blocks composed of clearly identifiable neoformed nerves.

In regard to carriers, regeneration took place either outside them, or around their perimeters, or in their lumens.

Neoformed sprouts present in the boundaries of the tube, for which a good systematization was achieved, were present *in all 96 specimens* (Fig. 6). The four specimens from the SHAM group were negative, which may indicate an important correlation with cell transfer. The number of sprouts varied from 1 to 6 per section, as shown in Table 3.

With respect to covering of the perimeter of carriers, nerve growth was observed *in 96 out of 100 specimens*. One of the specimens could not be evaluated. In the vast majority, there was great covering; exceptions were the three nerves operated on in the SHAM group that only underwent alignment of nerve stumps, and which exhibited low nerve proliferation.

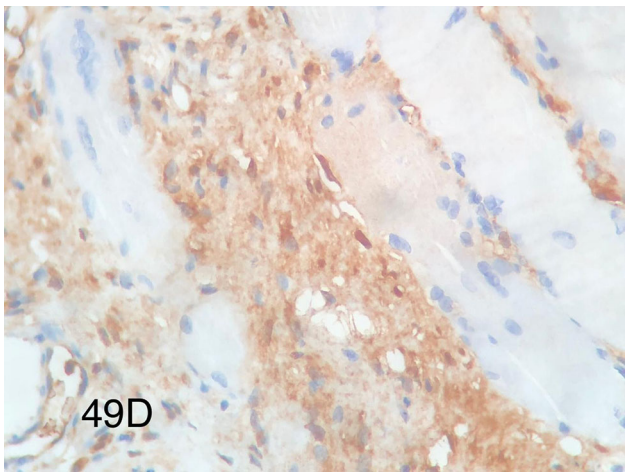

Fig. 4 Positive IH in DPSC/DPSCN group at 2 weeks

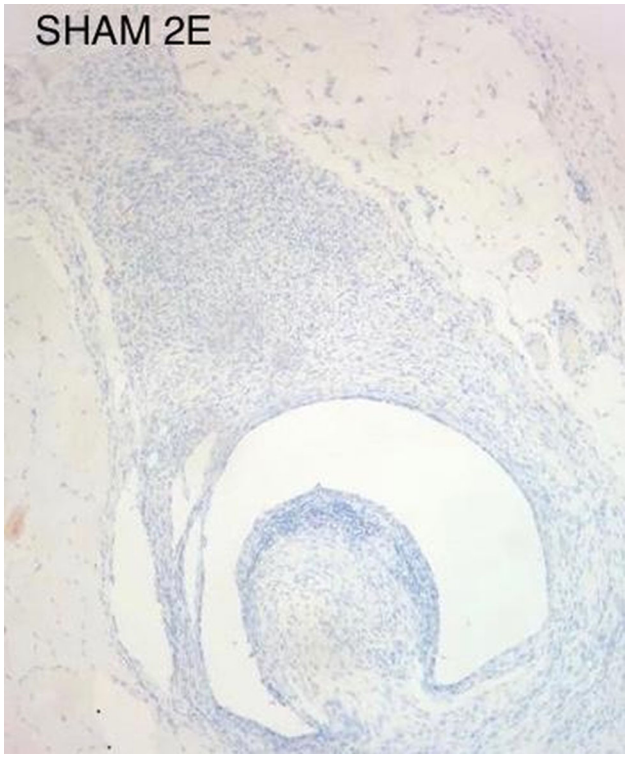

Fig. 5 Negative IH in the SHAM 02—Blank

Table 2 Immunohistochemical analyzes

Immunohistochemical analyses					
Inclusions	Weeks				
	02	04	06	08	12
Blank	+	−	−	−	
DPSCs	+	−	−	−	
DPSCNs	+	−	−	−	
SHAM		−			−

Blank (empty tube), dental pulp stem cells (DPSCs), dental pulp stem cells induced to neural lineage (DPSCNs)

Regarding radial neoformation of nerve tissue with a minimum of 0.5 mm around the perimeter of the carrier, *58 positive cases were found out of 100* (SHAM group was negative).

Concerning the presence of nerve tissue in the lumen of carriers, *46 positive cases were found* (SHAM group was negative).

Gait analysis, which represents the search for the desired clinical outcome, is shown in Fig. 7.

