Bone marrow- and adipose-derived stem cells show expression of myelin mRNAs and proteins

Aims: PNS myelin is formed by Schwann cells (SCs). In this study, we applied an in vitro model to study myelin formation, using bone marrow mesenchymal stem cells and adipose-derived stem cells differentiated into SC-like cells and co-cultured with dissociated adult dorsal root ganglia neurons. Methods: Immunocytochemistry, reverse transcription-PCR and western blotting techniques were used to investigate the expression of myelin proteins at both the transcriptional and translational level. Results: Transcripts for protein zero, peripheral myelin protein 22 and myelin basic protein were detected in differentiated stem cells following co-culture with neuronal cells. Furthermore, protein zero, peripheral myelin protein 22 and myelin basic proteins were recognized in the co-cultures. These results were consistent with immunostaining of myelin proteins and with observation by electron microscopy. Conclusion: Both types of adult stem cells differentiated into SC-like cells have potential to myelinate neuronal cells during regeneration, being functionally identical to SCs of the PNS.

KEYWORDS: adipose mesenchymal myelin stem cells

Recent progress has markedly expanded our knowledge of the molecular mechanisms behind the proliferation and differentiation processes of Schwann cells (SCs) – the myelin-forming cells of the PNS. Myelin is composed of four integral membrane glycoproteins. Protein zero (P0), peripheral myelin protein (PMP)22 and myelin basic protein (MBP) are components of compact peripheral nerve myelin [1], whereas myelin-associated glycoprotein (MAG) relates to myelin compaction between the PNS and CNS [2].

Protein zero is a 28 kDa protein confined to the compact portion of mature myelin and is believed to stabilize the intraperiod line of compact PNS myelin by homophilic interactions [3]. PMP22 is a glycoprotein comprising 2–5% of PNS myelin protein [4] and belongs to the tetra-family transmembrane proteins. MBPs include several isoforms of membrane proteins with different molecular weights ranging from 14 to 22 kDa [5,6]. Their function is still unknown, but MBPs are essential for myelin compaction and formation of the major dense line in the PNS [7]. MAG is a 100-kDa transmembrane glycoprotein and a minor constituent of myelin, selectively localized in peri-axonal SCs and in membranes of myelin-sheath oligodendrocytes [8]. The small MAG isoform is responsible for maintaining the integrity of myelinated fibers in the PNS [2].

Schwann cells are essential for nerve regeneration and myelin formation, and we have demonstrated that mesenchymal stem cells (MSCs), isolated from bone marrow [9,10], and adipose-derived stem cells (ADSCs) [11] can differentiate to SC lineage with functional properties and growth factor synthesis activities that are similar to those of SCs [9,12]. It would also be valuable to demonstrate that differentiated stem cells are capable of myelinating the regenerating axons. Previous studies demonstrated that co-cultures of embryonic dorsal root ganglia (DRG) and SCs can reproduce in vitro formation of myelin [13,14]. In this study, co-cultures of adult neuronal cells and differentiated stem cells were used as an in vitro model of axonal growth and regeneration to demonstrate that differentiated MSCs and ADSCs are able to express the myelin proteins found in the PNS. This is of crucial importance if MSCs or ADSCs are to be used in tissue-engineered nerve conduits to promote and enhance peripheral nerve regeneration.

Materials & methods

SC culture
Animal procedures were performed according to the UK Animal Scientific Procedures Act 1986. SCs were harvested from the sciatic nerves of 1–2-day-old neonatal rats, as previously described [15,16]. Briefly, sciatic nerves were collected into chilled Dulbecco’s modified Eagle’s medium (DMEM; Invitrogen, Paisley, UK). The nerve was digested in fresh DMEM containing 0.25% collagenase type I ( Worthington, OH, USA) and 0.8% trypsin (Invitrogen) at 37°C x 15 min. The
medium was aspirated and the process repeated three more times using fresh enzyme solution. Subsequently, cell growth medium (DMEM plus 10% (v/v) fetal calf serum; Invitrogen and 0.1% (v/v) penicillin–streptomycin) was added to neutralize the enzymes and undigested tissue fragments were removed with a 70 µm filter (BD Falcon; Oxford, UK). The suspension was centrifuged at 600 g × 5 min and the medium aspirated from the pellet, which was resuspended in fresh growth medium. The cells were plated on poly-d-lysine (Sigma-Aldrich; Dorset, UK) coated flasks (BD Falcon) and incubated at 37°C, 5% CO₂. The medium was replaced after 24 h with medium containing 100 µM cytosine-β-d-arabinoside (Sigma) to minimize fibroblast contamination. After 3 days, the medium was replaced with medium enriched with 4.1 µg/ml forskolin (Sigma) and 63 ng/ml GGF-2 (Acorda Therapeutics; NY, USA).

