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Surface modified electrospun nanofibrous scaffolds for nerve tissue engineering

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Abstract

The development of biodegradable polymeric scaffolds with surface properties that dominate interactions between the material and biological environment is of great interest in biomedical applications. In this regard, poly-ε-caprolactone (PCL) nanofibrous scaffolds were fabricated by an electrospinning process and surface modified by a simple plasma treatment process for enhancing the Schwann cell adhesion, proliferation and interactions with nanofibers necessary for nerve tissue formation. The hydrophilicity of surface modified PCL nanofibrous scaffolds (p-PCL) was evaluated by contact angle and x-ray photoelectron spectroscopy studies. Naturally derived polymers such as collagen are frequently used for the fabrication of biocomposite PCL/collagen scaffolds, though the feasibility of procuring large amounts of natural materials for clinical applications remains a concern, along with their cost and mechanical stability. The proliferation of Schwann cells on p-PCL nanofibrous scaffolds showed a 17% increase in cell proliferation compared to those on PCL/collagen nanofibrous scaffolds after 8 days of cell culture. Schwann cells were found to attach and proliferate on surface modified PCL nanofibrous scaffolds expressing bipolar elongations, retaining their normal morphology. The results of our study showed that plasma treated PCL nanofibrous scaffolds are a cost-effective material compared to PCL/collagen scaffolds, and can potentially serve as an ideal tissue engineered scaffold, especially for peripheral nerve regeneration.

(Some figures in this article are in colour only in the electronic version)

1. Introduction

Nerve tissue engineering is a rapidly expanding area of research providing a new and promising approach to nerve repair and regeneration. The treatment of peripheral nerve defects after traumatic injury involves the surgical transfer of a nerve from an uninjured part of the patient's body to the injured site. This necessitates the sectioning of a viable nerve, causing a sensory defect at the donor site along with prolonged operative time for donor nerve harvesting [1]. Alternatively, fabrication of a biodegradable and implantable scaffold/nerve guide to replace an autologous nerve graft offers an improved approach to patient care. Such bridges implanted at the lesion function to maintain a continuous path for nerve regeneration, promoting the infiltration of cells to secrete inductive factors for axonal elongation reducing scar formation [2, 3]. Schwann cells (SCs) play a crucial role during nerve regeneration through the production of growth factors and the secretion of extracellular matrix (ECM) proteins [4, 5]. After nerve injury, SCs undergo proliferation and phenotypical changes to prepare the local environment for axonal regeneration [6, 7].

Electrospinning is a unique and versatile technique that produces fibers with diameters as small as 5 nm and serves as a method to produce nanofibrous scaffolds that mimic the architecture of natural ECM [8, 9]. Cells attach and organize well around fibers with diameters smaller than the diameter of cells [10]. Synthetic aliphatic polyesters and copolyesters such as poly-L-lactic acid (PLLA), poly-ε-caprolactone (PCL), polyglycolic acid (PGA), polylactic-co-glycolic acid (PLGA), polyurethane (PU) and poly(organo)phosphazenes are the

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most promising biodegradable materials utilized for the preparation of hollow neural guides to enhance nerve tissue regeneration [11–15]. Moreover, these synthetic biodegradable materials including PCL have an advantage in their flexibility, since tailoring their chemical and mechanical properties can further alter their degradation properties, biocompatibility and mechanical strength. Additionally, the surface properties of these materials can be further altered to make them more effective as biomaterials, including modifying their hydrophobicity by covalent surface modification techniques and synthesis of novel graft copolymers with desired functional groups.

Plasma treatment is a convenient and cost-effective method for altering the surface properties of polymeric materials by introducing desired functionalities onto the surface and improving the hydrophilic properties and permeability of polymer surfaces [16, 17]. The extent of cell attachment and growth on polymer surfaces are important factors that directly influence the capacity of cells to proliferate and differentiate on these polymeric scaffolds. A few research groups have utilized PCL film for immobilization of biologically active molecules like fibronectin, laminin, collagen etc for tissue replacement [18, 19]. To the best of our knowledge, no reports are available on the utilization of plasma treated PCL nanofibrous scaffolds (p-PCL) for nerve tissue engineering. Therefore we compared the efficacy of p-PCL nanofibrous scaffolds with unaltered PCL scaffolds using SCs for peripheral nerve regeneration.

Electrospun PCL nanofibers modified by plasma treatment were analyzed by contact angle measurement and surface composition was studied using x-ray photoelectron spectroscopy (XPS). The potential application of surface modified electrospun PCL nanofibrous scaffolds for nerve guide repair was assessed by *in vitro* growth and proliferation tests with SCs. The results of SC proliferation on PCL scaffolds were compared with the proliferation behavior of SCs on p-PCL scaffolds, and further comparison was made between p-PCL and PCL/collagen nanofibers, a previously studied biocomposite material containing the natural protein collagen. Our study shows improved cell adhesion and proliferation of SCs on p-PCL nanofibers, proving them to be potential substrates for peripheral nerve regeneration.

2. Materials and methods

2.1. Materials

Poly-ε-caprolactone with molecular weight of 80 000, chloroform, methanol, 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) and glutaraldehyde were purchased from Sigma-Aldrich (St Louis, MO, USA). 'CellTiter 96 Aqueous One solution assay' was purchased from Promega (Madison, WI, USA).