Among more than 1000 parameters evaluated in different topics using the 100 collected specimens, those that possessed both statistical significance and relevance were displayed. Conditions that were present in all groups but were not differential, such as foreign body reaction,

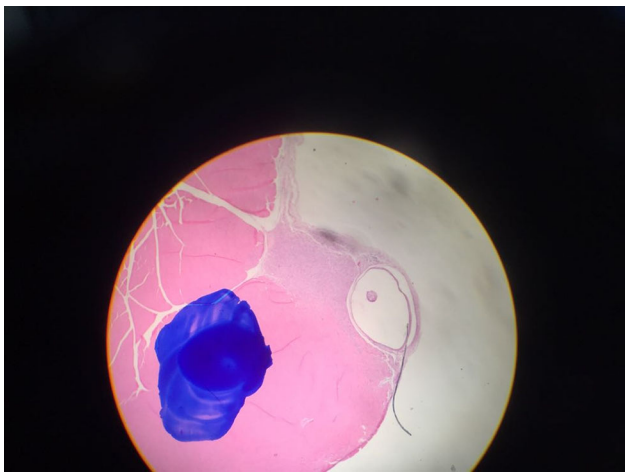

Fig. 6 Carrier and neural neoformation sprout

changes in the carrier structure and inflammatory reaction, were discarded.

Discussion

The possibility of developing a nerve repair-inducing guide manufactured on demand in a specialized laboratory unveils a perspective for treatment of nerve lesions. Choosing the materials and the adequate cells for the proposed therapy, expanding them and delivering them in suitable conditions for transport is an idea that seemed utopian not long ago.

In this study, the sciatic nerve was chosen for experimentation. This option was due to its favorable anatomy, located in a low-motion area along the femur. In a pilot study, it was observed that the relative stiffness of the PDO tube required a stability that could not be obtained in high-motion areas, mainly those near joints.

Surgical procedures lasted from 6 to 30 min each. (Delays occurred mainly due to photographic documentation.) A bias was detected, since operating with the rat's dorsum facing the right-handed surgeon led to faster procedures on the right side.

In the laboratory research phase at the Stem Cell Laboratory, while seeking to load the carriers with stem cells, it was noted that these cells showed negative tropism toward the PDO tube, due to antagonistic polarities, which leads them to quest for a more favorable environment, such as the recipient site; based on these observations, it was found adequate to place the tube parallel to the nerve so that cell migration might occur toward the nerve stumps, having the former bed of the sciatic nerve to be occupied, if that was the case, with the tube serving as a guide.

Table 3 Comparison between groups for "Grimaldi Index"

Grimaldi Index	Mean	Median	Standard deviation	Q1	Q3	N	CI	*P* value
2 weeks								
SC/SN	2.13	2.0	0.64	2	2.25	8	0.44	0.034*
Blank/SC	3.00	3.0	1.31	2	4	8	0.91	
Blank/SN	3.50	3.5	0.93	3	4	8	0.64	
4 weeks								
SC/SN	2.75	3.0	0.71	2	3	8	0.49	0.113
Blank /SC	2.63	2.5	1.06	2	3.25	8	0.73	
Blank /SN	3.75	3.5	1.16	3	5	8	0.81	
6 weeks								
SC/SN	3.13	3.0	0.35	3	3	8	0.24	0.239
Blank /SC	3.88	4.0	1.46	2.75	5	8	1.01	
Blank /SN	3.00	3.0	0.00	3	3	8	X	
8 weeks								
SC/SN	2.75	3.0	0.46	2.75	3	8	0.32	0.805
Blank /SC	2.75	3.0	0.71	2	3	8	0.49	
Blank /SN	2.88	3.0	0.99	2.75	3.25	8	0.69	
All groups								
SC/SN	2.69	3.0	0.64	2	3	32	0.22	0.039*
Blank /SC	3.06	3.0	1.22	2	4	32	0.42	
Blank /SN	3.28	3.0	0.92	3	4	32	0.32	

Dental pulp stem cells (SC), dental pulp stem cells induced to neural lineage (SN), Blank (empty tube), confidence interval (CI)

