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Running title: PAMs and MIAMI cells for Parkinson's disease

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ABSTRACT

Multipotent marrow stromal cells (MSCs) raise great interest for brain cell therapy due to their ease of isolation from bone marrow, their immunomodulatory and tissue repair capacities, their ability to differentiate into neuronal-like cells and to secrete a variety of growth factors and chemokines. In this study, we assessed the effects of a subpopulation of human MSCs, the marrow-isolated adult multilineage inducible (MIAMI) cells, combined with pharmacologically active microcarriers (PAMs) in a rat model of Parkinson's disease (PD). PAMs are biodegradable and non-cytotoxic poly(lactic-co-glycolic acid) microspheres, coated by a biomimetic surface and releasing a therapeutic protein, which acts on the cells conveyed on their surface and on their microenvironment. In this study, PAMs were coated with laminin and designed to release neurotrophin 3 (NT3), which stimulate the neuronal-like differentiation of MIAMI cells and promote neuronal survival. After adhesion of dopaminergic-induced (DI)-MIAMI cells to PAMs *in vitro*, the complexes were grafted in the partially dopaminergic-deafferented striatum of rats which led to a strong reduction of the amphetamine-induced rotational behavior together with the protection/repair of the nigrostriatal pathway. These effects were correlated with the increased survival of DI-MIAMI cells that secreted a wide range of growth factors and chemokines. Moreover, the observed increased expression of tyrosine hydroxylase by cells transplanted with PAMs may contribute to this functional recovery.

Keywords: laminin, mesenchymal stromal cells, tissue engineering, neurotrophin 3, Parkinson's disease, Pharmacologically Active Microcarriers

INTRODUCTION

Parkinson's disease (PD), mainly resulting from the degeneration of the nigrostriatal dopaminergic system, is a progressive neurodegenerative disorder that affects 2 % of the population over 65 years of age. Currently the most efficient therapeutic treatment, L-DOPA, aims at replenishing the amount of dopamine missing in the striatum. However, this strategy slowly becomes less effective after long-term treatment and shows undesirable side effects [1, 2]. Cell therapy is an alternative strategy to treat PD and many clinical studies using foetal dopaminergic cells or dopamine-producing cells, such as adrenal chromaffin cells and human retinal pigment epithelium have been performed [3-5]. These studies gave encouraging results that have provided the proof of principle for cell therapy in PD. However, the outcome was also highly variable between patients and the foetal grafts raised ethical and practical concerns [6], with a poor survival after transplantation [7-10].

Stem cells, that can self renew and further differentiate into dopaminergic precursors are currently the most studied candidates for cell therapy in PD. However, due to the difficulties in obtaining neural stem cells from adults and the inherent ethical problems to the use of foetal cells or of embryonic stem cells, multipotent mesenchymal stromal cells (MSCs), may represent an alternative cell source to repair the nervous system [5]. Indeed, as they are easily isolated from the bone marrow, autologous grafts can be performed avoiding ethical and availability concerns. MSCs may differentiate into progeny of the three embryonic layers *in vitro*, including neuronal-like cells, under the influence of matrix molecules and growth factors [11-14]. Using appropriate driving cues, these cells that may partially originate from the neural crest [15], can be further directed toward a dopaminergic phenotype [16-18]. Recently, mesenchymal stem-like cells from endometrial origin have also been described as an interesting source of cells for PD therapy due to their ability to produce TH and restore dopamine level in parkinsonian mice [19]. In addition to their neuronal differentiation potential, MSCs posses immunomodulatory properties [20, 21] and have the ability to migrate towards sources of lesions in the brain [22-26]. Furthermore, some studies showed a functional improvement in the rotational behavior of hemi-parkinsonian rats upon transplantation of rat or human MSCs [27-30]. As only a very small number of neuronal-like cells were observed in the brain [29], these effects were mostly attributed to the ability of MSCs to secrete various growth factors that protected the degenerating neuronal fibres. These reports encourage further studies with MSCs for cell therapy of PD, but also highlight the need to enhance MSC cell engraftment.

Tissue engineering, which combines cells with a supportive scaffold providing a 3D structure, may help to improve cell engraftment after transplantation [5, 31]. In this way, microcarriers transporting foetal ventral mesencephalic (FVM) cells or adrenal chromaffin cells improved their long-term survival after intracerebral transplantation in hemi-parkinsonian rats [32-34]. A clinical trial has also reported the safety and efficacy of gelatine microcarriers conveying human retinal pigment epithelial cells for the treatment of PD [35, 36]. Scaffolds providing a biomimetic surface of different extracellular matrix (ECM) molecules or their derived peptides, that stimulate cell survival and differentiation, may further enhance cell engraftment [5, 37]. In this regard, various studies report that laminin (LM) enhances neurite outgrowth of neurons [38] as well as neural stem cell proliferation [39]. In addition, LM may also improve the integration of transplanted cells and their tissue regeneration potential in an *ex vivo* model of Parkinson's disease [40]. Finally, this ECM molecule is known to affect stem cell motility [41] and differentiation of MSCs towards a neuronal phenotype, in terms of morphology [42] but also of marker expression [43].

Another way to improve the efficiency of cell grafts is to engineer a scaffold that not only provides a biomimetic surface but also delivers a relevant bioactive growth factor that is released in a controlled manner, further affecting the fate of both transplanted and host cells (see for review [5, 31, 37]). Indeed, synergistic effects between adhesion and growth factor signals to guide and enhance cell differentiation have now been described [44]. In this sense, we have developed an adaptable and efficient device for tissue engineering, the pharmacologically active microcarriers (PAMs). They are biodegradable and non-cytotoxic polymeric microcarriers made of poly(lactic-co-glycolic acid) (PLGA), that with a functionalized surface provide an adequate 3D support for cell culture and/or for their administration. Their microcarrier role, the biomimetic surface and the programmed delivery of an appropriate therapeutic factor may act synergistically to induce and further maintain the survival and/or differentiation of the transplanted cells and their microenvironment, therefore enhancing their engraftment after complete degradation of the vector [45, 46]. The efficacy of this tool was previously demonstrated in a rat PD paradigm using PAMs conveying PC12 cells and releasing nerve growth factor [47], but also with FVM cells attached to PAMs releasing glial cell line-derived neurotrophic factor [48]. In both cases, the PAMs stimulated cell survival and differentiation leading to an improved behavior of the animals.

