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Nanofibers Support Oligodendrocyte Precursor Cell Growth and Function as a Neuron-Free Model for Myelination Study

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Abstract

Nanofiber-based scaffolds may simultaneously provide immediate contact guidance for neural regeneration and act as a vehicle for therapeutic cell delivery to enhance axonal myelination. Additionally, nanofibers can serve as a neuron-free model to study myelination of oligodendrocytes. In this study, we fabricated nanofibers using a polycaprolactone and gelatin co-polymer. The ratio of the gelatin component in the fibers was confirmed by energy dispersive x-ray spectroscopy. The addition of gelatin to the polycaprolactone (PCL) for nanofiber fabrication decreased the contact angle of the electrospun fibers. We showed that both polycaprolactone nanofibers as well as polycaprolactone and gelatin co-polymer nanofibers can support oligodendrocyte precursor cell (OPC) growth and differentiation. OPCs maintained their phenotype and viability on nanofibers and were induced to differentiate into oligodendrocytes. The differentiated oligodendrocytes extend their processes along the nanofibers and ensheathed the nanofibers. Oligodendrocytes formed significantly more myelinated segments on the PCL and gelatin co3polymer nanofibers than those on PCL nanofibers alone.

Keywords

Oligodendrocyte precursor cells; nanofibers; myelination; differentiation

INTRODUCTION

The repair of an injured spinal cord presents a significant medical challenge. The development of biomaterial constructs has offered a promising solution for the treatment of wounded neural tissue. In neural regeneration studies of animal models, the transected or partially wounded spinal cord has been reconstructed by bridging the gap with neural conduits or filling the defect with hydrogels.[1–3] The contact mediated guidance provided by

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Author Contributions

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biomaterial scaffolds may steer axonal regrowth across the site of injury into the distal host tissue and could potentially result in functional recovery.

Oligodendrocytes (OLs) undergo both necrosis and apoptosis shortly after spinal cord injury (SCI). In response to demyelination, oligodendrocyte precursor cells (OPCs) are recruited from gray and white matter and migrate to the lesion to myelinate the regenerated axon; however, this process is limited by the number of endogenous OPCs.[4, 5] Focal delivery of OPCs provides an alternative approach for axonal myelination. A biomaterial scaffold can simultaneously mediate the contact guidance of neural tissue growth and act as a vehicle for therapeutic cell delivery to enhance axonal myelination.[6–9] The nanofibers and continuous porous structure generated by electrospinning may enhance neural regeneration because the nanofibers mimic the extracellular matrix and provide guidance for axonal growth at nano-levels. Previous studies have shown that electrospun aligned fibers can guide neurite growth in vitro[10–12] and can be applied in spinal cord and peripheral nerve regeneration.[13–16] Polycaprolactone (PCL) has been fabricated as a biomaterial scaffold for neural regeneration because of its biodegradable and biocompatible properties.[15, 16] PCL nanofibers can be generated by electrospinning, and their biocompatibility can be enhanced by adding extracellular protein to the electrospun fibers. Gelatin, a heterogeneous mixture of polypeptides formed by thermal denaturation of collagen, maintains the biological property of collagen and together with PCL has been reported to generate electrospun fibers.[17] However, whether PCL nanofibers can support oligodendrocyte precursor cells has not been studied previously.

Myelination is a critical step in neural regeneration. The axonal signaling to oligodendrocytes and the intimate interaction between axons and oligodendrocytes are complicated processes that direct myelination formation of the regenerated axons.[18–20] The co-culture of dorsal root ganglion (DRG) neurons and oligodendrocyte precursor cells is the general model for myelination studies. The neurotrophic factor is required for the survival of embryonic DRG neurons in the cell culture. [21,22] However, nerve growth factor (NGF) in the cell culture medium can negatively affect the myelination function of oligodendrocytes to the axons.[18,19] Additionally, the cell culture medium containing fetal bovine serum can significantly increase the non-specific cells in the co-culture of DRGs and OPCs, which can make the analysis of myelination complicated.[19] It was recently revealed that oligodendrocytes can myelinate electrospun fibers of synthetic polymers, and these fibers can serve as a simplified neuron-free model for myelination study,[23, 24] We hypothesize that the function of a particular biological molecule in the myelination process can be studied by incorporating it into the synthesized fibers. The OPCs will interact with nanofibers in a relatively isolated environment using the optimal cell culture medium targeting myelination process. Such a neuron-free model will allow the myelination study to focus on the interaction of particular biological molecules and the OPCs. In this study, we fabricated PCL nanofibers and PCL and gelatin co-polymer nanofibers. First, we studied the growth and differentiation of OPCs on both electrospun PCL nanofibers as well as PCL and gelatin co-polymer nanofibers. Second, we investigated the function of gelatin as a biological molecule on oligodendrocyte myelination for the nanofibers. Our work was the first trial to study the effect of biological molecules in the nanofiber on OPC myelination and this study provided new insight for the investigation of biological molecules in axonal myelination.

MATERIALS AND METHODS

Generation of PCL and Gelatin Electrospun Nanofibers

PCL (molecular weight of 70,000, Scientific Polymer Products, Inc., New York) nanofibers were generated by electrospinning a PCL solution (35 wt% in acetonitrile). To fabricate the PCL-gelatin nanofibers, gelatin (Sigma-Aldrich, St. Louis, MO) and PCL (mixing ratio: 50

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wt% of gelatin to PCL) in a mixture of acetic acid and acetonitrile (50:50 (v/v)) were used to generate fibers by electrospinning. The solvent-to-solute ratio for the mixtures was 85:15 by weight. Fibers were collected on a stationary collector placed 20–30 cm from the infusion syringe at an infusion speed of 2 ml/hour at 20–25kV. To study the growth and differentiation of the OPCs, films of dense fibers were electrospun onto aluminum foil or round glass coverslips. Loose nanofibers were also fabricated on coverslips to study myelination of the differentiated OPCs to the fibers.

Contact Angle of Nanofibers

The contact angle values of the electrospun nanofibers were measured with an optical contact angle goniometer (CAM 100, KSV Instruments Ltd., Helsinki, Finland). This compact video-based instrument measures contact angles between 1° and 180° with an accuracy of ±1°. Computer software provided by KSV Instruments Ltd. precisely recorded and measured the contact angles, and also took pictures of the measured contact angle values on the surfaces of the nanofibers.