2.2. Electrospinning of nanofibers

PCL (12 wt%) was dissolved in chloroform/methanol (1:3) for a day under stirring conditions. Collagen (8 wt%) and PCL/collagen (1:1) were dissolved in HFIP for the electrospinning process. Each polymer solution was loaded into a 5 ml plastic syringe fitted with a needle with a tip diameter of 0.4 mm. Polymer solutions were fed to the needle tip using a syringe pump (KD Scientific, Holliston, MA, USA) at a flow rate of 1 ml h^{-1}. A positive voltage of 12 kV was applied to the needle using a high voltage power supply (Gamma High Voltage Research, Ormond Beach, FL, USA). An aluminum sheet, used as the collector, was placed at a distance of 12 cm from the needle tip. On applying high voltage, the polymer solution formed a Taylor cone at the needle tip and a positively charged jet was sprayed on the collector. Nanofibers collected on round cover slips with 15 mm diameter were dried overnight under vacuum before being used for experiments.

2.3. Surface modification of electrospun nanofibers

Air plasma treatment of electrospun PCL nanofibrous scaffolds was carried out by using PDC-001 plasma cleaner (Harrick Scientific Corporation, Ossining, NY, USA). Nanofibers were placed in the chamber of the plasma cleaner and plasma discharge was applied for 1 min with radio frequency power set as 30 W under vacuum mode.

2.4. Physicochemical and mechanical characterization of nanofibers

Electrospun nanofibrous scaffolds were sputter coated with gold (JEOL JFC-1200 Fine Coater, Tokyo, Japan) and visualized under field emission scanning electron microscope (FEI-QUANTA 200F, the Netherlands) at an accelerating voltage of 10 kV. Fiber diameters were measured using Image J software (Image analysis, National Institutes of Health, Bethesda, MD, USA).

The XPS spectra of nanofibers were obtained on VG-Escalab 2201-XL Base System (Thermo VG Scientific, UK) with a take off angle of 90°. Survey scan spectra were taken and the ratio of C 1s and O 1s served as an indication of improvement in hydrophilicity of p-PCL nanofibers compared to PCL nanofibers. For biocomposite PCL/collagen nanofibers, the XPS N1S signal served as an indication of the presence of amino groups from collagen.

The water contact angle for PCL nanofibrous scaffolds before and after plasma treatment were measured using a VCA Optima XE Video Contact Angle System (Crest Technology, Singapore), mounted with a CCD camera. Scaffolds were placed on the sample stage and a drop of distilled water was dropped to the surface for contact angle measurement.

Mechanical testing was carried out using a 5848 microtester (Instron, Canton, MA, USA) at a stroke rate of 10 mm min^{-1}. Rectangular specimens were cut from the as-spun membranes of thickness 60–70 μm and used for mechanical testing studies. The ends of the rectangular specimens were mounted vertically on mechanical gripping units of the tensile tester. The obtained results were plotted to obtain the stress–strain curve for different scaffolds.

2.5. In vitro culture of Schwann cells

Schwann cells (RT4-D6P2T) obtained from ATCC (USA) were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin/amphotericin-B (Invitrogen Corp., USA). Cells were maintained in a humidified CO_2 incubator at 37 °C until confluency and fed with fresh medium every 3 days. Before seeding, cells were detached from the cell culture flask with trypsin-EDTA and viable cells were counted by trypan blue assay using a hemocytometer. The second passage culture was used for proliferation and scanning electron microscopy (SEM) experiments.

2.6. MTS assay for Schwann cell proliferation

Schwann cells were seeded (10 000 cells cm^{-2}) on PCL, p-PCL and PCL/collagen nanofibrous scaffolds in 24-well plates. Proliferation of cells were monitored after 2, 4, 6, 8 and 10 days by MTS assay [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt]. The number of cells on different scaffolds obtained by MTS colorimetric assay determines the SC adhesion and proliferation capacity on different nanofibrous scaffolds. Metabolically active cells react with tetrazolium salt in MTS reagent to produce a soluble formazan dye, measured at 490 nm. After designated time periods, the cell constructs were rinsed with phosphate buffered saline (PBS) and incubated with 20% MTS reagent in DMEM for 3 h. The color developed dye was further pipetted out in 96-well plates and absorbance was read using a spectrophotometric plate reader (FLUOstar OPTIMA, BMG Lab Technologies, Germany).

2.7. Cell-scaffold constructs processing for SEM

Cells were seeded (10 000 cells cm^{-2}) on PCL, p-PCL and PCL/collagen nanofibrous scaffolds in 24-well plates. After 6 days of cell culture, the cell–scaffold constructs were rinsed in PBS and fixed with 3% glutaraldehyde for 3 h. Further, it was dehydrated through a series of graded alcohol solutions and air dried into hexamethyldisilazane (HMDS) overnight. The cellular constructs were observed under SEM at an accelerating voltage of 10 kV, after sputter coating with gold. The morphology of cells seeded on tissue cultured polystyrene plate (TCP) was also evaluated.