The main goal of this study was to improve MSC survival, differentiation and tissue repair function after implantation in the striatum of hemi-parkinsonian rats, using PAMs tailored for this application. We chose to work with a homogeneous and well characterized subpopulation of human MSCs that express pluripotent stem cells markers. These cells termed “marrow-isolated adult multilineage inducible” (MIAMI) cells, may generate mature cells derived from all three embryonic germ layers [49, 50]. EGF is now considered as an important factor able to enhance the therapeutic potential of MSCs (see for review Tamama *et al.*[51]). We recently demonstrated that exposing MIAMI cells to an EGF and bFGF pre-treatment enhances their neural specification and response to neuronal commitment [11]. MIAMI cells further differentiate toward the neuronal lineage on a fibronectin surface in a NT3-dependent manner [16] and this molecule increases β3-Tubulin expression by MIAMI cells [11] and MSCs [52] during *in vitro* neuronal differentiation. These results appoint NT3 as an ideal protein to encapsulate into PAMs and to use in combination with EGF-bFGF pre-treated MIAMI cells induced towards a dopaminergic phenotype. In order to choose the appropriate biomimetic surface for PAMs, we first screened the panel of integrins expressed by MIAMI cells by RT-qPCR and flow cytometry. We then studied the effect of LM, compared to fibronectin (FN), on the *in vitro* neuronal differentiation potential of MIAMI cells in terms of cell proliferation, cell length and expression of neuronal markers. Based on these results, PAMs with a

biomimetic surface of LM and poly-D-lysine (PDL) were formulated and their total charge as well as the homogeneity of the LM biomimetic surface was characterized by zetametry and immunofluorescence imaging. PAMs delivering NT3 were formulated and the NT3 release kinetics characterized *in vitro*. These combined properties should act together to stimulate the survival and differentiation of the grafted MIAMI cells toward a neuronal phenotype. After adhesion of MIAMI cells to PAMs, these complexes were first characterized *in vitro*. Next, using a rat partial progressive 6-OHDA model of PD their effects on MIAMI cell survival and differentiation as well as on the motor behavior of the animals and tissue repair/protection were further evaluated.

MATERIALS AND METHODS

Bone marrow harvesting, selection & expansion of MIAMI cells

Whole bone marrow was obtained from vertebral bodies (T1–L5) of a 3 year old male cadaveric donor following guidelines for informed consent set by the University of Miami School of Medicine Committee on the Use of Human Subjects in Research. As previously described [49], isolated whole bone marrow cells were plated at a constant density of 10^5 cells/cm^2 in DMEM-low glucose, containing 5 % fetal bovine serum (FBS) (Hyclone, South Logan, Utah) and antibiotics (AB) (100 U/mL penicillin, 0.1 mg/mL streptomycin, 0.25 µg/mL amphotericin B, Sigma, St-Quentin Fallavier, France) on a FN (Sigma), substrate under hypoxic conditions (3 % O_2, 5 % CO_2, 92 % N_2). Fourteen days later, the non-adherent cells were removed and pooled colonies of adherent cells were selected and plated at low density for expansion (100 cells/cm²) on 1.25 ng/cm² FN substrate in DMEM-low glucose (Gibco, Cergy Pontoise, France) containing 3 % FBS and AB (40 mL/175 cm² flask) under hypoxic conditions. Cells were fed every 2-3 days by changing half the medium and split every 5 days, keeping 1/4 of old medium.

Neuronal differentiation in vitro

To assess the effects of the extracellular matrix molecules on the 3-step *in vitro* neuronal differentiation of MIAMI cells (passage 4-5) they were first expanded for 10 days in DMEM-low glucose with 20 ng/mL of both EGF and bFGF, 5 µg/mL heparin and a mixture of lipids (working concentration of 510 nM lipoic, 70 nM linolenic and 150 nM linoleic acid, all from Sigma) (pre-treatment step). As previously described [11], the protocol continued with the 3-step neuronal differentiation by plating the cells at 3000-4000 cells/cm^2 on either FN or LM (from human placenta, Sigma) coated dishes (all at 2 µg/cm²) in DMEM-F12 (GIBCO) medium supplemented with 20 % FBS, 20 ng/mL of both EGF-bFGF, 5 µg/mL heparin, antibiotics and cultured for 24 h in a normoxic atmosphere (Neural specification, step 1). Coating molecules were diluted in Dulbecco's phosphate buffered saline (DPBS) (without Ca and Mg) and coating was made in presence of 1 mM $CaCl_2$ for LM as it was previously reported to enhance its stabilization [53]. When plated on glass slides, LM coating was made at 0.5 µg/cm² as it allowed a sufficient attachment of cells during differentiation. At the end of step 1, cells were washed and neuronal commitment (step 2) was induced by exposing the cells to 1 mM β-mercaptoethanol (Sigma), 30 ng/ml NT3 (R&D Systems) for 2 days. Neuronal differentiation (step 3) was induced by washing and then exposing the cells to 100 µM butylated hydroxyanisole, 25 mM KCl, 2 mM valproic acid, 4 µM

forskolin, 1 µM hydrocortisone, 5 µM insulin, 5 mM Hepes, 4 µM forskolin, 10 µM rolipram (all from Sigma), 30 ng/mL NT3, 10 ng/mL NGF (R&D Systems), and 30 ng/mL BDNF (R&D Systems) for 3 days.

For transplantation studies, EGF-bFGF pre-treated cells were used as such in some cases, or were further induced toward a dopaminergic phenotype (DI-MIAMI cells). DI-MIAMI cells were obtained prior to their attachment to PAM biomimetic surface (see figure 3A). Pre-treated cells were plated (3000-4000 cells/cm²) on a 2 µg/cm² LM substrate in DMEM-F12 containing 20 % FBS, 20 ng/mL of both EGF-bFGF and 5 µg/mL heparin for 24 hours. Medium was then replaced by DMEM-F12 containing 200 ng/mL SHH and 100 ng/mL FGF8b for another 24 hours (both from R&D Systems). Finally, cells were exposed to 0.5 µM retinoic acid (Sigma) during the last 24 hours before attachment to PAMs.