Isolation and Culture of OPCs on Electrospun Nanofibers

The procedure for isolating the OPCs from neonatal rats was approved by the Institutional Animal Care and Use Committee (IACUC) and completed at Wichita State University, Wichita, KS. The culturing of OPC cells was performed as reported previously.[25] In brief, cerebral cortexes were isolated from the brains of neonatal rats (postnatal day P1-2 rats) after they were sacrificed. The cortex tissues were triturated gently through a 5 ml syringe with needle. The tissue suspension was passed through a 70-mm nylon cell strainer (BD Falcon™, Durham, NC), and the flow-through was collected with a 50-ml conical tube. The isolated cells were cultured for about 7 days. The OPCs were then isolated from the mixed cell culture layer by mechanically shaking the cell culture flasks for about 24 h in an incubator at 37°C. The collected OPCs were cultured in an OPC growth medium (DMEM, Lifetechnologies™, Grand Island, NY) with Sato media (DMEM, 100 μg/ml transferrin, 100 μg/ml BSA, 0.2 g=mM progesterone, 16 μg/ml putrescine, 40 ng/ml sodium selenite [Sigma-Aldrich, St. Louis, MO]), 1% penicillin-streptomycin, 2 mM L-glutamine (Lifetechnologies™, Grand Island, NY), 5 μg/ml insulin, 10 nM D-biotin, 1 mM sodium pyruvate, 5 g=mg/ml N-acetyl cysteine (Sigma-Aldrich, St. Louis, MO), trace elements B (1×, Mediatech Inc., Manassas, VA), 10 ng/ml PDGF, and 10 ng/ml bFGF (Peprotech, Rocky Hill, NJ). Then the OPCs were passaged or grown on the electrospun nanofibers.

Growth of OPCs on Electrospun Nanofibers and Cell Viability Assay

To study their growth and differentiation on nanofibers, OPCs were grown in cell culture wells or the films of dense electrospun nanofibers placed in 24-well plates with a cell density of 15,000 cells/well. The cells were either cultured in OPC growth medium or oligodendrocyte growth medium (OL medium) for OPC differentiation (DMEM with Sato media, 1% penicillin-streptomycin, 2 mM L3 glutamine, 5 μg/ml insulin, 10 nM D-biotin, 1 mM sodium pyruvate, 5 μg/ml N-acetyl cysteine, trace elements B, 15 nM triiodothyronine, and 10 ng/ml CNTF (Peprotech, Rocky Hill, NJ)). After 4 days, the cells were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) solution for the immunostaining assay.

The viability and proliferation of OPCs, which were seeded on the films of dense electrospun nanofibers, were studied by monitoring their metabolic activity using the alamarBlue assay (Pierce Biotechnology, Rockford, lL). To perform this assay, the films of dense electrospun nanofibers were cut into round shapes and placed in 24-well plates to cover the bottom of the wells. The cells, with a density of 25,000 cells/well, were cultured for 4 days. Then they were incubated with OPC culture medium containing 10% (v/v)

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alamarBlue reagent for 2 h. Absorbance was measured at a wavelength of 570 nm and 600 nm nm in a microplate reader (Synergy Mx Monochromator-Based Multi-Mode Microplate Reader, Winooski, VT).

Myelination Study of Oligodendrocytes for Nanofibers

To study the myelination of OPCs for electrospun fibers, coverslips with PCL or PCL-gelatin nanofibers were placed in the 24-well plates for the culture of OPCs. The OPCs (20,000 cells/well) were seeded on these coverslips for 3 or 8 days. The medium for the myelination study was reported previously.[24, 25] In brief, the myelination medium consists of DMEM containing 1× B27, 1× N2 (Lifetechnologies™, Grand Island, NY), 5 μg/ml of N-acetyl cysteine (NAC, Sigma-Aldrich, St. Louis, MO), and 5 μM of forskolin (Minneapolis, MN). The cell culture medium was changed every 3 days. After 3 or 8 days of culturing, the cells cultured on the fibers were fixed with 4% paraformaldehyde in a PBS solution for immunostaining.

The myelination segment of oligodendrocytes for the nanofibers was quantified by counting the number of anti-MBP antibody labeled segments. The myelination segment was defined as the complete ensheathment of a fiber with a length greater than 30 μm. [24] The percentage of wrapping cells was quantified as the ratio of the number of cells that formed myelination segment to the total number of MBP positive cells. The myelination segment and wrapping cells were quantified by analyzing images taken from at least three experiments.

Immunocytochemistry

The phenotype of the cultured OPCs was determined with anti-A2B5 antibody (generated in Dr Q. Richard Lu's lab, Cincinnati Children's Medical Center) and anti-PDGFR3-α antibody (Santa Cruz Biotechnology, Inc., Dallas, Texas). The differentiated OPCs were labeled with anti-O4 antibody (Dr Q. Richard Lu's lab, Cincinnati Children's Medical Center). The myelination of OPCs for the nanofibers was labeled with anti-myelin basic protein (MBP) antibody (Millipore, Billerica, MA). Images were taken with a Zeiss Axio Observer microscope (Carl Zeiss Microscopy, LLC, Thornwood, NY).

Scanning Electron Microscopy (SEM) for Nanofibers

The images of the nanofibers were taken by scanning electron microscopy (SEM) with a model of ZEISS SIGMA VP and energy dispersive spectroscopy (EDS) (Carl Zeiss Microscopy, LLC, Thornwood, NY). These images were used to measure the fiber diameter using NIH ImageJ software (National Institutes of Health, Bethesda, MD). To quantify the fiber diameter, 150 fibers from each type of fiber were measured. The nanofibers with oligodendrocytes were washed with PBS and placed in 2% glutaraldehyde-PBS for 2 h. The samples were then dehydrated with graded ethanol and dried with hexamethyldisilazane. The scaffolds were air-dried and subsequently coated with gold. Then the oligodendrocyte morphology and ensheathed nanofibers were analyzed using the SEM images.

Energy Dispersive X-Ray Spectroscopy for Nanofibers

EDS was used to analyze the composition of the nanofibers. An electron beam that scans across the surface during SEM causes shell transitions that result in the emission of an x-ray. Detection and measurement of the energy from the emitted x-ray permit qualitative and quantitative elemental analyses. Gelatin is a heterogeneous mixture of polypeptides formed by thermal denaturation of collagen. These polypeptides primarily contain glycine, proline, and 4-hydroxyproline residues. Polycaprolactone is a biodegradable polyester with the molecular formula $C_6H_{10}O_2$. PCL contains more carbon per oxygen than gelatin. An increase of PCL in nanofibers would result in an increase in carbon.

