In Vitro Biocompatibility of Schwann Cells on Surfaces of Biocompatible Polymeric Electrospun Fibrous and Solution-Cast Film Scaffolds

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The in vitro responses of Schwann cells (RT4-D6P2T, a schwannoma cell line derived from a chemically induced rat peripheral neurotumor) on various types of electrospun fibrous scaffolds of some commercially available biocompatible and biodegradable polymers, i.e., poly(3-hydroxybutyrate) (PHB), poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV), polycaprolactone (PCL), poly(L-lactic acid) (PLLA), and chitosan (CS), were reported in comparison with those of the cells on corresponding solution-cast film scaffolds as well as on a tissue-culture polystyrene plate (TCPS), used as the positive control. At 24 h after cell seeding, the viability of the attached cells on the various substrates could be ranked as follows: PCL film > TCPS > PCL fibrous > PLLA fibrous > PHBV film > CS fibrous ≈ CS film ≈ PLLA film > PHB film > PHBV fibrous > PHB fibrous. At day 3 of cell culture, the viability of the proliferated cells on the various substrates could be ranked as follows: TCPS > PHBV film > PLLA film > PCL film > PLLA fibrous > PHB film ≈ PCL fibrous > CS fibrous > CS film > PHB fibrous > PHBV fibrous. At ∼8 h after cell seeding, the cells on the flat surfaces of all of the film scaffolds and that of the PCL nanofibrous scaffold appeared in their characteristic spindle shape, while those on the surfaces of the PHB, PHBV, and PLLA macrofibrous scaffolds also appeared in their characteristic spindle shape, but with the cells being able to penetrate to the inner side of the scaffolds.

1. Introduction

Attempts at repairing peripheral nerve injury have been explored for several hundred years, with a major problem associated with such treatments being the bridging of a large gap between the two ends of a transected nerve.[1,2] Autograft is a standard procedure for peripheral nerve restoration, by taking nerve tissues from a donor site and grafting into the injured site.[1,2] However, this may not work well because of the loss of nerve functions at the donor site and the difficulty in getting the implanted nerve cells to line up and reconnect.[1−5] The use of a biological or a synthetic scaffold to bridge the gap has been one possible solution to such limitations.[1] Several biomaterials have been developed as artificial nerve guides.[5] However, the sole use of scaffolding materials may not be sufficient for bridging a large gap of an injured nerve, likely as a result of the lack of neurotrophic factors that help to promote the cellular ingrowth.[1−5] A promising alternative for peripheral nerve restoration is through the use of an artificial scaffold that is preseeded with Schwann cells.[6−8]

Recently, a number of biological and synthetic biomaterials have been investigated for their suitability as matrixes for nerve regeneration: they are, for example, acellular nerve grafts,[9] chitosan nerve grafts,[8] hyaluronan-based nerve guides,[10] tubular collagen nerve grafts,[11] cross-linked gelatin nerve conduits,[12] nerve grafts from 1,3-trimethylene carbonate (TMC) and ϵ-caprolactone homopolymers or their copolymers,[7] and poly(L-lactide-*co*-glycolide) (PLGA) bioresorbable nerve guides.[6,13,14] For successful utilization as nerve scaffolds, these materials should promote the ingrowth of Schwann cells and either allow nutrients or prohibit inhibitory molecules to go from the outer to the inner side of the tubular structure.[1−2] In the past decade, a new technique, electrostatic spinning or electrospinning, has been introduced as a means for fabricating fibrous materials resembling the fibrous structure of collagens in connective tissues.[15−17]

Electrospinning is a process capable of fabricating ultrafine fibers with average diameters in the submicrometer to nanometer range. In this process, a continuous strand of a polymeric liquid (i.e., solution or melt) is ejected from the open end of a spinneret by a high electrostatic force to deposit randomly on a grounded collector as a nonwoven mat. These fibers exhibit several interesting characteristics, e.g., a high surface area to mass or volume ratio, a small interfibrous pore size with high porosity of the obtained fiber mat, vast possibilities for surface functionalization, etc.[18,19] These advantages render electrospun polymeric fibers good candidates for a wide variety of applications, including filters,[20] composite reinforcements,[21,22] drug delivery vehicles,[23,24] and tissue-engineered scaffolds.[15−17,25−29]

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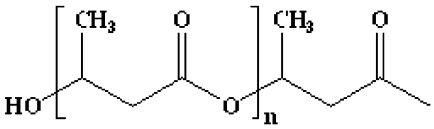
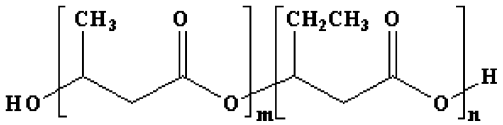
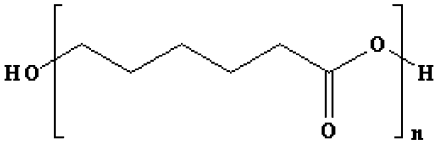
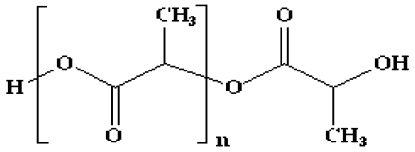
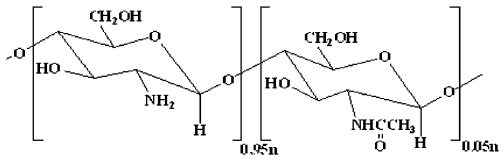
Table 1. Weight-Average Molecular Weight and Chemical Structure of the Polymers Investigated in the Present Work

material	weight-average molecular weight (g mol^{-1})	chemical structure
poly(3-hydroxybutyrate) (PHB)	300 000	
poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV)[a]	680 000	
polycaprolactone (PCL)	80 000	
poly(L-lactic acid) (PLLA)	80 000	
chitosan (CS)[b]	70 000	

[a] The content of 3-hydroxyvalerate was 5 mol %. [b] The degree of deacetylation was 95%.