Immunocytofluorescence & cell length analyses in vitro

Cells were plated on coated glass slides for NFM and β3-Tubulin immunocytofluorescence staining at the end of the neuronal differentiation protocol. After washing twice with DPBS, cells were fixed with 4 % paraformaldehyde (PFA) (Sigma) at 4°C for 15 min and then permeabilized for 5 min with DPBS containing 0.2 % Triton X-100 (DPBS-T) (Sigma). Slides were blocked with DPBS-T, 10 % normal goat serum (NGS) (Sigma), 4 % bovine serum albumin (BSA) (Sigma) at room temperature (RT) for 45 min. After 3 washes in DPBS, slides were then incubated overnight at 4°C with anti-NFM (1:50, clone NN18, Sigma) or anti-β3-Tubulin (1:1000, clone SDL.3D10, Sigma) antibodies in DPBS-T-4 % BSA. Controls were made without primary antibody and with isotypic IgG1k (clone MOPC-31C, BD Biosciences) or IgG2bk (clone 27-35, BD Biosciences). After 3 further washes in DPBS, slides were incubated with 2.5 µg/mL secondary biotinylated anti-mouse antibody (Vector, Burlingame, CA, USA) in DPBS-T-4 % BSA for 1 hour at RT. Finally, after washing 3 times in DPBS, and following incubation with 20 µg/mL streptavidin FITC (Dako) in DPBS for 40 min at RT, slides were mounted and observed with a fluorescence microscope (Axioscop, Carl Zeiss, Le Pecq, France), a CoolSnap ES camera (Photometrics, Tucson, Arizona) and Metamorph™ software (Roper Scientific, Evry, France). Immunocytofluorescence results are presented as the mean ± average deviation of averaged object intensities calculated from 6 different images. Metamorph™ was also used for cell length analyses and results are presented as the average length of the 3 longer cells of 5 different images ± average deviation.

Reverse transcription and real-time quantitative PCR

Design of primers specific for human genes and PCR were performed as described elsewhere [11] (table 1). Cells were lysed in a 1 % β-mercaptoethanol containing buffer and RNA extracted following treatment by DNAse to remove any traces of genomic DNA (Total RNA isolation Nucleospin® RNA II, Macherey Nagel, Hoerdt, France). First strand cDNA synthesis was performed with a Ready-To-Go You-Prime First-Strand Beads® kit in combination with random hexamers (Amersham Biosciences, Orsay, France) using 1 µg RNA according to the manufacturer's guidelines. Following first strand cDNA synthesis, cDNAs were purified (Qiaquick PCR purification kit, Qiagen, Courtaboeuf, France), eluted in 50 µL RNAse free water (Gibco). Five microliters of cDNA (1:20) were mixed with iQ SYBR Green Supermix (Biorad) and primer mix (0.2 µM) in a final volume of 15 µL. Amplification was carried on a Chromo4 thermocycler (Biorad) with a first denaturation step at 95°C for 3 min and 40 cycles of 95°C for 10 s, 55°C for 15 s and 72°C for 15 s. After amplification, a melting curve of the products determined the specificity of the primers for the targeted genes. A mean cycle threshold value (Ct) was obtained from 2 measurements for each cDNA. Several housekeeping genes, Glyceraldehyde-3-phosphate dehydrogenase (Gapdh, NM_002046), Hypoxanthine phosphoribosyltransferase 1 (Hprt1,NM_000194), Beta actin (Actb, NM_001101), 30S ribosomal protein S18 (Rps18, NM_001093779) and Heat shock 90 kD protein 1 beta (Hspcb, NM_007355) were tested for normalization. The GeNorm™ freeware (http://medgen.ugent.be/~jvdesomp/genorm/) was used to determine that Gapdh, Hprt1 & Hspcb were the three most stable housekeeping genes. The relative transcript quantity (Q) was determined by the delta cT method Q=E (Ct min in all the samples tested-Ct of the sample) where E is related to the primer efficiency (E=2 if the primer efficiency=100 %). Relative quantities (Q) were normalized using the multiple normalization method described in Vandesompele et al. [54]. Q normalized = Q/(geometric mean of the 3 most stable housekeeping genes Q).

Integrin screening by flow cytometry

Cells were washed with DMEM-low glucose and detached with 10 mL Versene (Lonza) for 20 min at 37°C. After pelleting at 295 g for 10 min, cells were washed twice before distribution in 296 well plates (10^5 cells/50 µL). 50 µL of 10 µg/mL mouse monoclonal anti-integrin antibodies (CD29, CD49e, CD49c and CD49b, all from BD Biosciences, Le Pont De Claix, France) or IgG1k isotypic antibody (BD Biosciences) solutions diluted in DPBS, 5 % FBS, 0.02 % azide were added and incubated for 1 h at 4°C. After washing, 20 µg/mL FITC-conjugated anti-mouse antibody (Dako) was added for 30 min at 4°C. Cells were rinsed 3 times before a final addition of DPBS, 0.02 % azide and transfer to tubes containing DPBS, 0.02 % azide, 0.7 % formaldehyde.

Every washing step was performed with DPBS, 5 % FBS, 0.02 % azide. Fluorescent signal was acquired using a FACScalibur flow cytometer (BD Biosciences) and data analysis was performed using the Cellquest™ software (BD Biosciences).

Formulation of NT3 solid particles and encapsulation within microspheres

PLGA microspheres of an average diameter of 60 µm were prepared using a s/o/w emulsion solvent extraction-evaporation process described in [55], with modifications. Polymer used was a poly(lactic-co-glycolic acid) (PLGA) copolymer with a lactic:glycolic ratio of 37.5:25 (MW: 25,000 Da) (Phusis, Saint Ismier, France). The protein loading was 1 µg of NT3 (Abcys, Paris, France) with 5 µg of human serum albumin (HSA) (Sigma)/mg of microspheres. First, NT3 and HSA were precipitated separately using a process previously described [55] and adapted to each protein. Briefly, 1.077 g of cold glycofurol was added to 10 µl of a NaCl solution (0.3 M) containing 50 µg NT3 and 1 mg poloxamer 188 (Lutrol F68, BASF). After 30 min on ice, the protein particles were harvested by 30 min centrifugation at 10000 g. HSA solid particles were produced in a similar manner, adding 1.077 g of cold glycofurol on 25 µl of a NaCl solution (0.3 M) containing 250 µg HSA and 5 mg poloxamer. After supernatant removal, the NT3 and HSA solid particles were mixed with 670 µL of organic phase made of 50 mg PLGA dissolved in a 3:1 methylene chloride:acetone solution. This organic phase was emulsified in a poly(vinylalcohol) (Mowiol® 4-88, Kuraray Specialities Europe, Frankfurt, Germany) aqueous solution (90 ml, 4 % w/v) maintained at 1°C and mechanically stirred at 550 rpm for 1 min (Heidolph, RZR 2041, Merck Eurolab, Paris, France). After addition of 100 ml of deionized water and stirring for 10 min, the resulting o/w emulsion was added to 500 mL deionized water and stirred for a further 20 min to extract the organic solvent. Finally, the microparticles were filtered on a 0.45 µm filter (HVLP type, Millipore SA, Guyancourt, France), washed five times with 100 ml of deionized water and freeze-dried. The average volume diameter and the size distribution of the resulting microspheres were evaluated using a Multisizer™ Coulter Counter (Beckman Coulter, Roissy, France). Encapsulation yield was determined after dissolution of microspheres in acetone, centrifugation and evaporation, using a NanoOrange® protein quantification kit (Invitrogen, Cergy Pontoise, France) and following the manufacturer's guidelines. To assess the effects of NT3, microspheres without NT3 were also formulated and covered with a biomimetic surface. Thus, in the following, we will distinguish PAMs encapsulating NT3 (PAMs-NT3) and PAMs without NT3 (PAMs-Blank). When no precision is given, the term PAMs refers to both PAMs-NT3 and PAMs-Blank. For technical reasons, microspheres with a reduced diameter (mean of 25 µm) were also formulated to perform zeta potential

measurements. We produced these microspheres using the above protocol with increased poly(vinylalcohol) concentration (6 % w/v) and agitation speed (1000 rpm).