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Statistical Analysis

Statistical analysis was conducted using a two-tailed Student's t-test. A p value of 0.05 was considered to be statistically significant. Data are expressed as means ± standard deviation.

RESULTS

Characterization of Electrospun Fibers of PCL and Gelatin Co-Polymer

SEM images of films of dense PCL fibers and PCL-gelatin fibers are shown in Figure 1A and B. The fiber diameter for the PCL fibers and PCL-gelatin fibers are 163.5 ± 126.8 nm and 214.9 ± 58.7 nm, respectively (Figure 1C). EDS assay was used to analyze the composition of the PCL-gelatin nanofibers. Carbon is the major element in both gelatin and PCL. The carbon atomic ratio and the carbon weight ratio of gelatin fibers, PCL fibers, and PCL-gelatin fibers were measured. The carbon atomic ratio and carbon weight ratio of PCL-gelatin fibers are 60.2 ± 0.9% and 54 2 ± 0.7%, respectively, which are close to the mean ratio of PCL fibers (69.7 ± 1.0% and 59.7 ± 1.1%, respectively) and gelatin fibers (50.0 ± 3.5% and 38.4 ± 2.3%, respectively) (Figure 1D).

The contact angles of the fiber surface were measured to show the hydrophilicity of the fibers. Gelatin in the electrospun fibers decreased the surface contact angle. The contact angles for the PCL fibers, PCL-gelatin fibers, and gelatin fibers are 133.5° ± 2.3°, 78.6° ± 3.1°, and 63.3° ± 2.2°, respectively. The decrease in contact angle indicates the increase of hydrophilicity of the electrospun fibers.

Electrospun Nanofibers Support OPCs Growth and Differentiation

OPCs were seeded on the films of dense nanofibers and cultured with OPC culture medium for 4 days. The OPCs grown on nanofibers were labeled with anti-A2B5 and anti-PDGFα antibodies. Immuno-labeling showed that the OPCs grown on both PCL nanofibers and PCL-gelatin nanofibers expressed A2B5 and PDGFRα (Figure 3). OPCs were also seeded on the films of dense nanofibers and cultured with OL medium for 4 days. Immnostaining with anti-O4 antibody on the nanofibers showed that OPCs differentiated into oligodendrocytes and developed multiple processes (Figure 3F, J).

The alamarBlue assay showed that OPCs proliferated on both films of dense PCL nanofibers and PCL-gelatin nanofibers; no significant difference in OPC proliferation was detected. The reduction of alamarBlue reagent for OPCs grown on PCL nanofibers, PCL-gelatin nanofibers, and tissue culture plates are 47.9 ± 3.4%, 42.6 ± 5.6%, and 54.9 ± 6.2%, respectively (Figure 4).

Differentiated Oligodendrocyte Ensheathed Nanofibers

To study the ability of OPC differentiation and wrapping of the nanofibers, OPCs were grown on coverslips with PCL nanofibers and PCL-gelatin fibers (Figures 5 and 6). The PCL nanofibers and PCL-gelatin nanofibers were 731 ± 198.9 nm and 801.2 ± 188.4 nm, respectively (Figure 5). To study the myelin formation around the individual nanofibers, loose PCL or PCL-gelatin fibers were spun on the coverslips by electrospinning. The coverslips with electrospun fibers were placed in 24-well plates, and the OPCs were seeded on the nanofibers with a cell density of 20,000 cells/well. After the OPCs were cultured on PCL nanofibers and PCL-gelatin nanofibers with myelination medium for 3 days, PDGFα positive cells developed short processes, some of which were associated with the nanofibers (Figure 5). After the OPCs were cultured on PCL nanofibers and PCL-gelatin nanofibers with myelination medium for 8 days, the OPCs differentiated and developed multiple processes. For both PCL nanofibers and PCL-gelatin nanofibers, differentiated OPCs wrapping the nanofibers were observed (Figure 6). The OPCs formed significantly more

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myelin segments for PCL-gelatin nanofibers than those for PCL nanofibers. The percentages of MBP positive cells wrapping nanofiber and myelin segments for PCL-gelatin nanofibers are 70.7 ± 8.8% and 48.9 ± 10.9%, respectively, which are significantly higher than those for PCL nanofibers alone (27.7 ± 7.2% and 15.4 ± 3.2, $p < 0.01$). SEM images showed the morphology of oligodendrocytes and the myelination formation surrounding the nanofibers. Oligodendrocytes extended multiple processes on coverslips along electrospun fibers (Figure 7A). The electrospun fibers were wrapped by oligodendrocytes. The ensheathed fibers and the blank nanofiber segments are shown (Figure 7).

DISCUSSION

In this study, we generated PCL nanofibers and PCL-gelatin co-polymer nanofibers. The composition of gelatin in the nanofibers was confirmed by EDS testing. To fabricate nanofibers of a PCL and gelatin co-polymer, we dissolved the PCL and gelatin in acetic acid and acetonitrile. In this work, acetic acid was used to dissolve the gelatin. We found the fibers generated in this method were not toxic to the OPCs. The growing OPCs on the fibers were labeled with OPC-specific antibodies (anti-A2B5 and anti-PDGFα), and the OPCs were differentiated into oligodendrocytes on the nanofibers. The fiber diameter of the PCL fibers and PCL-gelatin fibers were determined by measurement of SEM images for the fibers. To perform the cell growth and myelination studies, we generated PCL nanofibers and PCL-gelatin co-polymer nanofibers with a similar diameter. The cell viability of OPCs on PCL fibers and PCL-gelatin fibers did not show a significant difference.

Although PCL is a biocompatible and biodegradable material for tissue engineering applications, the electrospun PCL nanofibers showed a hydrophobic property. Previous studies have suggested that this hydrophobic property of the PCL fibers is unfavorable for cell growth.[26] The nanofiber became hydrophilic when the PCL and gelatin co-polymer were electrospun. We showed that gelatin in the electrospun fibers of the PCL-gelatin co-polymer decreased the contact angle of the fibers. The cell viability and proliferation of OPCs on hydrophobic PCL nanofibers and hydrophilic PCL-gelatin nanofibers did not show a significant difference. In this study, we observed that differentiated OPCs wrapped the nanofibers after 8 days of cell culture in the myelination medium. Different from previous reports, [24, 25] the differentiated OPCs wrapped the electrospun nanofibers without a coating of Poly-D,L-ornithine (Sigma-Aldrich, St. Louis, MO). Additionally, we found that the myelination segments for the PCL-gelatin nanofibers and the number of cells wrapping the PCL-gelatin nanofibers were significantly higher than those of the PCL fibers alone. The results suggest that the incorporation of the biological molecules to the synthetic polymer fibers can significantly increase myelination formation by OPCs.