**Bone marrow MSCs**

Mesenchymal stem cells were harvested from the long bones of adult rats, as previously described [9]. The marrow was flushed from each bone using 5 ml MSC growth medium (α-minimal essential medium [MEM] containing 10% (v/v) fetal calf serum and 0.1% (v/v) penicillin–streptomycin). The medium was triturated three times through a 21 G needle, passed through a 70 µm filter and the cell suspension centrifuged at 600 g × 5 min. The supernatant was aspirated; the cell pellet resuspended in MSC growth medium, plated on 75 cm² culture flask and incubated at 37°C, 5% CO₂. Nonadherent cells were removed by daily washes with α-MEM to leave adherent bone marrow MSCs, which were grown to confluence.

**ADSCs**

Adipose-derived stem cells were isolated from adult rats, as previously described [11]. Visceral fat was enzymatically dissociated at 37°C × 60 min using 0.15% (w/v) collagenase type I (Invitrogen). The solution was passed through a 70 µm filter and the cell suspension centrifuged at 600 g × 5 min. The supernatant was aspirated; the stromal cell pellet was resuspended in MSC growth medium, plated on poly-d-lysine (Sigma-Aldrich; Dorset, UK) coated flasks (BD Falcon) and incubated at 37°C, 5% CO₂. The medium was replaced with growth factors (as previously described) or differentiated ADSCs (dADSCs), which were directly seeded over the neurons. Culture medium comprising 50% of either stem cell differentiation medium or SC medium enriched with growth factors (as previously described) plus 50% BS medium was added and, after 24 h, 100 µg/ml brain-derived neurotrophic factor

**DRG neurons**

Dorsal root ganglia were harvested from adult rats, as previously described [9,17]. DRG were dissociated in 0.125% (w/v) collagenase type IV (Worthington) in F12 medium (Invitrogen) for 1–2 h and then incubated in 0.25% trypsin (w/v) (Worthington) in F12 medium for 30 min. Trypsin was inactivated by the addition of 30% (v/v) fetal bovine serum (Invitrogen) in F12 medium, then the DRG were washed three times in F12 medium to remove all traces of serum. DRG were mechanically dissociated by gentle trituration using a glass pipette, passed through a 70 µm filter (BD Falcon) and centrifuged (200 g × 10 min). These steps were repeated to ensure the DRG were fully dissociated. Cells were resuspended in F12 medium and centrifuged (600 g × 10 min) with 15% (w/v) bovine serum albumin. The cells’ debris supernatant was removed, leaving the bolus of dissociated neurons. The neurons were resuspended in 1 ml modified Bottenstein and Sato’s medium (BS medium) containing 0.1 mg/ml transferrin, 20 nM progesterone, 100 µM putrescine, 30 nM sodium selenite, 1 mg/ml bovine serum albumin, 0.01 mM cytosine arabinoside and 10 µM insulin (all Sigma-Aldrich). Finally, the cells were suspended in 12 ml BS medium. Neurons were plated on six-well plates (BD Falcon) precoated with poly-d-ornithine (Sigma) and laminin-1 (2 µg/ml, Sigma-Aldrich). After 2 h, 150 ng/ml NGF (Sigma) was added to allow neurons to develop neurites for 3 days.

After 3 days of culture, neurons were co-cultured with SCs, differentiated MSCs (dMSCs) or differentiated ADSCs (dADSCs), which were directly seeded over the neurons. Culture medium comprising 50% of either stem cell differentiation medium or SC medium enriched with growth factors (as previously described) plus 50% BS medium was added and, after 24 h, 100 µg/ml brain-derived neurotrophic factor
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(BDNF; Autogen Bioclear; Wiltshire, UK) and 50 µg/ml ascorbate (Sigma) were also added and left to activate the myelination process for 14 days, at which point either RNA or protein was extracted from the cells.