Formulation of PAMs

To obtain PAMs, PLGA microspheres were coated with LM. A combination of LM with the highly charged PDL (Sigma) molecules was used to favor cell attachment on PAM surface. 6 mg of microspheres were resuspended in DPBS and sonicated upon full dispersion of the microspheres. Coating solutions were prepared in DPBS, mixed to the microsphere suspension (final volume: 12 mL) and placed under rotation at 15 rpm at 37 °C during 4 hours. After coating, PAMs were washed 3 times in distilled sterile water, lyophilized and finally kept at -20°C for long term storage. Every tube was covered with sigmacote ® (Sigma) to prevent product loss on the tube walls. The final concentration of the coating molecules was 40 µg/mL of LM or of a mixture of PDL/LM. For optimization of the PDL/LM coating, the ratio PDL-LM of 60/40 was changed to 40/60 after zeta potential evaluation, in order to maximize the quantity of LM adsorbed to the surface. The incubation time was finally decreased to 1.5 hours as well as the total coating molecules concentration (15 µg/mL).

Zeta potential

A Zetasizer 2000 (Malvern Instruments, Orsay, France) operating at 150 V at RT was used to assess PAM electrical surface charge variations depending on the coating used. Briefly, PAMs were redispersed in 10 mL of 1 mM NaCl and sonicated prior to every measurement. The chamber was washed with ultrapure water (Millipore) between every sample. Results are presented as the average ± standard deviation of three experiments and 10 measurements were performed in each experiment.

PAM immunofluorescence

Tubes containing 1 mg coated or uncoated lyophilized PAMs were resuspended in DPBS, 4 % BSA, 0.2 % Tween 20 and placed for 30 min at RT under 15 rpm rotation. Samples were then washed three times with DPBS followed by a centrifugation step at 9000 g for 5 min and supernatant removal. Incubation with mouse monoclonal anti-laminin antibody (Sigma) diluted at 100 µg IgG/mL in DPBS, 4 % BSA, 0.2 % Tween 20 was performed at 37°C for 1.5 hours under rotation. For negative controls, incubation was performed in the same solution without anti-laminin antibody. After incubation, samples were washed again 4 times before incubation with anti-mouse biotinylated antibody (Vector) diluted at 2.5 µg/mL in DPBS, 4 % BSA, 0.2% Tween 20 for 1

hour at RT under rotation. After three washes, samples were incubated with streptavidine-fluoroprobe 547 (Interchim, Montluçon, France) diluted 1:500 in DPBS for 40 min at RT under rotation. Samples were finally washed three times with DPBS and observed under confocal microscopy (Olympus Fluoview^{TM} TU 300, Rungis, France). Every condition was observed in triplicate and 3 independent experiments were performed.

In vitro NT3 release profile and bioactivity of the released protein

NT3 was labeled with ^{125}I by the iodogen method as previously described [56]. Briefly, 50 µL of $Na^{125}I$ (4.625 MBq) was added in a tube coated with 0.4 µg of iodogen (1,3,4,6-tetrachloro-3a,6a-diphrenylglycouril, Sigma) and mixed at RT during 3 minutes. 50 µg of NT3 was dissolved in 100 µL of phosphate buffer (67 mM KH_2PO_4, 33 mM Na_2HPO_4 $12H_2O$; pH=6.8) and then added to the tube containing $Na^{125}I$ and mixed during 45 minutes at RT. Free ^{125}I were separated from ^{125}I-labeled NT3 using a PD10 column (GE Healthcare) prior to precipitation and encapsulation within PAMs. An *in vitro* kinetic release was performed by placing 5 or 50 mg of PAMs in 500 µL of DPBS containing 0.1 % BSA and 0.02 % sodium azide. At different times, PAMs-NT3 were centrifugated for 5 min at 800 g and radioactivity measured in the supernatant using a gamma counter Minaxi AutoGammas 5000 (Packard, Australia). To assess the bioactivity of the released NT3, MIAMI cells were plated on fibronectin at 3000 cells/cm² in DMEM-F12 containing 20 % FBS and 10 ng/mL bFGF. After 24 hours, medium was changed with DMEM-F12 containing 1 mM β-me and diluted kinetics samples. After 48 hours, the increased proliferation rate when exposed to NT3 was measured using the Cyquant cell proliferation assay® (Invitrogen), following the manufacturer's guidelines. NT3 concentration in the kinetics samples was estimated by comparing the obtained cell density with density of MIAMI cells exposed to known concentration of NT3. Presented radioactivity count results were obtained from a representative experiment and bioassay results expressed as average NT3 release ± standard deviation.

Formation of PAMs/DI-MIAMI cells complexes

DI-MIAMI cells were washed with DMEM-F12, detached with 0.16 % trypsin (Sigma), 0.02 % EDTA (Lonza) solution, and pelleted at 295 g for 10 min. Cell pellets were resuspended in culture medium supplemented with 3 % FBS. 0.75 mg lyophilized microspheres were resuspended in coated Eppendorf tubes (Sigmacote, Sigma) containing DMEM-F12, 3 % FBS for 15 min. PAM suspension was sonicated and briefly vortexed prior to addition of 0.5 mL cell suspension ($2x10^5$ cells/0.75 mg PAMs). The mixture was then gently flushed and plated in 1.9 cm² Costar ultra low cluster plate (#3473, Corning, Avon, France). Plates were

incubated at 37°C during 4 hours to allow cell attachment on PAM surface. PAMs/cell aggregates were recovered, washed with DMEM-F12 and pelleted by centrifugation at 200 g for 2 min. Cell adhesion to PAM surface was assessed by microscopic observation and cells adhered to PAMs were quantified using the Cyquant cell proliferation assay®. A vehicle solution of CMC-Na-Tween 80-Mannitol (final concentration 0.125 %, 0.125 % and 0.5 %, respectively) was added to the aggregates in a final volume of 10 µL just prior to transplantation. To allow cell tracking after transplantation, DI-MIAMI cells were labeled with the membrane dye PKH26 (Sigma) before attachment to PAMs. Briefly, cells were washed with medium after harvesting and resuspended in 0.5 mL diluent C. PKH26 dye diluted in 500 µL diluent C (1 µL PKH26/ 1 million cells) was then added to the cell suspension and carefully mixed before incubation at 37°C for 5 min. Labeling reaction was stopped by washing three time with serum containing medium before proceeding to attachment onto PAMs.