Polystyrene is biocompatible and generally used to treat tissue culture plate for cell attachment and growth. Previous studies showed that the myelination formation on polystyrene fibers by cultured OPCs. [23, 24] However, polystyrene is very slow to biodegrade. PCL is a biodegradable polymer and has been approved by the Food and Drug Administration (FDA) in applications used in the human body as implantable material. PCL has also shown significant potential in the application of neural regeneration. Additionally, the biological function of PCL can be enhanced by adding biomacromolecules in PCL to generate implantable scaffolds with the co-polymer. In this study, we fabricated PCL-gelatin nanofibers and investigated their function to support OPC growth and differentiation. We also investigated the myelination formation of oligodendrocytes on these fibers as a neuron-free model. The nanofibers have a high surface-to-volume ratio and the high surface area can enhance the capacity for cell adhesion. [27] The fibers with smaller diameters can generate a higher surface area. In this study, the smallest-diameter PCL fibers and PCL-gelatin fibers generated were used in this study to investigate the growth and differentiation of OPCs. It

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was also reported that oligodendrocytes can form better myelination segments on nanofibers with diameters in the range of 0.4-0.8 μm or larger, compared with fibers having diameters lower than 0.4 μm. [22] To study the myelination of oligodendrocytes for fibers with a high surface area and optimal myelination segments, we fabricated PCL nanofibers and PCL-gelatin nanofibers with average fiber diameters of 0.73 μm and 0.8 μm, respectively. We observed that differentiated OPCs wrapped the electrospun nanofibers. The percentage of cells that formed myelin segments around the nanofibers is about 70%. A previous study showed that the differentiated OPCs myelinated the electrospun polystyrene nanofibers, and about 60% MBP positive cells wrapped the polystyrene nanofibers (0.4–0.8μm).[24] We observed that the percentage of cells forming myelin segments around the PCL nanofibers is about 30%. These observations suggest that the fiber composition and coating can affect the efficiency of OPC differentiation and fiber wrapping. The nanofiber may serve as a simple model to study the myelination of different molecules in axonal regeneration. It should be noticed that the biological molecules can change the fibers' biological function and hydrophilicity. The latter may also affect the myelination of oligodendrocytes for the nanofibers. To study the effect of a biological molecule on myelination using the nanofiber model, factors such as fiber diameter and hydrophilicity should be controlled.

CONCLUSION

We fabricated nanofibers using a PCL and gelatin co-polymer. The ratio of PCL and gelatin components in the fibers was confirmed by EDS testing. We showed that gelatin in the PCL-gelatin co-polymer for electrospinning decreased the fiber contact angle, which in turn increased the hydrophilicity of the electrospun fibers. We also showed that both PCL nanofibers and PCL-gelatin nanofibers can support OPC growth and differentiation. The OPCs maintained their phenotype and viability on both PCL and PCL-gelatin nanofibers and were able to differentiate into oligodendrocytes when cultured with an OL medium. The differentiated OPCs associated with the nanofibers and formed myelin along the nanofibers. The differentiated OPCs formed significantly more myelinated nanofiber segments for PCL-gelatin nanofibers than for PCL nanofibers. This study may open up new possibilities to repair the injured spinal cords and other nerves.

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ABBREVIATIONS

OLs	oligodendrocytes
OPCs	oligodendrocyte precursor cells
PCL	polycaprolactone
IACUC	Institutional Animal Care and Use Committee
SEM	scanning electron microscopy
EDS	energy dispersive spectroscopy
PBS	phosphate-buffered saline

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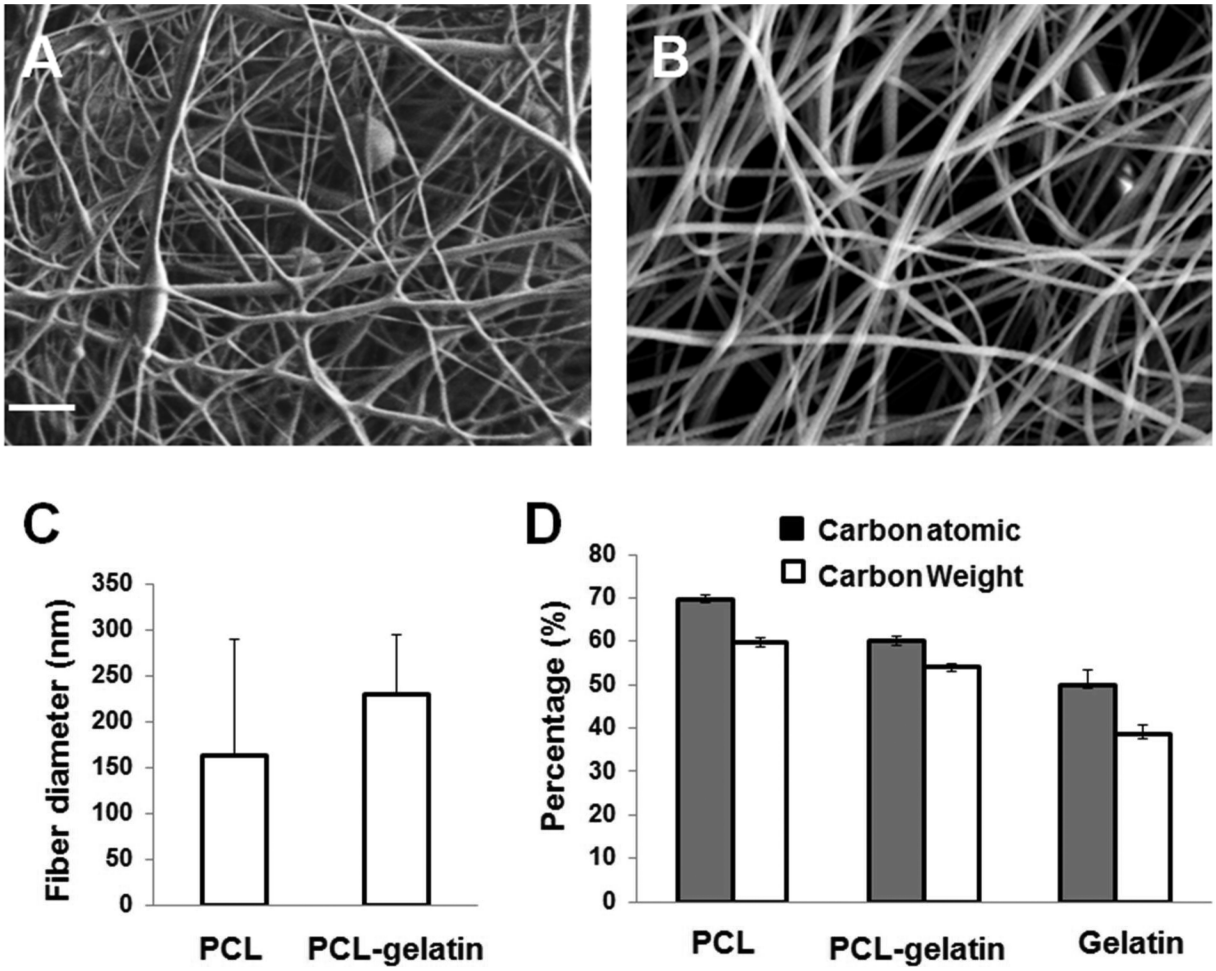