**Reverse transcription-PCR analysis**

Total RNA was extracted from cells or the sciatic nerve (positive control) according to the manufacturers’ protocol (Qiagen RNeasy; Crawley, UK) and RNA concentration was determined by UV spectrophotometry at 260 nm. Oligonucleotide primers (Invitrogen) were used to amplify the transcripts of P0, MBP, PMP22 and the housekeeping gene transcript β-actin (Table 1). Optimum annealing temperature for each primer pair was determined experimentally. A master mix was prepared in RNase-free water to a final concentration of 1× Qiagen OneStep reverse transcription (RT)-PCR buffer, 400 µM each deoxyribonucleotide triphosphate, 0.6 µM forward primer, 0.6 µM reverse primer, 5–10 units Qiagen OneStep enzyme mix and 1 ng template RNA or RNase-free water (negative control).

MJ Research PTC-200 (MJ Research Inc., MA, USA) thermal cycler was used for all reactions. Cycling parameters were as follow: a reverse transcription step (50°C × 30 min), a nucleic acid denaturation/reverse transcriptase inactivation step (95°C × 15 min) followed by 35 cycles of denaturation (95°C × 30 s), annealing (65°C × 30 s) and primer extension (72°C × 1 min), followed by final extension incubation (72°C × 5 min). Qualitative assessment of quantity and linear size of amplicons was determined by gel electrophoresis through 2% (w/v) agarose stained with 0.5 mg/ml ethidium bromide (Sigma); images were captured using AlphaImager 2200 (Alpha Innotech, CA, USA) gel documentation system with UV trans-illumination. For sequencing the amplicons were isolated and purified using the QIAquick gel extraction protocol (Qiagen) according to manufacturer’s protocol. Briefly, under UV trans-illumination, individual DNA bands were cut out from the gel, purified and the amplicons were eluted into water. DNA sequence of each amplicon was confirmed using Big Dye™ Terminator sequencing kit protocol (Applied BioSystems Inc., CA, USA) followed by sequence analysis on Prism 3100 Genetic Analyzer (Applied Biosystems Inc.).

**Western blotting**

Whole cell lysates were prepared from confluent cells that were scraped with 150 µl lysis buffer (100 mM PIPES, 5 mM MgCl, 20% (v/v) glycerol, 0.5% (v/v) Triton-X, 5 mM EGTA per well, with 0.005% (v/v) protease inhibitors [Sigma]). Lysates were collected in microfuge tubes, left for 15 min on ice then centrifuged at 600 g × 5 min. Protein concentration of the supernatant was determined using the DC Protein Assay (BioRad, CA, USA). Samples were diluted in Laemmli buffer and heated to 95°C × 5 min. Equal amounts of protein (10 µg/well) were loaded onto 15% (w/v) SDS-polyacrylamide gels and electrophoresed at 120 V × 1.5 h. Separated proteins were transferred to Hybond ECL™ nitrocellulose membranes (GE Healthcare, Buckinghamshire, UK) at 80 V × 90 min. The membranes were gently agitated for 2 h in blocking buffer containing 5% (w/v) nonfat milk powder in TBS Tween® (10 mM Tris pH 7.5, 100 mM NaCl, 0.1% (v/v) Tween-20 [Sigma]) and incubated with primary antibody (anti-MBP mouse monoclonal, 1:1000 [Serotec, Kidlington, UK]; anti-MAG mouse monoclonal, 1:1000 [Chemicon, Hampshire, UK]; anti-PMP22 goat polyclonal, 1:500 [Santa-Cruz Biotech., UK]; anti-P0 goat polyclonal, 1:500 [Santa-Cruz Biotech.]) overnight at 4°C. Following incubation with primary antibodies, eight × 5 min washes were performed using TBS-Tween. The membranes were incubated for 1 h with horse radish peroxidase-conjugated secondary

<table>
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<th>Rat primers</th>
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<td>Myelin basic protein</td>
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<td>K00512</td>
<td>[26]</td>
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<td>GGACACATCGGCGCTG</td>
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All sequences are shown in 5’ to 3’ orientation and supplied by Invitrogen (Paisley, UK).
antibodies (horse anti-mouse [Sigma] for MBP and MAG and horse anti-goat 1:1000 [Sigma] for P0 and PMP22). After a further eight washes with TBS-Tween, the membranes were treated with ECL chemilluminescent substrate (Amersham Biosciences, Buckinghamshire, UK) for 1 min and exposed to Kodak X-OMAT light sensitive film (Sigma).