2.8. Statistical analysis

Data were obtained at least in triplicate, averaged and expressed as mean ± standard deviation (SD). Each experiment was repeated at least three times. Statistical analysis was carried out using one way analysis of variance (ANOVA). A value of $p \leqslant 0.05$ was considered statistically significant.

3. Results and discussion

Polycaprolactone (PCL) is a biocompatible and bio-resorbable polymer that has been studied extensively as a polymer substrate in tissue engineering [20, 21]. It has low degree of swelling and degrading properties, suitable for the fabrication of a nerve guide for tissue regeneration. Minimum swelling characteristics are preferred for a nerve guide material since swelling of nerve guides can result in nerve compression and impede the outgrowth of regenerating nerves [22, 23]. However, poor hydrophilicity together with their lack of functional group often results in low cell adhesion and proliferation on these scaffolds. Different types of physico-chemical and post-processing surface modification techniques (photo/radiation/cerium-induced graft polymerization, aminolysis etc) have been attempted to solve the problem of cell adhesion and proliferation on such hydrophobic surfaces [24, 25]. For example, the photo-oxidation method introduces peroxide group onto polymer surfaces by immersing the material in hydrogen peroxide solution and the peroxide group is further used to initiate graft polymerization under UV irradiation [26, 27]. Hydrophilic polymers such as poly(acrylamide) grafted on PCL surfaces likewise showed improved cell adhesion [28]. As an alternative to performing a covalent modification on the polymer, a reactive group (carboxyl, amino or hydroxyl) must be present, but is usually not present in hydrophobic polymers. Poly(acrylic acid) or poly(methacrylic acid) are frequently grafted to yield carboxyl groups on inert polymeric surfaces, followed by the activation of carboxyl groups with carbodiimide, whereby the final reaction of carboxyl group with amino groups of the target protein occurs [29]. Improvement in cell proliferation of PCL can also be achieved by harsh surface treatment methods, cross-linking or co-polymerization with different monomers and proteins or by blending with high cost ECM and growth factors [30, 31]. Wet chemical surface modification methods are harsh surface treatment methods that utilize concentrated corrosive solutions for an extended period and generate hazardous chemical waste [31]. Plasma treatment, however, serves as an effective substitute for such modification procedures, since it uses no solvents nor generates chemical waste, with less polymer degradation [31–33]. Enhancement of surface hydrophilicity can be induced by having oxygen-containing groups (–OH, –COOH etc) on the surface of polymers. In this respect, plasma treatment is a rapid, non-solvent surface modification process with short treatment times, which can create active sites on the surface of polymeric materials [34].

3.1. Chemical and mechanical properties of electrospun scaffolds

Polycaprolactone solution (12 wt%) produced uniform and randomly oriented nanofibers using the electrospinning process and the SEM micrographs of different electrospun nanofibrous scaffolds are shown in figure 1. PCL nanofibers were found to have a fiber diameter of 350 ± 83 nm, while PCL/collagen nanofibers had a fiber diameter of 245 ± 80 nm. High magnification SEM images of PCL nanofibers before and after plasma treatment were also taken to evaluate the morphological changes. However, SEM images did not show any changes in the surface morphology of PCL nanofibrous scaffolds after plasma treatment (figure 1). The natural ECM exists in fibrous form and the electrospun nanofibers serve as an excellent matrix suitable for tissue engineering applications.

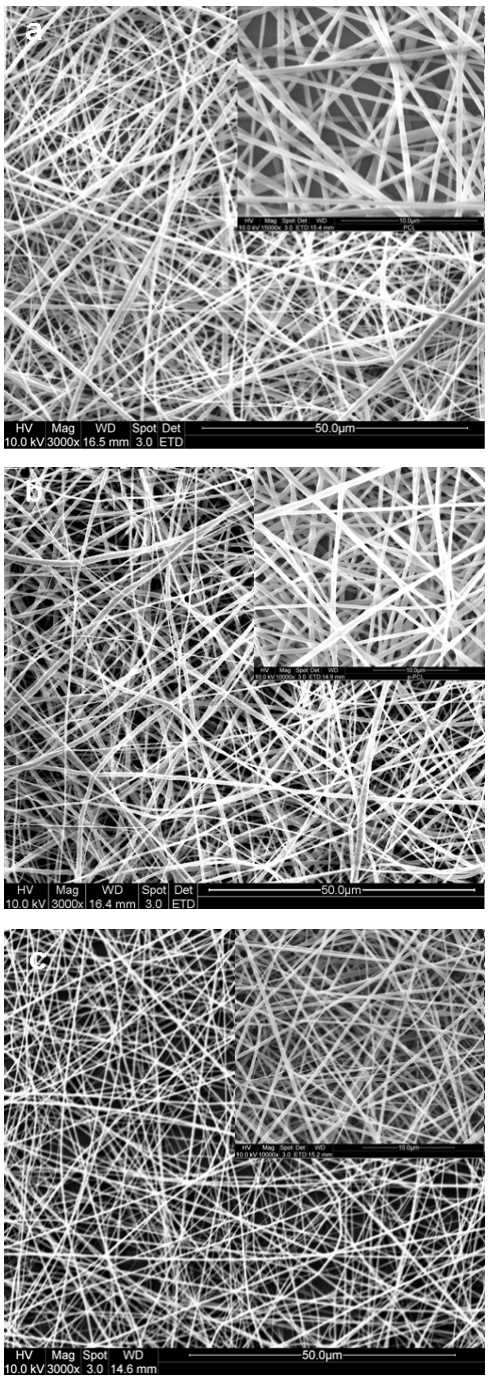

Figure 1. SEM micrographs of electrospun (a) PCL, (b) p-PCL and (c) PCL/collagen nanofibers. Inset figures are for the respective nanofibers at high magnification (×10 000).