Figure 1.
Characterization of electrospun fibers. (A) SEM image of electrospun PCL fibers. (B) SEM image of PCL-gelatin fibers. (C) Quantification of fiber diameter. (D) Carbon atomic and carbon weight ratio in nanofibers. Scale bar: 2 μm.

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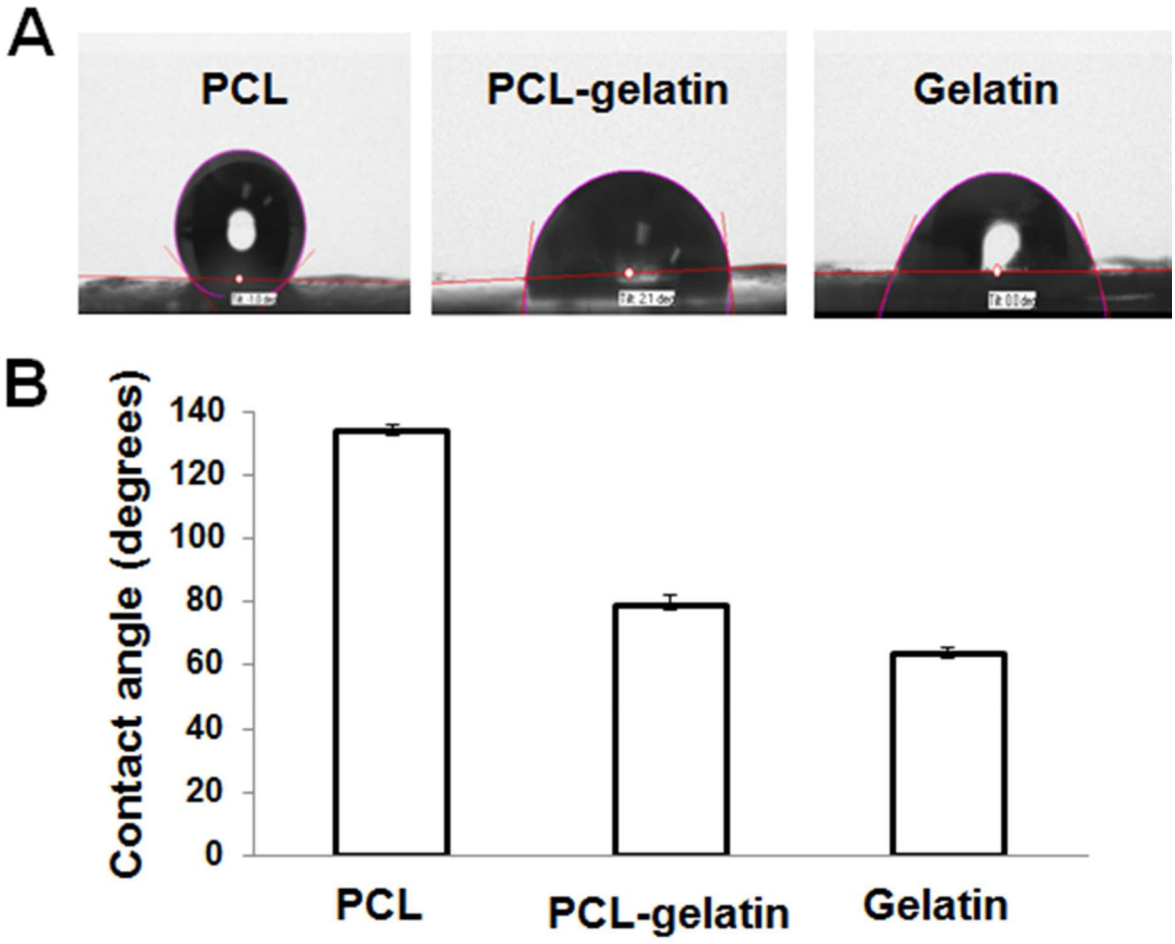

Figure 2.
Measurement of contact angle of PCL nanofibers, PCL-gelatin nanofibers, and gelatin nanofibers. (A) Droplet profiles and contact angle evaluation on different films of dense PCL nanofibers, PCL-gelatin nanofibers, and gelatin nanofibers. (B) Quantification of contact angle of PCL nanofibers, PCL-gelatin nanofibers, and gelatin nanofibers.