**Immunocytochemistry**

Dorsal root ganglia neuron/stem cell co-cultures were established and maintained for 2 weeks on slide flasks with the same type of medium described previously, and then were fixed in 4% (w/v) paraformaldehyde at room temperature for 30 min. The samples were blocked for 1 h in normal rabbit serum (1:100 [Dako; Cambridge, UK]) and normal goat serum (1:100, Sigma) and incubated overnight (4°C) with primary antibodies for P0 (1:50, goat polyclonal [Santa Cruz; CA, USA]) and β-tubulin (1:500, mouse monoclonal [Abcam; Cambridge, UK]). DRG–dADSC co-culture were also blocked in normal goat serum (1:100) and normal donkey serum (1:100, Sigma) and incubated overnight (4°C) with primary antibodies for S100 (1:500, rabbit polyclonal) and β-tubulin (1:500, mouse monoclonal).

The cells were then incubated for 2 h with secondary antibodies; fluorescein isothiocyanate-conjugated rabbit anti-goat (1:100 [Vector Laboratories; Peterborough, UK]) for P0, fluorescein isothiocyanate-conjugated goat anti-rabbit (1:100) for S100 and Cy3-conjugated mouse anti-goat (1:200; GE Healthcare) for β-tubulin. The slides were mounted and examined under a fluorescence microscope (Olympus BX60).

**Scanning-electron microscopy analysis**

For scanning-electron microscopy (SEM) studies, DRG co-cultures with SCs, dMSCs or dADSCs were established as described previously and kept in culture for 2 weeks. The samples were then fixed with 2.5% (v/v) glutaraldehyde (Sigma) in phosphate buffer (0.1 M, pH 7.4 at 4°C × 30 min). Subsequently, the samples were dehydrated in a graded series of ethanol solutions (50, 75 and 100%) and coated with silver particles prior to examination with a scanning-electron microscope (SEM Philips 52M).

**Results**

**Myelin protein transcripts**

Reverse transcription-PCR showed transcripts for P0 (190 bp amplicon), MBP (230 and 306 bp amplicons) and PMP22 (636 bp amplicon) in both DRG–dMSC and DRG–dADSC co-cultures, and in the DRG–SC co-culture (positive control) (Figure 1). RT-PCR amplification efficacy of mRNA was confirmed by amplification of the β-actin (510 bp amplicon) housekeeping gene transcript. Individual cultures of DRG, SCs, dMSCs and dADSCs alone were also analyzed. SCs expressed the transcript for P0, whereas DRG, dMSCs and dADSCs did not (Figure 1). Furthermore, DRG neurons cultured separately (negative control), but using the same conditioned medium as for co-cultures, did not express transcripts for any myelin proteins. SCs, dMSCs and dADSCs all showed a positive band for the PMP22 transcript. MBP transcripts were present in isolated cultures of dMSCs and SCs, but not in dADSC cultures alone. MBP transcript was always present as a double band. Sequencing of the two MBP bands confirmed that they originate from two splice variants Genbank NM_001025291.1 (variant 1) and NM_001025292.1 (variant 2); the sequencing of both amplicons confirmed that they were alternatively spliced MBP transcripts. Taken together,
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**Protein expression**

Western blotting using P0-, PMP22- and MBP-specific antibodies showed that all co-cultures expressed myelin protein P0 (28 kDa), PMP22 (22 kDa) and MBP (22 kDa) (Figure 2), consistent with the results of the RT-PCR. The addition of ascorbate to the culture medium did not influence myelin expression at either the transcript or protein level (data not shown). Isolated DRG neuron cultures alone did not express significant levels of myelin proteins (Figure 2), which taken together with the RT-PCR results, confirms that dissociated neuronal cells did not display any contamination with satellite cells, which might have given rise to spurious results. Isolated SCs, dMSCs and dADSCs expressed the proteins P0, PMP22 and MBP (Figure 2). MBP was present as a single band in western blotting of co-cultures, isolated SCs and stem cells, possibly indicating the translation of only one splice variant of the transcript.