Plasma treatment plays a critical role in improving the hydrophilicity of polymer surfaces. The XPS survey scan spectra of PCL, p-PCL and PCL/collagen nanofibrous scaffolds are shown in figure 2. The atomic ratio of carbon to oxygen on the surface of the scaffolds can be calculated from the peak area ratios of C 1s to O 1s for a particular scaffold. It was found that the proportion of oxygen for p-PCL scaffolds increased because of the introduction of oxygen atoms on these scaffolds after air plasma treatment. Moreover, with the introduction of oxygen atoms there was a corresponding decrease in the percentage of saturated hydrocarbon C 1s peak at 285.0 eV for p-PCL scaffolds. Atomic ratios (%) of O 1s and C 1s on the sample surfaces with different binding energies before and after plasma treatment are also listed in table 1. On the other hand, the XPS results of PCL/collagen nanofibrous scaffolds showed a new peak corresponding to N 1s, confirming the presence of collagen in addition to C 1s and O 1s peaks (figure 2). Air plasma treatment is a surface selective process that influences the surface wettability of polymeric scaffolds and even reduces the surface friction and tackiness. After plasma treatment, the PCL nanofibrous scaffolds showed rapid penetration of water drops into the scaffolds. The surface contact angle of p-PCL scaffolds was lower than that of PCL scaffolds, showing an improved hydrophilicity compared to PCL scaffolds (table 2). The effect of water contact angle for p-PCL scaffolds on improved hydrophilicity was also consistent with XPS results, showing an increased atomic ratio of oxygen atoms on these scaffolds.

Table 1. Atomic ratios of C 1s, O 1s and N 1s on the surface of PCL, p-PCL and PCL/collagen nanofibrous scaffolds, as determined by XPS.

Nanofibers	C atomic ratio (%)	O atomic ratio (%)	N atomic ratio (%)
PCL	74.90	25.10	—
p-PCL	62.76	37.24	—
PCL/collagen	73.62	25.38	1.00

The mechanical properties of different electrospun scaffolds, including their tensile strength and ultimate strain, were evaluated and are shown in figure 3. The tensile strength of PCL and p-PCL nanofibrous scaffolds was 3.89 and 1.75 MPa, respectively. The hydrophilicity of p-PCL scaffolds decreased their tensile strength. With regard to elongation, the p-PCL scaffolds did not have a reduced ability to extend, but instead maintained their stability. However, PCL/collagen scaffolds were found to have a much lower elongation at break of 24% (table 2).

3.2. Schwann cell proliferations on different electrospun scaffolds

Cultured SCs on electrospun scaffolds might serve as a model for denervated SCs in the distal stump of an injured nerve [35]. Reports by Chew *et al* stated the possible enhancement in maturation of SCs in contact with electrospun nanofibers, providing enhanced sciatic nerve regeneration [9]. Guenard *et al* seeded SCs into the guidance channels, whereby the initial regenerative events were bypassed, and suggested an accelerated regeneration [36]. Because of their importance in nerve repair, SCs have also been loaded in both synthetic and natural nerve guide conduits by different research groups as a method of developing tissue engineered conduits for peripheral nerve repair [37, 38]. However, any nerve graft material needs to function as a suitable growth substrate for SCs guiding the nerve tissue regeneration. We evaluated the potential application of surface modified electrospun PCL nanofibers as

Table 2. Water contact angle and tensile properties of different electrospun nanofibrous scaffolds.

Property	PCL	p-PCL	PCL/collagen
Water contact angle (deg)	122 ± 5	0	65 ± 7
Tensile strength (MPa)	3.89 ± 0.20	$1.75^{a} \pm 0.07$	$2.00^{a} \pm 0.10$
Elongation at break (%)	101.0	97.0	24.0^{a}

[a] Significant differences in measurement at $p \leqslant 0.05$ when compared to PCL nanofibers.