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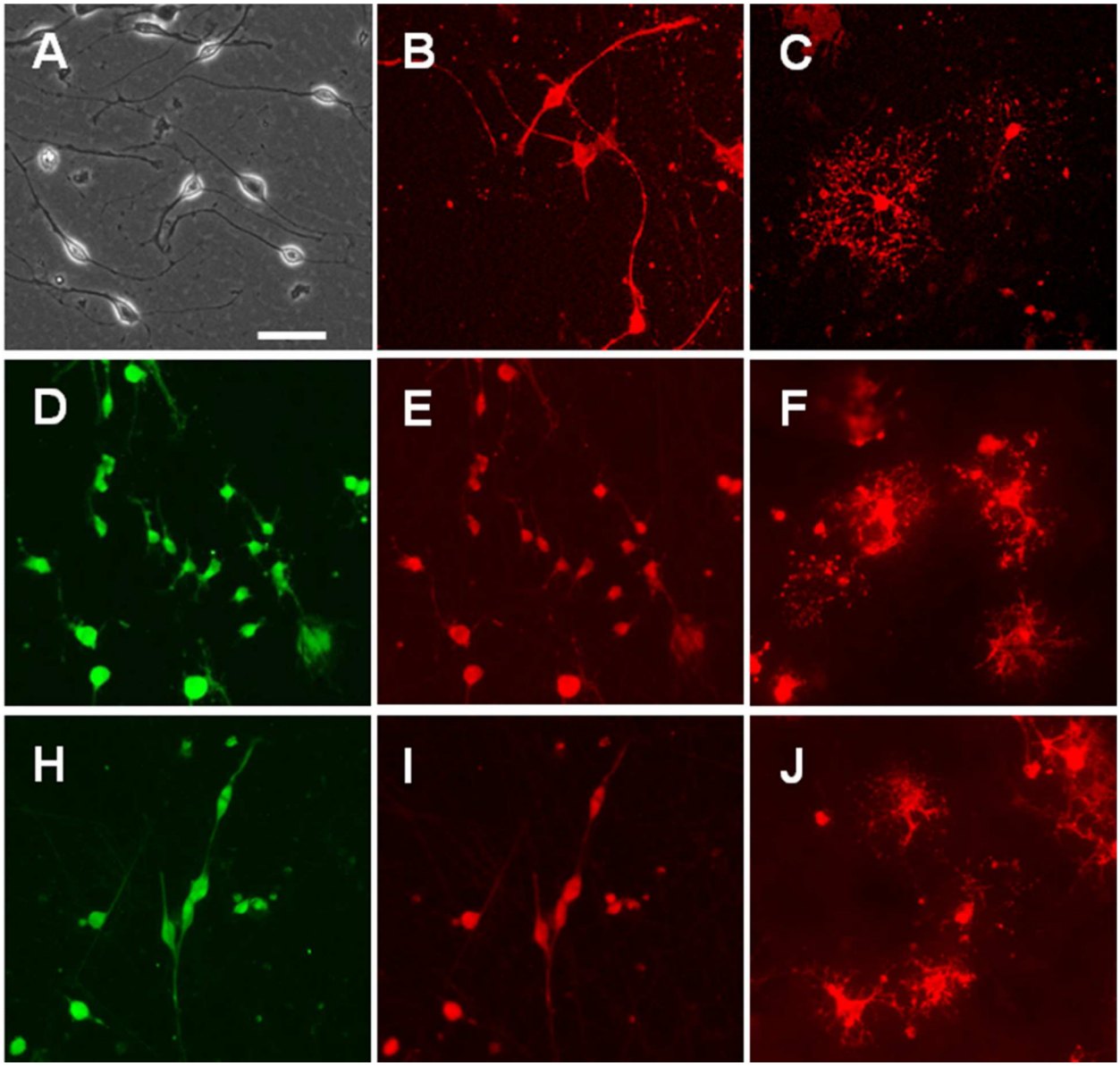

Figure 3.
Growth and differentiation of OPCs on films of dense electrospun nanofibers. Growth of OPCs on tissue culture plate (A, B), PCL nanofibers (D, E), and PCL-gelatin nanofibers (H, I). OPCs differentiated into OL cells on tissue culture plate (C), PCL nanofibers (F), and PCL-gelatin nanofibers (J). OPCs were labeled with anti-PDGFα antibody (D, H) or anti-A2B5 antibody (B, E, I). OL cells are labeled with anti-O4 antibody (C, F, J). Scale bar: 100 μm.

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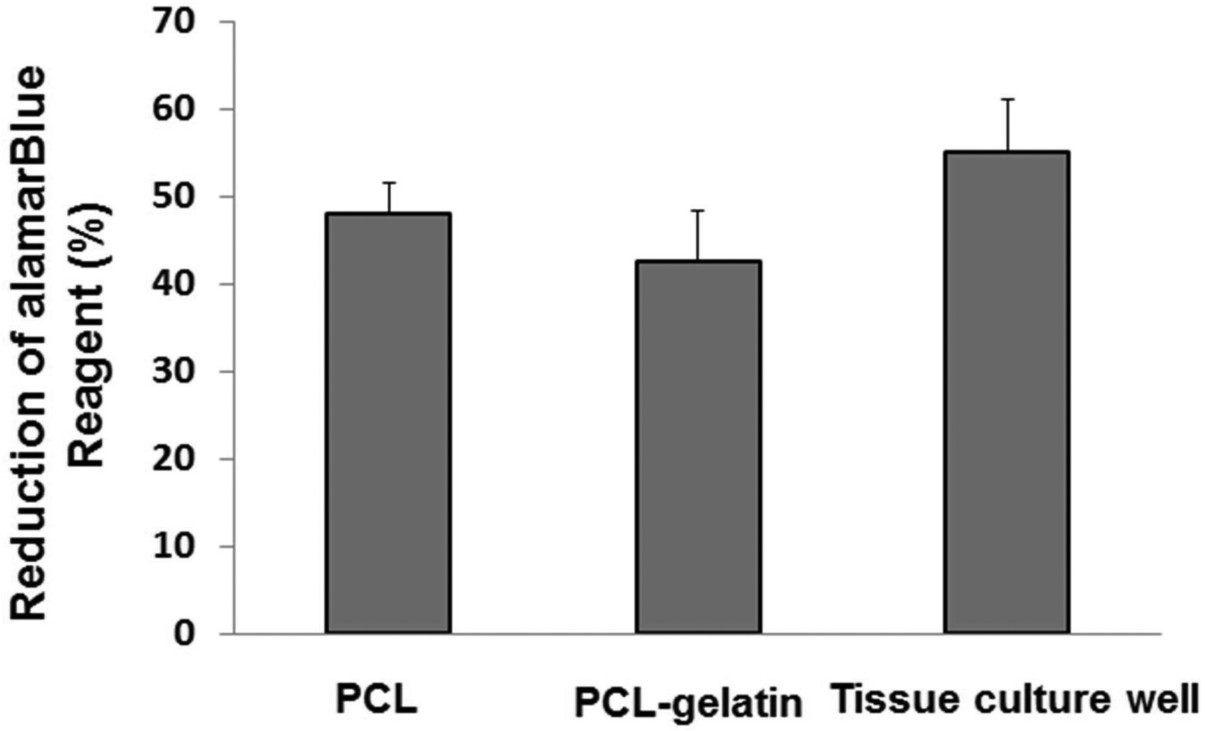

Figure 4.
alamarBlue cell viability assay of OPCs growing on the films of dense electrospun PCL nanofibers, PCL-gelatin nanofibers and gelatin nanofibers.