**Morphological Investigations**

Immunostaining for neuronal and glial markers showed the close relationship between neurons and differentiated stem cells in co-culture (Figure 3B). The results demonstrated close alignment of S100-positive dADSCs along neurites extending from neuronal cell bodies after 2 weeks in co-culture. Consistently, immunostaining for P0 of dMSCs and dADSCs showed positive cells closely associated with the neurites (Figure 3A). Such alignment was also corroborated by the SEM result, which showed dMSCs closely aligned to DRG neurites (Figure 4). The SEM results also confirmed that the size and shape of the differentiated stem cells was consistent with those of SCs. The alignment of dADSCs along the neurites was similar to the results seen with DRG–SC co-cultures (data not shown), reflecting the spatial relationship between the two cell types in vivo. These results are further evidence of the functional similarity between SCs and differentiated stem cells.

**Discussion**

This study presents good evidence that dMSCs and dADSCs, co-cultured with DRG neurons, upregulate the myelin transcripts characteristic of the myelin produced by SCs in peripheral nerves. The results indicate that dMSCs and dADSCs, co-cultured with DRG neurons, upregulate the myelin transcripts characteristic of the myelin produced by SCs in peripheral nerves.

In our co-culture model, we recreated the type of environment that stimulates SCs to produce myelin whilst they are in contact with neuronal cells. Using this model we have been able to demonstrate the expression of the major myelin proteins, P0, MBP and PMP22 by dMSCs and dADSCs. We have established that when adult differentiated stem cells are co-cultured with adult DRG neurons they are able to express transcripts and proteins of the peripheral nerve myelin. Although electron microscopy showed the alignment of differentiated stem cells along the neurites, we were unable to demonstrate the formation of myelin around the neurites. The lack of myelin formation is probably due to the short time that these co-cultures can be kept; this short co-culture period allows expression of myelin protein, but it is not sufficient for the glial cell to fully envelope the neurites and develop myelin sheets. Clearly, as the bone marrow- and adipose-derived stem cells are of different origins, they present substantial differences; however, both types of stem cell can be differentiated into SC-like cells and, once differentiated, demonstrate many similarities to SCs at both the gene transcription and protein expression level. Myelin formation was demonstrated in the PC12 neuronal cell line and the dMSC co-cultures at 2 weeks [18], but myelin formation was highly dependent on culture conditions. Furthermore, the use of neuronal cells derived from a tumor and the use of dissimilar differentiation and culture medium makes the
comparison with our results difficult. In addition, expression of different myelin proteins was not examined.

Myelin is a complex structure produced by SCs in the PNS and its development is strictly correlated with the interactions of SCs and axons. In a DRG–SC co-culture model, it was shown that neurotrophins enhance myelin formation [13] with NGF and BDNF being positive modulators of myelination during embryogenesis. Similarly, in our study, the addition of NGF and BDNF was essential to stimulate neurite extension and myelin protein expression, and this was confirmed by experiments where omission of either or both of these neurotrophins resulted in a negative outcome (results not shown). Recently, it was speculated that embryonic DRG neurons could induce phenotypic transdifferentiation of MSCs into SC-like morphology and stimulate the expression of antigens, such as S100 [14], but the co-culture of these cell types was not sufficient to elicit myelin expression in vitro.

In vitro, the myelination process is based on the strict association between myelinating SCs and axons involving different reactions, including formation of basal lamina. Previous in vitro studies demonstrated that the ability of SCs to assemble basal lamina and proceed to myelination is dependent upon culture conditions, including the requirement for ascorbate [19]. Varying ascorbic acid concentrations affected MSC differentiation, proliferation, cytotoxicity and extracellular matrix production, and ascorbate is a potent stimulator of MSC proliferation, without reducing their differentiation capacity [20].