*Statistical significance

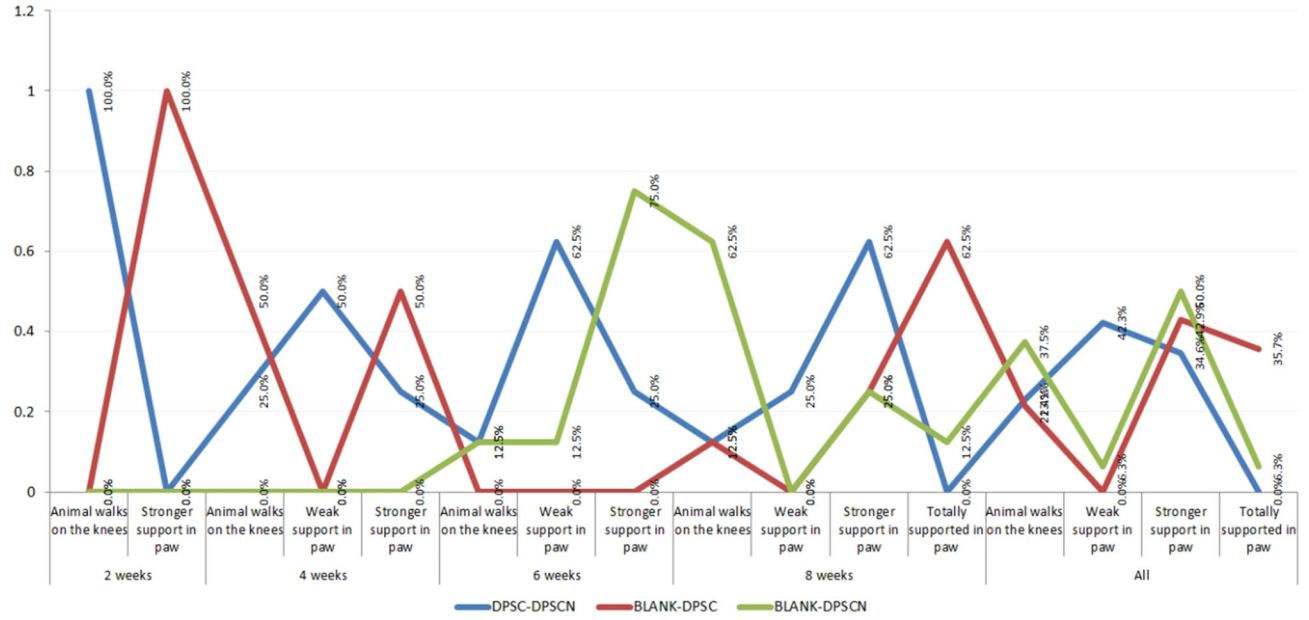

Fig. 7 Comparison between groups for "gait evaluation" distribution

This study was focused on observing some clear aspects:

1. To evaluate the possibility of dealing with a specific type of tube to transport biomaterials at a distance, which would also be easy to store, of low immunogenicity and readily available at cell and tissue banks, to be used at emergency situations. In summary, a ready biological repair tool may be kept at room temperature and immediately utilized.
2. To evaluate the use of stem cells from two different lineages as inducers of nerve regeneration.

Regarding the first item, it became evident that the multiperforated PDO tube is efficient as a biomaterial carrier. The presence of human cells in the rats was clearly demonstrated through immunohistochemical tests with the MAB1281, showing that the PDO carriers are a reliable alternative for transporting stem cells at room temperature, since the only way that labeled human cells could be present in the rats would be through transplantation. At the end of the study period, the remnants of the cell-loaded carriers were analyzed by an automated cell viability counter that attested the good condition of cells.

This study confirmed the already described tropism that stem cells carry for inflamed environments [31–34], even at a distance, since implantation of these cells was detected in blank carriers that were placed contralaterally to cell-loaded carriers. The literature shows us that this strong tropism does occur when cells are injected at a distant location, which can be determined through bioluminescence [35].

The second week shows a significant source of analyses, which coincides with the identification of human cells that were still present. At this time, the following aspects could be evaluated: the presence of cells on the external wall of carriers, in their lumen or outside the carriers.

The fact that cells were present in the outer parts of carriers suggests that these cells tend to use them as a natural regeneration guide, while their presence in the lumen of carriers indicates a more comfortable reproductive habitat. The existence of cells outside the carriers points to either an intense rejection toward the carrier or a tendency of cells to reproduce in a living environment, with the possibility of expansion.

The group that combined DPSCs and DPSCNs displayed total rejection toward the lumen of carriers (0% presence, $p = 0.045$), with low proliferation of neoformed sprouts around the carriers: Grimaldi Index with $p = 0.034$ when compared to the other groups; however, a high adhesion (100%) to the perimeter of the carriers was observed, showing a significant neoformation in this region, with sprouts larger than 0.5 mm ($p = 0.005$). That means that this coculture [36] left the lumen of the carrier and attached to its exterior wall, where it proliferated using the carrier as a regeneration guide.