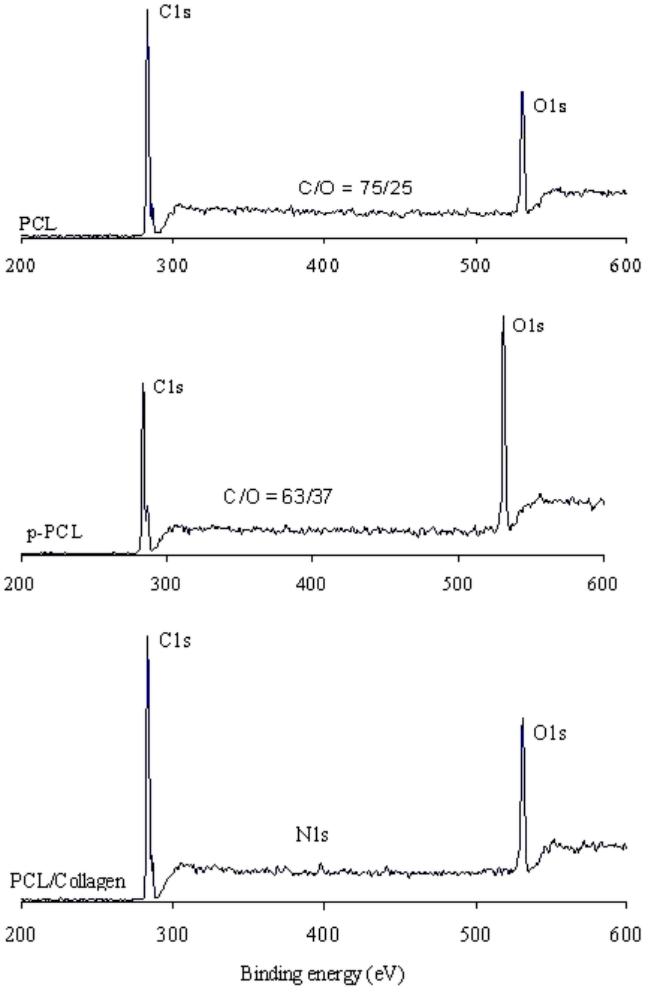

Figure 2. XPS scan of PCL, p-PCL and PCL/collagen nanofibrous scaffolds. Atomic ratios of carbon to oxygen (C/O) are shown in the figure.

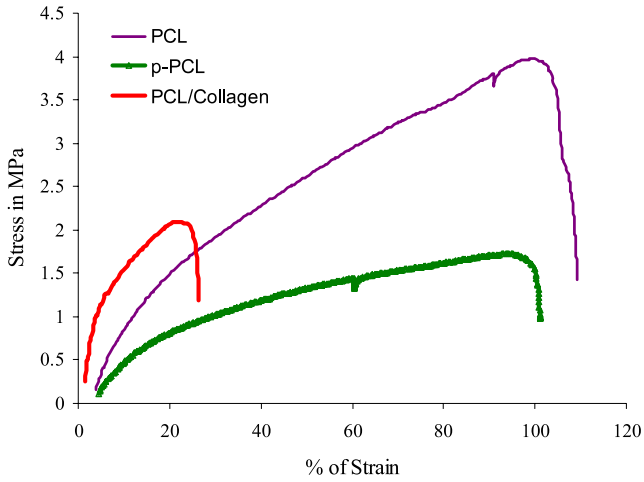

Figure 3. Tensile profile of electrospun scaffolds of PCL, p-PCL and PCL/collagen under loading conditions.

a scaffold material for nerve regeneration *in vitro* using rat SCs during this study.

Proliferation of SCs cultured on different electrospun nanofibrous scaffolds was determined by MTS assay after culturing for 2, 4, 6, 8 and 10 days. Schwann cells were found to attach on PCL, p-PCL and PCL/collagen nanofibrous scaffolds in increasing numbers over a period of 10 days (figure 4). However, the cell growth on p-PCL nanofibrous scaffolds increased by 47% and 39%, respectively, after 8 and 10 days compared to PCL scaffolds. The results of MTS assay showed a significant increase in the proliferation of SCs on p-PCL compared to PCL nanofibrous scaffolds ($p \leqslant 0.05$). The proliferation of SCs on p-PCL nanofibrous scaffolds after 8 days of cell culture was also 17% higher than the SC proliferation on PCL/collagen scaffolds, implying a convenient and cost-effective option for scaffold selection. Studies by Schnell *et al* utilized collagen-blended PCL nanofibers [39], while PGA/collagen tubes and PCL/collagen beads have been utilized by other research groups [40, 41] to enhance the biological effects of PCL and improve cell growth and proliferation. Natural ECM proteins such as collagen are usually blended, coated or adsorbed [42] on nanofibers to introduce cell recognition sites for enhancing cell–biomaterial interactions [43], and even to promote and maintain long term functional recovery of transected and injured nerves [44, 45]. However, concerns about the feasibility of obtaining large amounts of collagen for clinical applications persist for fabrication of such collagen containing scaffolds. Our studies showed that the number of SCs attached to p-PCL scaffolds was not only higher than the number attached to PCL scaffolds but also higher than the number of SCs attached on PCL/collagen scaffolds. Better cell attachment on p-PCL scaffolds suggest that the O-containing groups play an important role in cell attachment and proliferation. Results of our study showed that the surface modification of PCL nanofibrous scaffolds by plasma treatment serves as an effective and convenient method for improving the affinity of SCs for these polymeric scaffolds.