Protein zero is the most abundant protein expressed by myelinating SCs in the PNS, but a low basal level of P0 expression in SCs and neuronal crest cells is seen in early embryonic development [21]. The development of myelin implies an active interaction between neuronal and glial cells. Consistently, our RT-PCR data demonstrate that dMSCs and dADSCs do not express P0 transcripts when cultured in the absence of neuronal contact, while immunocytochemical staining of the DRG–dMSC co-cultures provided evidence that contact is a fundamental requirement to stimulate expression of P0 protein. The upregulation of P0 by SCs during myelination appears to involve, as yet, unidentified signals from the axon, interactions with the basal lamina and elevated levels of intracellular cAMP [22]. In our study, we have shown that PMP22 is also expressed by dMSCs and dADSCs. This supports the hypothesis that expression of P0 and PMP22 is linked during the formation of the PNS myelin. The PMP22 protein appears to play a role both in formation and maintenance of myelin [23].

Myelin basic protein is reported to be essential for myelin compaction and formation of the myelin dense line [7], and in the CNS, there is a strict correlation between the expression level of MBP and myelin thickness [24]. However, MBP absence does not prevent the formation of compact myelin in the PNS as this absence seems to be compensated by another myelin protein, possibly P0. It has been observed that P0 and MBP can play interchangeable roles during myelination formation in the dense line [25]. The results of our study demonstrated expression of two isoforms of the MBP transcript whereas western blot showed only one protein to be present. Following sequencing of the two transcripts, we concluded that only one isoform is expressed. The disparity between the number of MBP isoform transcripts and the number of detectable MBP proteins remains to be clarified.

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**Figure 3. Immunocytochemical staining to show differentiated mesenchymal stem cell and differentiated adipose-derivated stem cell morphology and their close association with dorsal root ganglia neurites in co-culture. (A)** Dorsal root ganglia (DRG) differentiated mesenchymal stem cell co-culture immunostained for protein zero (P0; fluorescein isothiocyanate; green) and βIII-tubulin (Cy3; red). Only a proportion of differentiated mesenchymal stem cells are P0 positive, possibly owing to a lack of contact between some of the two cell types, which is necessary for P0 expression. Merger of the two colours (yellow) shows the overlapping of the two structures. **(B)** Co-culture of DRG–differentiated adipose-derivated stem cells (dADSCs) immunostained with fluorescein isothiocyanate-conjugated anti-S100 (green) and Cy3-conjugated anti-βIII-tubulin (red) antibodies. dADSCs show typical Schwann cell-like spindle shape and positivity for S100 glial marker. Of particular note is the alignment of the dADSCs along the neurites; contact between the DRG and dADSCs is fundamental for the myelin formation (arrows).
but the strong expression of P0 might be indicative of a compensatory effect for the absence of one of the two isoforms of MBP.

These results confirm that adult dMSCs and dADSCs can transcribe and translate myelin protein in a similar manner to SCs. This co-culture strategy will be a useful tool to further investigate the myelination of differentiated stem cells under controlled micro-environmental conditions in order to understand the mechanisms by which these cells might contribute to peripheral nerve regeneration. These results clearly indicate a functional similarity of differentiated adult stem cells to mature SCs. This will allow the development of adult stem cell application in bioengineered nerve repair in the near future, and it also opens up the possibility for the application of this technology to spinal cord repair in the future.

Financial & competing interests disclosure
The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research
The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary
- Schwann cells have been shown to improve nerve regeneration, but this approach is limited by the sourcing of autologous cells.
- Myelin in the PNS is formed by Schwann cells, which wrap around one axon by a dynamic process of extension and spiraling of the cell plasma membranes.
- To avoid these problems attention has been turned towards stem cell transplantation and, in particular, adult mesenchymal stem cells (MSCs) and adipocyte-derived stem cells (ADSCs).
- In this study, we have used an in vitro model of myelination formation involving differentiated stem cell–neuronal interactions. MSCs or ADSCs were cultured with adult dorsal root ganglia (DRG) neurons in direct contact.
- Myelin formation was assessed using reverse transcription-PCR and western blotting. Immunocytochemistry staining and scanning-electron microscopy analysis were also used to assess the influence of stem cells on neurite outgrowth.
- The goal of this study was to develop an in vitro co-culture system comprising DRG–differentiated MSCs and DRG–differentiated ADSCs that closely resemble the peripheral nerve regeneration process in vivo in order to study, in a controlled microenvironment, all the variables that modulate the outgrowth of neurites and result in their myelination.

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