Animals and surgical procedures

All animal experiments were conducted in accordance with the "Direction des Services Vétérinaires", the "Ministère de l'Agriculture" of France and with the European Communities Council Directive of 24 November 1986 (86/609/EEC). A total of 50 female Sprague-Dawley rats, 12 week old and about 250 g in weight, were used in this study. Rats were anaesthetized with xylazine (7.7 mg/kg) and ketamine (41.5 mg/kg) and positioned in a Kopf stereotaxic instrument. Two injections of 10 µg of 6-hydroxydopamine (Sigma, France, in 5 µl saline supplemented with 0.1% ascorbic acid) were performed to induce a unilateral partial progressive and retrograde lesion of the nigrostriatal system, a lesion model previously described [57]. The lesion coordinates were: (1) AP: 0.5, L: -2.5, and V: -5 mm, (2) AP: -0.5, L: -4.2, and V: -5 mm relative to Bregma and ventral from dura, with the tooth-bar set at 0 mm. Only rats that showed more than 7, but less than 18 net ipsiversive turns per minute eleven days after the lesion were used. They were assigned to one of 6 treatment groups: animals receiving intrastriatal injection of vehicle solution only (carboxymethylcellulose (CMC)-Na-Tween 80-Mannitol), DI-MIAMI cells alone, PAMs conveying DI-MIAMI cells (PAMs-NT3 or PAMs-Blank), PAMs-NT3 alone and MIAMI cells only pre-treated with EGF-bFGF, without dopaminergic induction and without PAMs. On the day of transplantation, 2 weeks post-lesion, $1.5x10^5$ cells alone or attached to 0.75 mg of PAMs were stereotactically implanted with a 20 gauges Hamilton microsyringe (with a modified 25° bevel, to allow a better sample aspiration) in the lesioned striatum at the following coordinates: AP+0.5; ML–2.8; DV–5; tooth bar–3.3.

Behavioral study

Amphetamine-induced rotational behavior was measured in an automated rotometer 11 days after the lesion and 2, 4, 6 & 8 weeks after grafting. The animals were weighed prior to each rotation test. All tests were conducted in a blind manner. 5 mg/kg of D-amphetamine (dissolved in NaCl 0.9%) (Sigma, France) was administered intraperitoneally. Animals were individually placed in circular plastic boxes and attached to the rotational leash 15 min before injection for accustoming. Immediately after amphetamine injection, the test began and the data were recorded for 90 min using a computerized system. A net rotational asymmetry score was expressed as full-body turns per minute in the direction ipsilateral to the lesion. Results are presented as the mean rotations of 5-6 rats per group ± average deviation.

Histological study

Eight weeks after cell transplantation, the animals were anaesthetized and perfused through the heart with 150 ml of ice-cold 0.9 % saline, followed by 300 ml of ice-cold 4 % PFA, 2.5 % sucrose (Sigma) in DPBS pH 7.4. Brains were left 1.5 hours in the PFA solution, then transferred to 10 % sucrose solution gradually increased up to 30 % during the next 48 hours. Brains were finally frozen in cold isopentan (-40°C) before storage at -80°C. Striatal and substantia nigra sections, of 14 µm and 30 µm respectively, were made on a CM3050S cryotome (Leica Microsystems).

Immunohistochemistry was used to assess the extent of the striatal lesion using a mouse anti-tyrosine hydroxylase (TH) antibody (1:1000, clone 6D7, Covance, Emeryville, CA, USA). For the detection of DI-MIAMI cells, we used a human specific, mouse anti-mitochondria antibody (1:100, clone MTCO2, Abcam, Paris, France). A mouse anti-CD11b antibody (1:100, clone MRCOX42, Abd Serotec, Cergy Saint-Christophe, France) (specific for macrophage-microglia) and a mouse anti-β3-Tubulin (1:250, clone SDL.3D10, Sigma) were used to assess, respectively, the inflammatory reaction and the presence of neuronal cells. A polyclonal rabbit anti-human TH was also ordered from Eurogentec (AS-DOUB-LX immunization program, Liège, Belgium) after characterization of 2 sequences of peptides specific for human TH. Briefly, rabbits were immunized with both EDVRSPAGP & GTAAPAASYTPTPRS peptides. Serum was harvested 87 days later and used at a 1:250 dilution to detect human MIAMI cells expressing TH 8 weeks after grafting. Sections were first washed with DPBS, 0.1 % Triton X-100 (DPBS-T). For TH staining, quenching of peroxidases was made with 0.3 % H_2O_2 (Sigma) in DPBS-T, at RT for 15 min. After washing, blocking was performed with DPBST, 4 % BSA, 10 % NGS for 2 hours at RT. Sections were incubated overnight at 4 °C with the primary antibodies diluted in DPBS-

T, 4 % BSA. After washing, sections were incubated at RT for 2 h with the secondary biotinylated anti-mouse antibodies (1:200, Vector) diluted in DPBS-T , 4 % BSA. For TH staining, incubation with Vectastain® ABC reagent (Vector) in DPBS was made at RT for 1 h. Sections were washed and revealed with 0.03 % H_2O_2, 0.4 mg/mL diaminobenzidine (DAB) (Sigma) in DPBS (2.5 % nickel chloride was sometimes added to enhance the signal) and dehydrated before mounting. For human mitochondria and anti-CD11b staining, sections were incubated with Streptavidine-Fluoroprobes 488 ® (1:200, Interchim) diluted in DPBS for 40 min at RT before mounting with fluorescent mounting medium. Free floating TH staining of substantia nigra was performed with a similar treatment as for striatal TH staining, apart from the use of a polyclonal rabbit anti-TH (1:20000, Jacques Boy, Reims, France) and of a biotinylated anti-rabbit secondary antibody (1:500, Vector). At 8 weeks, human mitochondria fluorescent staining was used to quantify cell survival using the Metamorph^{TM} software. For each group, an average fluorescent intensity was calculated from 2 pictures taken in the center of the injection site of 3 different rats. The density of TH positive fibres was also quantified using the Metamorph^{TM} software in both ipsilateral and contralateral side of the striatum. The extent of the lesion was estimated by substracting the TH intensity of the contraleral side minus the intensity of the lesioned side. Results are presented as mean differences ± average deviation and were calculated from 3 slides taken from 5 different rats for each group, unless otherwise stated.