The DPSCN group followed the DPSC/DPSCN group in the analyzed aspects, except for Grimaldi Index, since the DPSC group was capable of producing sprouts outside the carrier.

The DPSC group behaved differently, with cells present partly in the lumen, partly in the external wall and partly outside the carrier, which shows that this group is the most adaptable to different environments.

Interestingly, those differences tend to vanish along the eight-week period.

Non-detection of human stem cells after the two-week period suggests that such cells may have a temporary role in the induction of regeneration, being either absorbed, modified or discarded after the initial therapeutic action, since they belong to different species. When studies carried out with humans and their own cells are made available, it should be expected that those cells will remain indefinitely, thus allowing for their use in volume augmentation, nerve regeneration, neovascularization, inoculation of antitumor cells in neoplasms, etc. In short, there is a promising array of options.

After performing statistical analysis of the parameters considered for evaluation, it is worth emphasizing the following items:

Behavior of stem cells according to the criterion "Nerve Tissue in the Lumen of the Carrier" shows that: while the presence of stem cells was determined by immunohistochemical tests, at two weeks of time there is zero occupation in the DPSC/DPSCN group, which carried the highest density of stem cells. After that period, with non-detection of stem cells, this occupancy arises to 75% of occupation in the lumen; meanwhile, there was a great increase in nerve neoformation larger than 0.5 mm. That means that the empty carrier started being occupied by neoformed nerve cells, no longer stem cells and the barrier of negative tropism ceased to exist; analysis of the nerve tissue in the lumen of the carriers showed strong activity, albeit with an aberrant morphology in 82.6% of specimens.

The "Percentage of the Circumferential Perimeter of the Carrier Covered with Neoformed Nerves" is already quite expressive in the second week; it occurred in 96.8% of all specimens. These nerves should not be related to the transferred cells, since they constitute ordinary spontaneous regeneration.

At gait analysis, the Blank/DPSC group displayed the greatest presence (57.1%) of intraluminal nerve tissue by the end of the second week, and it also was the group with greatest clinical recovery (62.5% of the best recovery cases).

This clinical analysis is the one most sought after regarding nerve regeneration. Despite different evaluations on this topic, it was possible to achieve 25% of very good results in the average of specimens analyzed after treatment of nerve gaps in the animals by the eighth week. Comparison to other studies that reported rates of 24.9% [36], 23.4% [17] and 21.6% [12] elicits the search for more answers within this subject. The use of DPSCs achieved an average of 37.5% of very good results by the end of the eighth week, which indicates that studies with greater sample sizes are warranted.

It is worth noting that the best recovery was observed with simple alignment of nerve stumps followed by immobilization by the carrier, which was performed in animal 02 from the SHAM group, which exhibited full mobility after 12 weeks.

Conclusion

It was possible to accomplish the main objective of this study, which was demonstrating the efficiency of the carrier as an alternative to transport human stem cells from a culture medium to an animal, at room temperature and at a distance, a fact that was confirmed by immunohistochemical analyses.

Stem cells migrated from their implantation site up to the regions with blank carriers, thus showing that they exhibit tropism for inflamed sites.

Although evaluations of nerve regeneration demonstrated a greater proliferation of nerve cells in the groups that contained stem cells induced to nerve regeneration, the best clinical results were observed in the groups that only had undifferentiated mesenchymal stem cells.

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Financial Support Acquisition of the rats and biological products (such as antibodies, antibiotics and analgesics) used in the study was funded by the senior author.

Compliance with Ethical Standards

Disclosure The main author has an Intellectual Property (Patent) of a biological carrier since 2008, in the USA. In August 2010, he signed a Collaboration Agreement with Aesculap/B. Braun in a potential production of the device. That is all to disclose.

Statement All applicable institutional and national guidelines for the care and use of animals were followed.