3.3. Evaluation of SC morphology

Figure 5 shows the SEM micrographs pertaining to the interactions of SCs and nanofibers after seeding the same

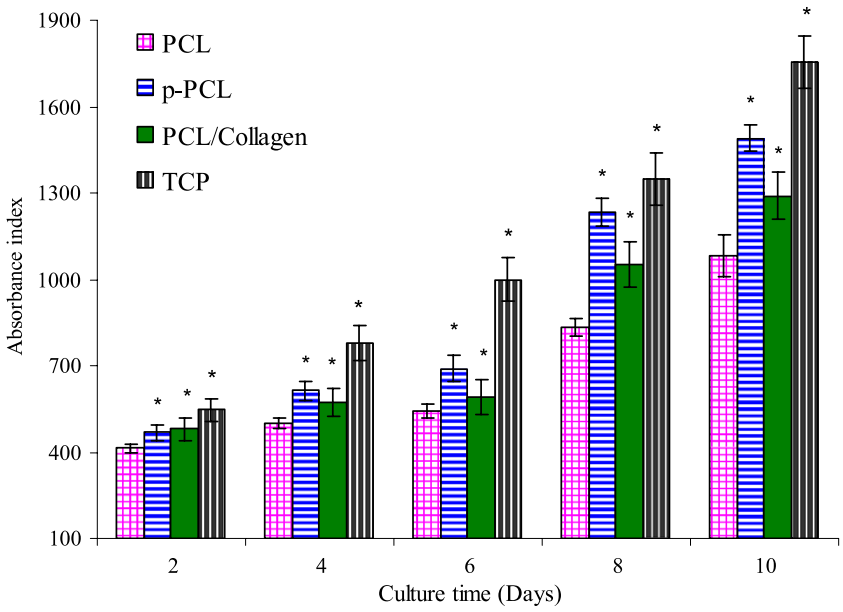

Figure 4. Proliferation of SCs on PCL, p-PCL, PCL/collagen nanofibrous scaffolds and TCP, as determined by MTS assay. * Significant against cell proliferation on PCL scaffolds at $p \leqslant 0.05$.

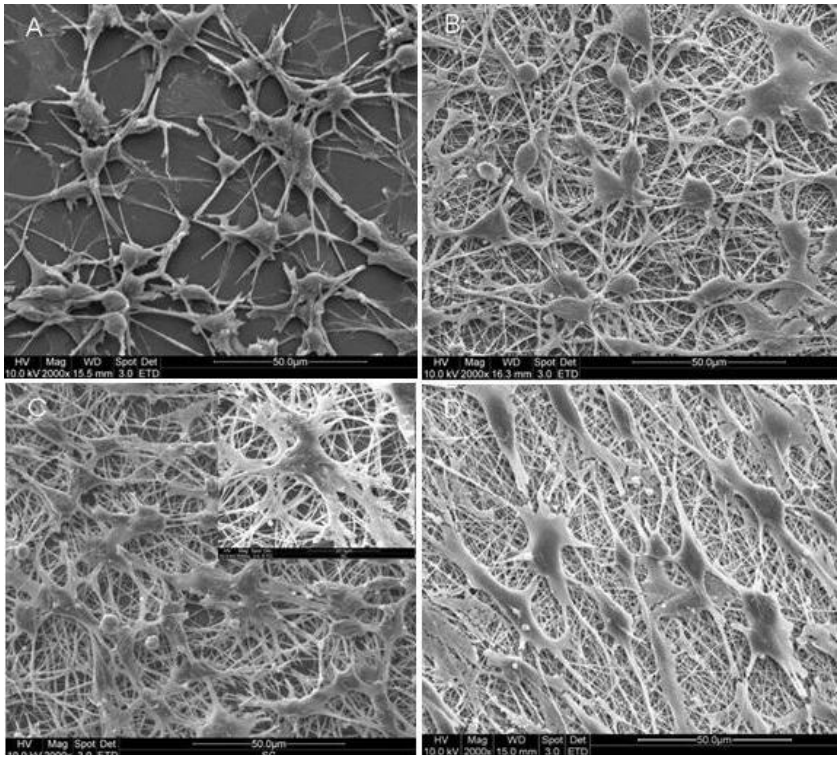

Figure 5. SEM images of SCs on electrospun (A) TCP, (B) PCL, (C) p-PCL and inset figure at high magnification and (D) PCL/collagen nanofibers, cultured for 6 days.

number of cells on different scaffolds. A normal phenotypic morphology of SCs was observed on different nanofibrous scaffolds in this study after 6 days of cell culture. Cells had a typical oval shaped cell body with long extensions giving an overall spindle shape. The lower cell proliferation and density on PCL scaffolds observed during this study was due to the slowed proliferation rate of SCs on PCL nanofibrous scaffolds compared to the cell proliferation on p-PCL nanofibrous scaffolds, as revealed by SEM (figure 5). Moreover, after seeding the same number of cells on different scaffolds, SEM micrographs of day 6 showed confluency in cell growth on p-PCL nanofibers compared to PCL/collagen nanofibers. The SCs were found to interact and integrate well with the surrounding fibers in p-PCL nanofibrous

scaffolds. Morphological evaluation of SCs on p-PCL nanofibrous scaffolds showed elongated bipolar extensions of SCs, retaining the normal cell phenotype observed on TCP (control). The success of a nerve guide in neural tissue engineering only occurs with the right combination of mechanical and biological properties of the scaffold, playing a crucial role in cell proliferation and the regeneration process. The present study attempted to improve the efficacy of PCL nanofibers by a short time plasma treatment process, thus enhancing the bioactivity of scaffolds, providing an ideal substrate for peripheral nerve regeneration.