Statistical analysis

Data are presented as the mean value of three independent experiments ± standard deviation (SD), unless otherwise stated. Significant differences between samples were determined using a Student's t-test modified for small samples. $t'_0=(m_1-m_2)/\sqrt{(s_1^2/n_1+s_2^2/n_2)}$, differences were considered significant if $|t'_0|> t_{k':0.05}$, with k' being the closer integer of the calculated $k=(s_1^2/n_1+s_2^2/n_2)^2/[(1/(n_1-1)(s_1^2/n_1)^2)+(1/(n_2-1)(s_2^2/n_2)^2)]$. Kruskal-Wallis test was used for multiple comparisons. Threshold P-value was set to 0.05, unless otherwise stated.

RESULTS

MIAMI cells integrin subunit screening

In preliminary experiments we observed a higher number of MIAMI cells if expanded on FN compared to other substrates such as collagen (data not shown). This result, together with the reported neuronal inducing effects of LM, prompted us to screen in MIAMI cells the expression of integrins that may interact with FN or LM. During expansion of MIAMI cells, integrin subunits beta 1, alpha 2, 3, 5, 11 and V were highly expressed when evaluated by RT-qPCR. Subunits beta 3, 4 and alpha 1, 4, 6-9 were also detected but at much lower level (data not shown). The high expression of subunits beta 1, alpha 2, 3 and 5 was confirmed by flow cytometry (figure 1A), thus showing that FN and LM are adequate molecules for the biomimetic surface of PAMs.

In vitro neuronal differentiation on laminin vs. fibronectin

We previously demonstrated [11] that during a neuronal induction protocol performed on a FN substrate, EGF-bFGF pre-treated MIAMI-derived neuronal-like cells showed a diminished proliferation rate, presented long neurites (figure 1B) and acquired an expression pattern consistent with a neuronal differentiation program. Using Trypan blue counting, we observed that differentiating the EGF-bFGF pre-treated cells on a substrate of LM instead of FN led to a further decrease of cell proliferation that was abolished most of the time (0.61 ± 0.06 doublings on FN *vs.* 0.26 ± 0.05 doublings on LM) during *in vitro* neuronal differentiation. Importantly, dead cells were never observed during differentiation on either substrate. In addition to a decreased proliferation rate, total cell length was increased in most experiments at the end of differentiation; with longer neurites when plated on LM compared to glass or FN (figure 1C).

Finally, we also observed by immunocytofluorescence that at the end of the induction protocol MIAMI-derived neuronal-like cells expressed significantly higher levels of β3-Tubulin as well as, to a certain extent, NFM, when plated on LM compared to glass or FN (figure 1D, E). We can note that all these trends were also obtained with MIAMI cells not pre-treated with 20 ng/mL of EGF-bFGF, but to a much lower extent (data not shown).

Characterization of PAM biomimetic surface

Uncoated PLGA microspheres and LM-coated PAMs (40 µg/mL) presented a negative zeta potential (figure 2A), which was not satisfactory for cell adhesion. Conversely, PDL-coated PAMs exhibited a positive zeta potential (49.7 ± 2.1 mV). We therefore used a blend of PDL with LM to combine the benefits of a

positively charged surface promoting cell adhesion and presenting the ECM molecule. The association of LM with PDL slightly decreased the zeta potential value compared to PDL alone. We next tested conditions allowing to optimize the ratio of PDL/LM, the concentration of coating molecules and the incubation time necessary for adsorption of the molecules on the surface. In this way, the ratio of LM was increased to 60 % instead of 40 %, the adsorption time decreased from 4 to 1.5 hours and the total quantity of coating molecules decreased from 40 µg/mL to 15 µg/mL. We next confirmed these optimized parameters did not impair the positive surface charge, which was 34.5 ± 2.6 mV (figure 2A, top right bar), and the homogeneity of the LM surface. Immunofluorescence imaging demonstrated that no background signal was observed on controls and uncoated microspheres (figure 2B, C) whereas the signal was intense and homogeneous all around the PAMs coated with a mixture of PDL/LM at a ratio of 40/60 (figure 2D, E). These optimized conditions were further used throughout this study.

NT3 encapsulation, in vitro release kinetics and bioactivity of the released protein

NT3 loaded microparticles presented a diameter of 57.4 ± 19.0 µm. The yield of the microencapsulation of NT3 was around 100 %, as measured using the NanoOrange® protein quantification kit. The radiolabeling assay demonstrated that around 50 % of the NT3 encapsulated was released in a sustained manner after 22 days, with only a small burst during the first hours. The bioassay performed with MIAMI cells confirmed that the NT3 was released under a bioactive conformation, meaning that the protein remains active through the precipitation/microencapsulation process and throughout the release (figure 2F). Thus, we could estimate that about 75 ng of bioactive NT3 could be released *in vivo* during the first 3 days upon transplantation of 0.75 mg of PAMs, the dose we used in our transplantation experiments.

In vitro characterization of PAMs/DI-MIAMI cell complexes

The LM biomimetic surface allowed the efficient attachment of $1.5x10^5$ MIAMI cells on 0.75 mg of PAMs prior to transplantation, as calculated using the Cyquant cell proliferation assay ®. Importantly, almost all the cells attached to the PAM biomimetic surface after 4 hours at 37°C, and no free floating cells were observed (figure 2G). RT-qPCR demonstrated that gene expression of *Nurr1*, *DAT* and *TH* was not modified upon attachment to PAMs as observed by RT-qPCR. Only low levels of these markers were detected in MIAMI cells, without significant differences upon early dopaminergic induction (data not shown).

Behavioral study

Amphetamine-induced rotational behavior of sham-treated rats continuously increased from the implantation day until the end of the experiment, at 8 weeks. Implantation of DI-MIAMI cells alone did not significantly reduce the number of ipsilateral rotations compared to sham-treated rats (figure 3B). However, transplantation of DI-MIAMI cells adhered onto PAMs-NT3 affected the rotational behavior, which was strongly, and significantly, decreased compared to sham-treated rats or rats implanted with cells alone. In addition, PAMs-Blank resulted in a less important, but still significant, decrease in the animal rotational behavior compared to PAMs-NT3. These observations show a behavioral improvement in rats treated with PAMs/DI-MIAMI cells complexes, while neither improvement nor deterioration was observed in control experiments when grafting PAMs-NT3, but without cells (data not shown).