References

1. Rodbell M (1964) Metabolism of isolated fat cells. Effects of hormones on glucose metabolism and lipolysis. J Biol Chem 239:357–380
2. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, Bwnhaim P, Lorenz HP, Hedrick MH (2001) Multilineage cells from human adipose tissue: implications for cell-based therapies. Tissue Eng 7:211–228
3. Zuk PA, Zhu M, Ashjian P, De Ugarte DA, Huang J, Mizuno H, Alfonso ZC, Fraser JK, Benhaim P, Hedrick MH (2002) Human adipose tissue is a source of multipotent stem cells. Mol Biol Cell 13:4279–4295

4. Gimble JM, Guilak F (2003) Adipose-derived adult stem cells: isolation, characterization and differentiation potential. Cytotherapy 5:362–369
5. Ogawa R, Mizuno H, Watanabe A, Migita M, Shimada T, Hyakusoku H (2004) Osteogenic and chondrogenic differentiation by adipose-derived stem cells harvested from GFP transgenic mice. Biochem Biophys Res Commun 313:871–877
6. Mitchell JB, McIntosh K, Zvonic S, Garrett S, Floyd ZE, Kloster A, Halvorsen YD, Storms RW, Goh B, Kilroy G, Wu X, Gimble JM (2006) Immunophenotype of human adipose-derived cells: temporal changes in stromal-associated and stem cell-associated markers. Stem Cells 24:376–385
7. Faroni A, Terenghi G, Reid AJ (2013) Adipose-derived stem cells and nerve regeneration: promises and pitfalls. Int Rev Neurobiol 108:121–136
8. Volpe BB, Santos Duarte AD, Ribeiro TB, Stocchero IN, Kharmandayan P, Olalla Saad ST, Bustorff-Silva JM, Malheiros Luzo AC (2014) Mesenchymal stromal cells from adipose tissue attached to suture material enhance the closure of enterocutaneous fistulas in a rat model. Cytotherapy 16:1709–1719
9. Tarasidis G, Watanabe O, Mackinnon SE, Strasberg SR, Haughey BH, Hunter DA (1997) End-to-side neurorrhaphy resulting in limited sensory axonal regeneration in a rat model. Ann Otol Rhinol Laryngol 106:506–512
10. Souza FI, Santos GB, Silva CF, Mattar R Jr, Zumiotti AV (2011) Avaliação Histológica da Neurorrafia Término-Lateral Estudo Experimental em Ratos. Acta Ortop Bras 19(3):129–131
11. Sasaki R, Aoki S, Yamato M, Uchiyama H, Wada K, Okano T, Ogiuchi H (2008) Tubulation with dental pulp cells promotes facial nerve regeneration in rats. Tissue Eng Part A 14(7):1141–1147
12. Santiago LY, Clavijo-Alvarez J, Brayfield C, Rubin JP, Marra KG (2009) Delivery of adipose-derived precursor cells for peripheral nerve repair. Cell Transplant 18(2):145–158
13. Sasaki R, Aoki S, Yamato M, Uchiyama H, Wada K, Ogiuchi H, Okano T, Ando T (2011) PLGA artificial nerve conduits with dental pulp cells promote facial nerve regeneration. J Tissue Eng Regen Med 5(10):823–830
14. Marconi S, Castiglione G, Turano E, Bissolotti G, Angiari S, Farinazzo A, Constantin G, Bedogni G, Bedogni A, Bonetti B (2012) Human Adipose-derived mesenchymal stem cells systemically injected promote peripheral nerve regeneration in the mouse model of sciatic crush. Tissue Eng Part A 18(11–12):1264–1272
15. Terada N, Bjursten LM, Papaloizos M, Lundborg G (1997) Resorbable filament structures as a scaffold for matrix formation and axonal growth in bioartificial nerve grafts: long term observations. Restor Neurol Neurosci 11(1–2):65–69
16. Walsh S, Midha R (2009) Practical considerations concerning the use of stem cells for peripheral nerve repair. Neurosurg Focus 26(2):E2
17. Orbay H, Uysal AC, Hyakusoku H, Mizuno H (2012) Differentiated and undifferentiated adipose-derived stem cells improve function in rats with peripheral nerve gaps. J Plast Reconstr Aesthet Surg 65(5):657–664
18. Patel M, Vandevord PJ, Matthew HW, Desilva S, Wu B, Wooley PH (2008) Functional gait evaluation of collagen chitosan nerve guides for sciatic nerve repair. Tissue Eng Part C Methods 14(4):365–370