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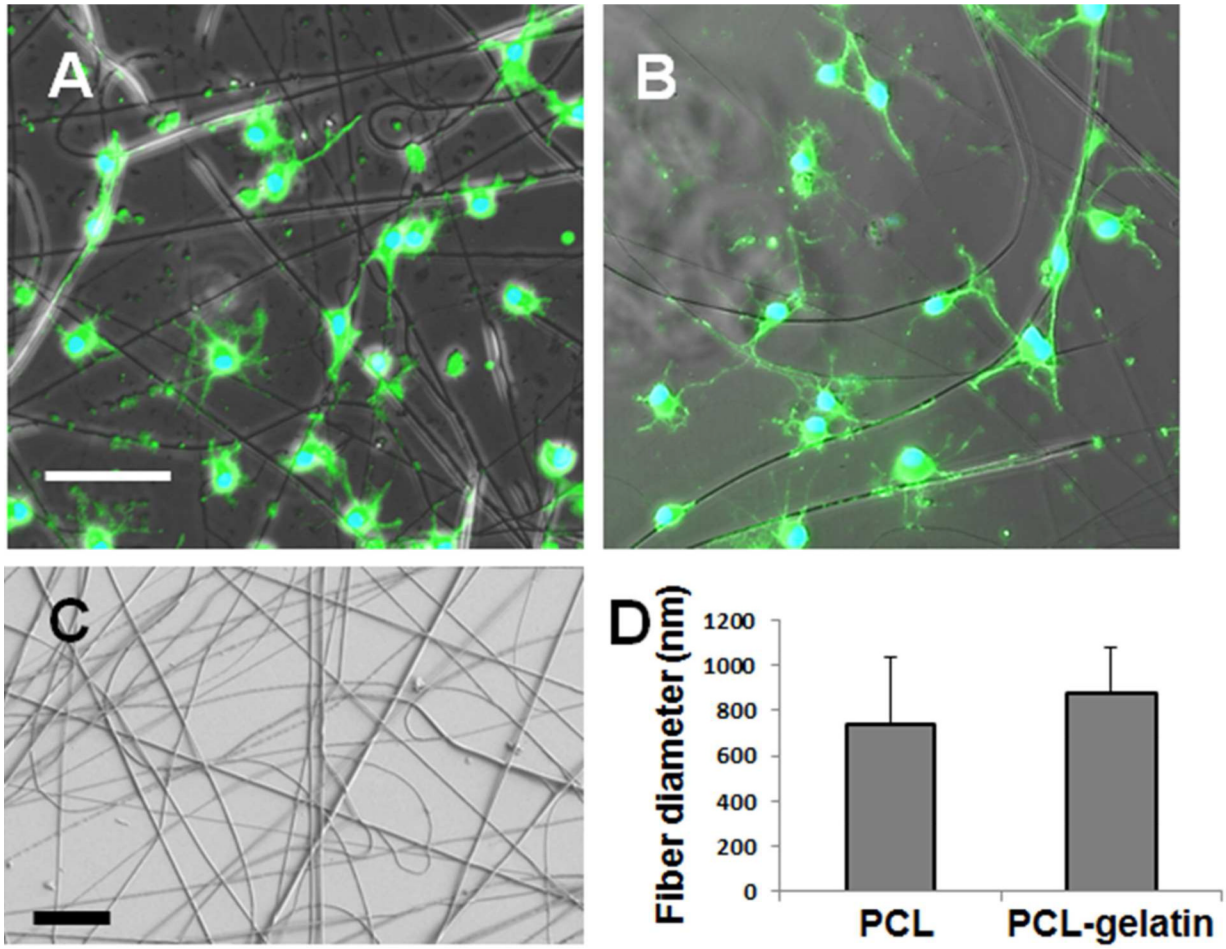

Figure 5.

Growth of OPCs on coverslips with electrospun nanofibers. After culturing for 3 days, OPCs developed short processes on coverslips with PCL nanofibers (A) and PCL-gelatin nanofibers (B). OPCs were labeled with anti-PDGFα antibody. Some cells were associated with nanofibers. Scale bar: 100 μm. (C) SEM images of PCL-gelatin nanofibers. Scale bar: 20 μm. (D) Analysis of nanofiber diameter.

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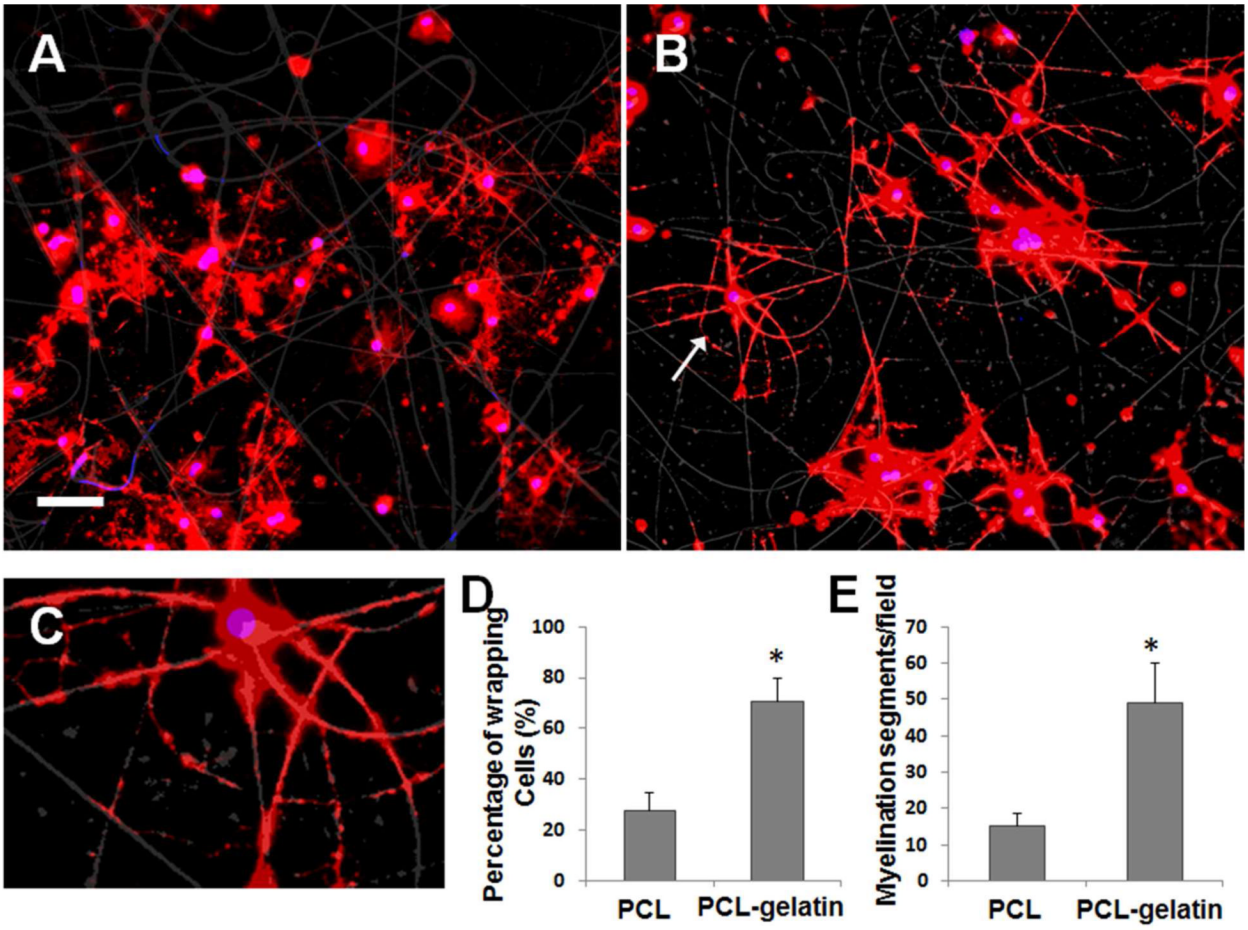