4. Conclusion

The surface properties of most synthetic polymers are sub-optimal in promoting cell adhesion. The major challenge in tissue engineering is to design and fabricate a biodegradable scaffold with desirable surface chemistry, suitable for cell attachment, proliferation and differentiation, that can guide the process of tissue formation. Evaluating the *in vitro* biocompatibility of plasma treated PCL nanofibrous scaffolds using SCs showed comparatively higher SC proliferation on p-PCL nanofibrous scaffolds than PCL and PCL/collagen scaffolds, retaining their normal phenotypic morphology. The surface modified p-PCL scaffolds showed more promising tissue engineered nerve grafts than the most widely utilized PCL/collagen scaffolds, which is a biocomposite material made of PCL and natural collagen protein. Results of our study suggested the potential application of simple plasma treatment of PCL nanofibrous scaffolds, making them ideal substrates for nerve tissue engineering. Plasma treatment might serve as a valuable method that can be applied as such for improving the affinity of polymeric scaffolds for cells.

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References

[1] Pannunzio M E, Jou I, Long A, Wind T C, Beck G and Balian G 2005 A new method of selecting Schwann cells from adult mouse sciatic nerve *J. Neurosci. Methods* **149** 74–81

[2] Schmidt C E and Leach J B 2003 Neural tissue engineering: strategies for repair and regeneration *Annu. Rev. Biomed. Eng.* **5** 293–347

[3] Zhang N, Yan H and Wen X 2005 Tissue-engineering approaches for axonal guidance *Brain Res. Rev.* **49** 48–64

[4] Fawcett J W and Keynes R J 1990 Peripheral nerve regeneration *Annu. Rev. Neurosci.* **13** 43–60

[5] Bunge R P 1994 The role of the Schwann cells in trophic support and regeneration *J. Neurol.* **241** S19–21

[6] Frostick S P and Kemp G J 1998 Schwann cells, neurotrophic factors and peripheral nerve regeneration *Microsurgery* **18** 397–405

[7] Scaravilli F, Love S and Myers R 1986 X-irradiation impairs regeneration of peripheral nerve across a gap *J. Neurocytol.* **15** 439–49

[8] Ma Z W, Kotaki M, Inai R and Ramakrishna S 2005 Potential of nanofiber matrix as tissue engineering scaffolds *Tissue Eng.* **11** 101–9

[9] Chew S Y, Mi R, Hoke A and Leong K W 2008 The effect of the alignment of electrospun fibrous scaffolds on Schwann cell maturation *Biomaterials* **29** 653–61

[10] Xu C Y, Inai R, Kotaki M and Ramakrishna S 2004 Aligned biodegradable nanofibrous structure: a potential scaffold for blood vessel engineering *Biomaterials* **25** 877–86

[11] Evans G R D *et al* 1999 *In vivo* evaluation of poly(L-lactic acid) porous conduits for peripheral nerve regeneration *Biomaterials* **20** 1109–15

[12] Widmer M S, Gupta P K, Lu L, Meszlenyi R K, Evans G R, Brandt K, Savel T, Gurlek A, Patrick C W Jr and Mikos A G 1998 Manufacture of porous biodegradable polymer conduits by an extrusion process for guided tissue regeneration *Biomaterials* **19** 1945–55

[13] Mackinnon S E and Dellon A L 1990 Clinical nerve reconstruction with a bioabsorbable polyglycolic acid tube *Plast. Reconstr. Surg.* **85** 419–24

[14] Rodriguez F J, Gomez N, Perego G and Navarro X 1999 Highly permeable polylactide-caprolactone nerve guides enhance peripheral nerve regeneration through long gaps *Biomaterials* **20** 1489–500

[15] Langone F, Lora S, Veronese F M, Caliceti P, Parnigotto P P, Valenti F and Palma G 1995 Peripheral nerve repair using a poly(organo) phosphazene tubular prosthesis *Biomaterials* **16** 347–53

[16] Greene G, Yao G and Tannenbaum R 2003 Wetting characteristics of plasma-modified porous polyethylene *Langmuir* **19** 5869–74

[17] Grace J M and Gerenser L J 2003 Plasma treatment of polymers *J. Dispers. Sci Technol.* **24** 305–41

[18] Schantz J T, Hutmacher D W, Ng K W, Khor H L, Lim T C and Teoh S H 2002 Evaluation of a tissue engineering membrane-cell construct for guided bone regeneration *Int. J. Oral Maxillofac. Implants* **17** 161–74

[19] Cheng Z and Teoh S H 2004 Surface modification of ultra thin PCL films using acrylic acid and collagen *Biomaterials* **25** 1991–2001

[20] Yoshimoto H, Shina Y M, Teraia H and Vacantia J P 2003 A biodegradable nanofiber scaffold by electrospinning and its potential for bone tissue engineering *Biomaterials* **24** 2077–82

[21] Venugopal J, Ma L L, Yong T and Ramakrishna S 2005 *In vitro* study of smooth muscle cells on polycaprolactone and collagen nanofibrous matrices *Cell Biol. Int.* **29** 861–7