Protection of nigrostriatal pathway

The rotational behavior was linked to the integrity of the lesioned nigrostriatal pathway, as observed using a rat specific anti-TH immunohistochemistry (figure 4). Only a few TH-positive fibres remained in the striatum of sham-treated rats 8 weeks after the lesion (figure 4A), suggesting that the retrograde neurodegeneration progressed in time concomitantly with the increased rotational behavior. The number of neurons in the ipsilateral substantia nigra (SN) was also importantly reduced compared to the contraletaral side. Transplantation of DI-MIAMI cells in combination with PAMs resulted in a significantly higher density of TH positive fibres in the lesioned striatum (figure 4B, C), therefore demonstrating their neurorepair properties. However, a higher number of dopaminergic neurons were also observed in the ipsilateral SN in 40 % of the animals, suggesting that a neuroprotection of the nigrostriatal pathway occurred in addition to a repair mechanism due to fibre outgrowth. Transplantation of DI-MIAMI cells without PAMs induced only a small, non significant, protection of the striatal dopaminergic fibres compared to sham-treated rats (data not shown).

Cell fate in vivo

In vitro, the cytoplasmic membrane marker PKH26 did not diffuse to surrounding cells during co-culture experiments and was consequently used to track transplanted DI-MIAMI cells *in vivo*. PKH26-positive cells co-localized nicely with the anti-human mitochondria-positive cells in the grafted area, therefore confirming that a significant fraction of cells survived and integrated within the parenchyma 2 months after transplantation (figure 5A). No strong inflammatory reaction was observed with OX42 (CD11b) staining and

only a small fraction of PKH26 dye colocalized with macrophage/microglia, suggesting phagocytosis occurred to a very limited extent (figure 5A). After 8 weeks, and as depicted by figure 5A, a higher number of MIAMI cells was observed by immunostaining upon transplantation with PAMs-NT3. PAMs were also still detected at that time (figure 5B). This increased survival was confirmed by semi-quantification of human mitochondria-positive cells within the striatum after 8 weeks (figure 5C). These data demonstrated that PAMs increased by 2-fold the survival of DI-MIAMI cells, an effect that was even more significant (3-fold) if NT3 was released by the PAMs. This may explain the differential functional recovery observed between PAMs-NT3 and PAMs-Blank. In comparison, DI-MIAMI cells alone poorly survived within the striatum. After 8 weeks, certain cells seemed to express a slight amount of β3-Tubulin in the striatum of rats. Most importantly, a fraction of DI-MIAMI cells expressed TH only if combined to PAMs (figure 6C, D), while this was almost not observed for DI-MIAMI cells alone (figure 6A, B).

EGF-bFGF pre-treated MIAMI cells, without dopaminergic induction

Interestingly, EGF-bFGF pre-treated MIAMI cells transplanted without PAMs survived as poorly as DI-MIAMI cells transplanted alone (figure 7A). However, and in opposition to DI-MIAMI cells alone, these cells did induce on their own a functional recovery and a high density of striatal TH-positive fibres, similarly to that observed upon PAMs/DI-MIAMI cell transplantation (figure 7B, C). This interesting effect of MIAMI cells only pre-treated with EGF-bFGF may be explained by their distinct profile of secretion of growth factors. Indeed, RT-qPCR demonstrated that MIAMI cells always expressed a low amount of the three neurotrophins (*NT3*, *NGF* and *BDNF*, data not shown), while EGF-bFGF pre-treated cells expressed a higher amount of molecules such as *GDNF* and *Stanniocalcin 1* (*STC1*) compared to DI-MIAMI cells, which may be one of the reasons for the benefits observed upon transplantation of EGF-bFGF pre-treated cells without dopaminergic induction. Noteworthy, adhesion onto PAMs tended to increase the expression profile of these molecules by DI-MIAMI cells, as observed by RT-qPCR (figure 7D, E). However, the potential role of these molecules in the observed functional recovery would need to be further examined as part of future studies.

DISCUSSION

Adult cells may be easily isolated from the patient body, in particular from accessible tissues (i.e., blood, skin, bone marrow), therefore permitting autologous grafts to be performed in the clinic without ethical problems. For this reason, as well as for their immunomodulatory and tissue repair capacities, their ability to differentiate into neuronal-like cells and to secrete a variety of molecules, the potential of MSCs to treat neurodegenerative disorders, and especially PD, has been recently investigated [5]. However, the percentage of MSCs that survive and express neural/neuronal markers after transplantation in the brain remains very low [22, 29]. Thus, functional improvements obtained in animal models of PD are thought to primarily derive from the action of growth factors and chemokines produced by transplanted MSCs [27, 28], which also seems to be the case in several other defects [58-60]. Tissue engineering may be of great interest to help repair lesioned tissues/organs due to the possible increase in grafted cell survival, differentiation, or secretory profile induced by the supportive element or scaffold. In this study, we used carriers providing a biomimetic support and the delivery of a growth factor, the PAMs, combined with MIAMI cells to maximize the resulting protective/reparative effects on hemi-parkinsonian rats. We here demonstrated the efficacy of this tool in a rat model of PD, as an important motor function recovery was observed upon grafting with PAMs/DI-MIAMI cell complexes, while no major effects were observed with DI-MIAMI cells alone. This behavioral recovery upon attachment of MIAMI cells to PAMs was correlated with a neuroprotection/repair of the nigrostriatal pathway. These effects were mainly due to the increased survival and engraftment of transplanted cells which secrete a variety of neurotrophic factors and chemokines. Moreover, a fraction of the cells transplanted with the PAMs expressed human TH, strongly suggesting a neuronal dopaminergic phenotype. Therefore, these cells may have produced dopamine, which could have contributed to functional improvement. We thus demonstrate that the PAMs enhance the stem cell repair capacity by improving their survival and differentiation, as we previously observed with PC12 cells and embryonic dopaminergic cells [47, 48].

To improve the known potential of MIAMI cells to differentiate toward a neuronal lineage [16][11], we studied their neuronal differentiation *in vitro* on 2 ECM molecules, FN and LM, for which the cells express a high level of integrin receptors [61]. Indeed, LM is known to induce Nestin expression, a marker of neuronal precursors, in MSCs [43] as well as morphological changes during neuronal differentiation, with a higher number of neurite-like branching [42]. In our hands, MIAMI cells differentiated on a substrate of LM *in vitro* exhibited longer neurite-like extensions together with an improved expression of neuronal proteins (β3-Tubulin and NFM, respectively) compared to FN. Noteworthy, no cell body retraction was observed upon culture on a

LM substrate, therefore confirming that the increased expression of β3-Tubulin and NFM were not artifacts due to cellular shrinkage as it has sometimes been suggested [62, 63]. Moreover, cell proliferation was almost abolished when cells were differentiated on LM. All these observations are commonly related to a normal stem cell differentiation process, thereby suggesting the bioactive signalling role of LM in the induction of MIAMI cells toward a neuronal lineage.