Figure 6.

Differentiated OPCs ensheath nanofibers. (A) Differentiated OPCs ensheath PCL fibers. (B) Differentiated OPCs ensheath PCL-gelatin fibers. Scale bar: 100 μm. (C) Magnified image of myelinated PCL-gelatin nanofibers, as indicated by arrow in (B). Cells were labeled with anti-MBP antibody. (D) Analysis of percentage of cells wrapping nanofibers (* indicates significant difference compared with differentiated OPCs myelinate PCL nanofibers, $p < 0.01$). (E) Analysis of percentage of myelinated nanofiber segments (* indicates significant difference compared with myelinated segments of PCL nanofibers, $p < 0.01$).

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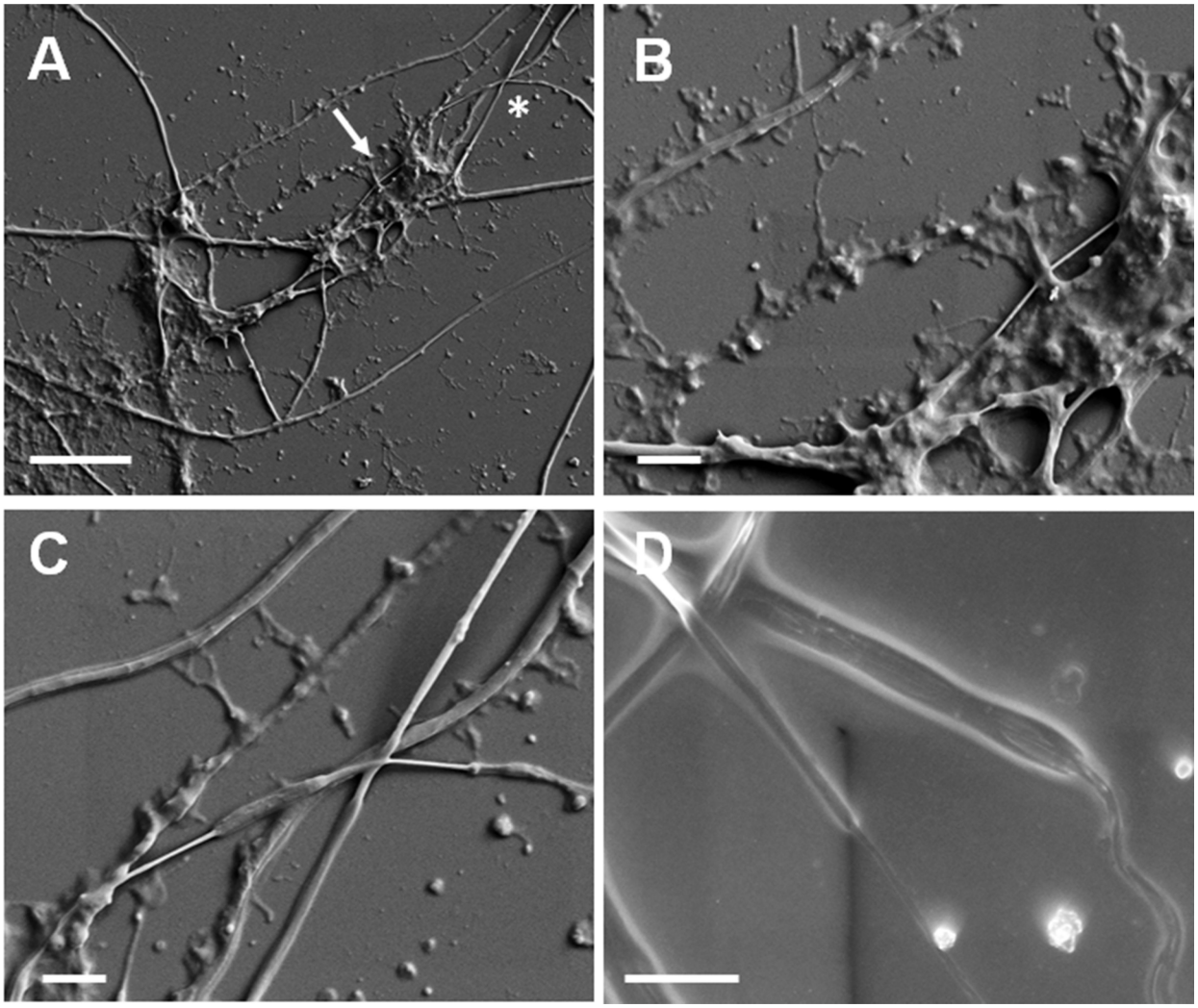

Figure 7.
SEM images showing differentiated OPC-ensheathed PCL-gelatin nanofibers. (A) Differentiated OPCs extend multiple processes. Scale bar: 10 μm. (B) Magnified image showing myelinated and unmyelinated fiber segments, as indicated by arrow in (A). Scale bar: 2 μm. (C) Magnified image showing myelinated and unmyelinated fiber segments, as indicated by asterisk in (A). Scale bar: 2 μm. (D) Magnified images show end of myelinated fiber segments. Scale bar: 2 μm.