[22] den Dunnen W F, vander Lei B, Robinson P H, Holwerda A, Pennings A J and Schakenraad J M 1995 Biological performance of a degradable poly(lactic acid-epsilon-caprolactone) nerve guide: influence of tube dimensions *J. Biomed. Mater. Res.* **29** 757–66

[23] Sundback C A, Shyu J Y, Wang Y, Faquin W C, Langer R S, Vacanti J P and Hadlock T A 2005 Biocompatibility analysis of poly(glycerol sebacate) as a nerve guide material *Biomaterials* **26** 5454–64

[24] Zhu Y, Gao C and Shen J 2002 Surface modification of polycaprolactone with poly(methacrylic acid) and gelatin covalent immobilization for promoting its cytocompatibility *Biomaterials* **23** 4889–95

[25] Ma Z, Gao C, Gong Y and Shen J 2005 Cartilage tissue engineering PLLA scaffold with surface immobilized collagen and basic fibroblast growth factor *Biomaterials* **26** 1253–9

[26] Guan J J, Gao C Y, Feng L X and Shen J C 2000 Functionalizing of polyurethane surfaces by photografting with hydrophilic monomers *J. Appl. Polym. Sci.* **77** 2505–12

[27] Ma Z W, Gao C Y, Juan J, Ji J, Gong Y H and Shen J C 2002 Surface modification of poly-L-lactide by photografting of hydrophilic polymers towards improving its hydrophilicity *J. Appl. Polym. Sci.* **85** 2163–71
[28] Zhu Y B, Gao C Y, Jian J and Shen J C 2002 Photo-induced grafting of poly(methacrylic acid) onto polycaprolactone towards promotion of cell compatibility *Chin. J. Mater. Res.* **16** 233–7
[29] Steffens G C M, Nothdurft L, Buse G, Thissen H, Hocker H and Klee D 2002 High density binding of proteins and peptides to poly(d,l-lactide) grafted with polyacrylic acid *Biomaterials* **23** 3523–31
[30] Lindberg T, Wirsen A and Albertsson A C 2000 Graft polymerisation of acrylamide onto PCL film by electron beam pre-irradiation in air or argon. Morphology in the final grafted state *Polymer* **41** 4099–111
[31] Desai S and Singh R P 2004 Surface modification of polyethylene *Long-term Properties of Polyolefins* ed A C Albertsson (New York: Springer) pp 231–93
[32] Chan C M, Ko T M and Hiraoka H 1996 Polymer surface modification by plasmas and photons *Surf. Sci. Rep.* **24** 3–54
[33] Lane J M and Hourston D J 1993 Surface treatments of polyolefins *Prog. Org. Coat.* **21** 269–84
[34] Yasuda H and Gazicki M 1982 Biomedical applications of plasma polymerization and plasma treatment of polymer surface *Biomaterials* **3** 68–72
[35] Mirsky R and Jessen P R 1999 The neurobiology of Schwann cells *Brain Pathol.* **9** 293–311
[36] Guenard V, Kleitman N, Morrissey T K, Bunge R P and Aebischer P 1992 Syngeneic Schwann cells derived from adult nerves seeded in semipermeable guidance channels enhance peripheral nerve regeneration *J. Neurosci.* **12** 3310–20
[37] Mosahebi A, Simon M, Wiberg M and Terenghi G 2001 A novel use of alginate hydrogel as Schwann cell matrix *Tissue Eng.* **7** 525–34
[38] Bryan D J, Wang K K and Chakalis-Haley D P 1996 Effect of Schwann cells in the enhancement of peripheral nerve regeneration *J. Reconstr. Microsurg.* **12** 436–9
[39] Schnell E, Kinkhammer K, Balzer S, Brook G, Klee D, Dalton P and Mey J 2007 Guidance of glial cell migration and axonal growth on electrospun nanofibers of poly-ε-caprolactone and a collagen/poly-ε-caprolactone blend *Biomaterials* **28** 3012–25
[40] Kiyotani T, Nakamura T, Shimizu Y and Endo K 1995 Experimental study of nerve regeneration in a biodegradable tube made from collagen and polyglycolic acid *ASAIO J.* **41** M657–61
[41] Bender M D, Bennett J M, Waddell R L, Doctor J S and Marra K G 2004 Multi-channeled biodegradable polymer/CultiSpher composite nerve guides *Biomaterials* **25** 1269–78
[42] Labrador L O, Buti M and Navarro X 1998 Influence of collagen and laminin gels concentration on nerve regeneration after resection and tube repair *Exp. Neurol.* **149** 243–52
[43] Mingyu C K, Gong J, Li Y, Gong N and Zhang X Z 2004 Surface modification and characterization of chitosan film blended with Poly-L-lysine *Biomat. Appl.* **19** 59–62
[44] Whitworth R A, Brown R A and Terenghi G J 1996 Nerve growth factor enhances nerve regeneration through fibronectin grafts *Hand Surg. [Br]* **21** 514–22
[45] Camarata P J, Suryanarayanan R, Parker R G and Ebner T J 1992 Sustained release of nerve growth factor from biodegradable polymer microspheres *Neurosurgery* **30** 313–9