A biomimetic scaffold bearing LM should therefore be advantageous to implement MIAMI cell therapy in a context where an efficient neuronal differentiation is required. PAMs covered with LM alone presented a weak cell adhesion property so we designed PAMs with a surface made of LM blended with the highly charged PDL molecules. Addition of PDL to the LM surface efficiently switched the zeta potential of PAMs toward positive value, without detriment to LM distribution homogeneity around PAMs. From a practical point of view, formulation of PAM surface was optimized for easier implementation within future potential clinical studies, resulting in a shorter (from 4 hours to 1.5 hours) but also less expensive protocol as less protein was used without affecting surface characteristics in terms of zeta potential and homogeneity. Moreover, reducing LM adsorption time prevented an excessive loss of NT3 during formulation as a small burst release was observed during the first hours, as is usually the case from PLGA microspheres [55]. In addition to the use of a LM biomimetic surface, PAMs may further enhance MIAMI cells survival and neuronal differentiation by releasing neurotrophin 3, a factor known to play a role in this process [16]. This neurotrophin was encapsulated with a high efficiency within PAMs (100 % encapsulation yield) and could be released in a prolonged manner under a bioactive conformation, with only a small burst during the first hours. Around 50 % of the encapsulated protein was released after 22 days, suggesting that NT3 could have a long term effect *in vivo.*

As reported earlier, PAMs may improve the integration of PC12 cells and embryonic dopaminergic cells within the brain parenchyma, after transplantation in hemi-parkinsonian rats, by improving their survival and differentiation [47, 48]. An improved differentiation of MSCs toward a chondrogenic phenotype in combination with TGFβ3 PAMs, has also recently been described *in vitro* and *in vivo* [46]. In a similar manner, use of PAMs-NT3 in our study led to a 3 fold increased survival of DI-MIAMI cells 8 weeks after transplantation in hemi-parkinsonian rats, while PAMs-Blank only induced a 2 fold increase in cell survival compared to DI-MIAMI cells grafted alone. This result suggests that bioactive NT3 is being released from the PAMs *in vivo*, which contributes to the survival and differentiation of MIAMI cells and their induced functional recovery. Thus, PAMs could be an easy way to improve the low survival usually observed in long term studies when grafting MSCs in PD rat brains [26-29]. In addition to an increased survival, PAMs induced the

differentiation of a fraction of DI-MIAMI cells, with TH expression observed 8 weeks after transplantation of the complexes, but not in the case of cells transplanted alone. Finally, these complexes successfully promoted a reduction of the amphetamine-induced rotational behavior, while cells alone did not induce a major recovery. A small fraction of TH-expressing human MSCs have already been described by another team in the context of PD [29], and the functional recovery they observed in the context of a total nigrostriatal lesion underlined the ability of human MSCs to produce dopamine *in situ*. Another study described functional improvements obtained by grafting dopaminergic-induced human MSCs in the same animal model of Parkinson's disease [27] as ours, despite an incomplete neuronal engagement *in vitro*, as is the case in our study. Thus, these authors concluded that the functional effects observed should mainly derive from secretion of growth factors, chemokines or cytokines from the transplanted cells, a property they previously observed *in vitro* [64]. This is also certainly the case in our study, although dopamine production by TH expressing cells in presence of PAMs may also have contributed to the functional effect we observed.

In addition to the increased survival/differentiation of grafted cells, the functional effect observed in our study was correlated with a neuroprotection/reparation of the nigrostriatal pathway. This neuroprotection/repair process cannot be observed in studies using total nigrostriatal lesion models [16, 29], in which the functional benefits are mainly derived from the secretion of dopamine by the transplanted cells. A repair of the nigrostriatal fibres has already been reported after GDNF striatal injection in not only pre-clinical but also clinical studies [65], and more recently with GDNF delivering microspheres (without cells) enabling the prolonged delivery of the factor [65-68]. Adhesion of DI-MIAMI cells to PAMs-NT3 induced an increased survival of cells but also resulted in an increased expression of *GDNF* and *Stanniocalcin 1* mRNAs. Therefore, in our study, a repair mechanism based on fibre sprouting and possibly driven by the secretion of growth factors such as GDNF may explain the high density of TH-positive fibres detected in the striatum with only a few TH-positive neurons in the substantia nigra. In comparison to the use of GDNF releasing scaffolds, our strategy advantageously results in the secretion of multiple growth factors and chemokines by surviving cells, with potential long-term effects. Importantly, combination of PAMs with DI-MIAMI cells not only led to repair but also to neuroprotection of the nigrostriatal pathway in 40 % of the animals, with TH-positive fibres and neurons surviving in the striatum and in the substantia nigra after 8 weeks. This protection was also probably due to the secretion of growth factors and chemokines by the transplanted cells. Accordingly, Stanniocalcin 1 may have a role in protecting surrounding cells from apoptosis [69].

In opposition to DI-MIAMI cells, MIAMI cells only pre-treated with EGF-bFGF efficiently promoted a functional recovery, despite a poor survival due to the absence of PAMs. This observation confirms the potential of EGF-bFGF pre-treated cells for brain cell therapy [11] and supports the hypothesis that functional recovery mainly derives from secretion of growth factors, chemokines or cytokines from the transplanted cells. For example, it is now known that EGF-bFGF treatment increase the secretion of BDNF, GDNF and NGF by adipocyte-derived MSCs, an ability supposed to be responsible for the functional recovery the authors observed [30]. In a similar manner, we demonstrated that EGF-bFGF pre-treated MIAMI cells did produce a significantly higher amount of GDNF and Stanniocalcin 1 compared to MIAMI cells that were furthermore differentiated toward the dopaminergic lineage.

CONCLUSION

To conclude, PAMs-NT3 transporting MIAMI cells induced a strong functional recovery in rat models of PD, mainly via an improved survival and differentiation of grafted cells. Moreover, the secretion pattern of relevant neuroprotecting/repairing factors by MIAMI cells was positively modified upon combination with PAMs. These factors, secreted by surviving cells, may be responsible for the neuroprotection/repair of the nigrostriatal pathway observed, while a possible secretion of dopamine by differentiated cells could have also contributed to the functional effects. This adult cell therapy study demonstrates the benefits of biomaterials combining the biomimetic strategy with the controlled delivery of a growth factor to treat PD [5]. After deeper characterization of the underlying mechanisms, this tissue engineering strategy may ultimately set the ground for pre-clinical studies with non-human primates to increase the efficiency of MSC therapy of the brain.

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DISCLOSURE OF INTERESTS

There is no disclosure of interest in this publication.

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