19. Ganga MVM, Coutinho-Netto J, Colli O, Marques W Jr, Catalão CHR, Santana RT, Oltramari MRP, Carraro KT, Lachat JJ, Lopes LS (2012) Sciatic nerve regeneration in rats by a nerve conduit engineering with a membrane derived from natural látex. Acta Cir Bras 27(12):885–894
20. Ghoreishian M, Rezaei M, Beni BH, Javanmard SH, Attar BM, Zalzali H (2013) Facial nerve repair with gore-tex tube and adipose-derived stem cells: an animal study in dogs. J Oral Maxillofac Surg 71:577–587
21. Stocchero IN, Stocchero GF, Stocchero GF, Fonseca ASF (2006) Liposuctionable fat: a hypothetic model. Plast Reconstr Surg 117(1):337–338
22. Stocchero IN, Stocchero GF (2011) Isolation of stem cells from human adipose tissue: technique, problems, and pearls. In: Illouz YG, Sterodimas A (eds) Adipose stem cells and regenerative medicine. Springer, Berlin, pp 13–18
23. Torres FC, Rodrigues CJ, Stocchero IN, Ferreira MC (2007) Stem cells from the fat tissue of rabbits: an easy-to-find experimental source Aesth. Plast Surg 31(5):574–578
24. Almeida KA, Campa A, Alonso-Vale MIC, Lima FB, Daud ED, Stocchero IN (2008) Fracción Vascular Estromal: Cómo Obtener Células Madre y su Rendimiento de Acuerdo a la Topografia de las Áreas Donantes: Estúdio Preliminar. Cir.plást.iberolatinoam 34(1):71–77
25. Rigotti G, Marchi A, Galiè M, Baroni G, Benati D, Krampera M, Pasini A, Sbarati A (2007) Clinical treatment of radiotherapy tissue damage by lipoaspirate transplant: a healing process mediated by adipose-derived adult stem. Cells Plast Reconstr Surg 119(5):1409–1422
26. Stocchero IN, Stocchero GHF, Stocchero GF, Stocchero VGZ, Fonseca ASF, Fonseca EFS (2009) Lipoinjerto laminar: un tratamiento prometedor con factores vasculares estromales para las vulvo-vaginitis crónicas. Cir.plást.iberolatinoam 5(3):179–186
27. Yoshimura K (2008) Clinical use of adipose-derived stem cells for breast volume enhancement. ISAPS News 2(3):6
28. Chen ZL, Yu WM, Strickland S (2007) Peripheral regeneration. Ann. Rev Neurosci 30:209–233
29. Nakashima M, Iohara K, Murakami M (2013) Dental pulp stem cells and regeneration. Endod Topics 28:38–50
30. Beigi M-H, Ghasemi-Mobarakeh L, Prabhakaran MP, Karbalaie K, Azadeh H, Ramakrishna S, Baharvand H, Nasr-Esfahani M-H (2014) In vivo integration of poly(e-caprolactone)/gelatin nanofibrous nerve guide seeded with teeth derived stem cells for peripheral nerve regeneration. J Biomed Mater Res Part A 4554–4567
31. Malek S, Kaplan E, Wang JF, Ke Q, Rana JS, Chen Y, Rahim BG, Li M, Huang Q, Xiao Y-F, Verheugt FWA, Morgan JP, Min J-Y (2006) Successful implantation of intravenously administered stem cells correlates with severity of inflammation in murine myocarditis. Pflug Arch Eur J Physiol 452:268–275
32. Lopatina T, Kalinina N, Karagyaur M, Stambolsky D, Rubina K, Revischin A, Pavlova G, Parfyonova Y, Tkachuk V (2011) Adipose-derived stem cells stimulate regeneration of peripheral nerves: BDNF secreted by these cells promotes nerve healing and axon growth de novo. PLoS ONE 6(3):e17899
33. Spaeth E, Klopp A, Dembinski J, Andreeff F, Marini F (2008) Inflammation and tumor microenvironments: defining the migratory itinerary of mesenchymal stem cells. Gene Ther 15:730–738
34. Rustad KC, Gurtner GC (2012) Mesenchymal stem cells home to sites of injury and inflammation. Adv Wound Care 1(4):147–152
35. Kidd S, Spaeth E, Dembinski JL, Dietrich M, Watson K, Klopp A, Battula VL, Weil M, Andreeff M, Marini FC (2009) Direct evidence of mesenchymal stem cell tropism for tumor and wounding microenvironments using in vivo bioluminescent imaging. Stem Cells 27:2614–2623
36. Dai LG, Huang GS, Hsu S (2013) Sciatic nerve regeneration by cocultured schwann cells and stem cells on microporous nerve conduits. Cell Transplant 22(11):2029–2039