Despite the numerous reports on the in vitro responses of numerous types of cells on various electrospun fibrous scaffolds,[15−17,25−29] a similar report on the in vitro responses of Schwann cells on any type of electrospun fibrous scaffolds is still lacking. In the present paper, the in vitro responses of Schwann cells on various types of electrospun fibrous scaffolds that were fabricated from a number of commercial biocompatible and biodegradable polymers, i.e., poly(3-hydroxybutyrate) (PHB), poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV), polycaprolactone (PCL), poly(L-lactic acid) (PLLA), and chitosan (CS), are evaluated and reported. The results are compared with those obtained for the corresponding solution-cast film scaffolds as well as the tissue-culture polystyrene plate (TCPS), used as the positive control.

2. Experimental Details

2.1. Materials. Five types of biocompatible and biodegradable polymers used to fabricate electrospun fibrous and solution-cast film scaffolds are PHB, PHBV, PCL, PLLA, and CS. These materials were purchased from Sigma-Aldrich and used without further purification. Table 1 summarizes the weight-average molecular weight (M_w) and the chemical structure of these polymers.

2.2. Preparation of Electrospun Fibrous and Solution-Cast Film Scaffolds. Electrospun fibrous scaffolds from the polymers listed in Table 1 were prepared according to the conditions previously described.[25,28−30] Briefly, the spinning solutions were prepared by dissolving the polymers in an appropriate solvent or a mixture of solvents, as summarized in Table 2, to obtain solutions with suitable concentrations for electrospinning. The as-prepared solutions were continuously stirred until clear solutions were obtained. Each of these solutions was fed into a glass syringe fitted with a flat-tipped gauge-20 stainless steel needle (o.d. = 0.91 mm), used as the nozzle. The as-spun fibers were collected on an aluminum sheet wrapped around a homemade rotating cylinder (width and diameter ~15 cm). The needle was connected to the emitting electrode of positive polarity from a Gamma High-Voltage Research ES30P-5W dc power supply. For each type of fibrous scaffold, a set of applied electrical potential, collection distance (i.e., the distance between the tip of the nozzle and the aluminum sheet), and collection time was fixed, as summarized in Table 2. The obtained fibrous scaffolds were dried in vacuo at room temperature prior to further investigation. Table 2 summarizes the solution conditions, the spinning conditions, and the physical information of the as-spun fibrous scaffolds.

For comparison purposes, the polymers were also fabricated into films by the solution-casting technique. The casting solutions were prepared by dissolving the polymers in a solvent, as summarized in Table 3, at suitable concentrations for casting. After being stirred until clear solutions were obtained, the solutions were cast on glass Petri dishes and dried in vacuo at room temperature prior to further investigation. Table 3 summarizes the solution conditions and the thickness of the as-cast film scaffolds.

Table 2. Solution Conditions, Spinning Conditions, and Physical Information of the Electrospun Fibrous Scaffolds

material	solvent system	solution concn (%, w/v)	solution temp (°C)	appl electrical potential (kV)	collection distance (cm)	thickness(μm)	fiber diam (μm)
PHB	chloroform	14	50−60	12	10	80 ± 5	3.7 ± 1.7
PHBV	chloroform	14	50−60	12	10	80 ± 5	2.3 ± 2.1
PCL	50:50 (v/v) dichloromethane (DCM)/*N*,*N*-dimethylformamide (DMF)	12	room temp	21	10	∼130	0.95−1.26
PLLA	70:30 (v/v) DCM)/tetrahydrofuran (THF)	15	room temp	15	10	∼120	4.3 ± 0.5
CS	70:30 (v/v) trifluoroacetic acid (TFA)/DCM	7	room temp	25	20	75 ± 3	0.13 ± 0.01

Table 3. Solutions Conditions and Thickness of the Solution-Cast Film Scaffolds

material	solvent	solution concn (%, w/v)	solution temp (°C)	thickness (μm)
PHB	chloroform	5	60	70 ± 5
PHBV	chloroform	5	60	70 ± 5
PCL	chloroform	2	room temp	∼130
PLLA	chloroform	2	room temp	∼125
CS	1% acetic acid	2.5	room temp	72 ± 5

2.3. Cell Culture and Cell Seeding. To evaluate the potential for use of the as-spun fibrous scaffolds as nerve grafting materials, their biocompatibility in terms of cytotoxicity, cell adhesion, and cell proliferation toward Schwann cells (RT4-D6P2T, a schwannoma cell line derived from a *N*-ethyl-*N*-nitrosourea (ENU)-induced rat peripheral neurotumor) was evaluated in comparison with that of the corresponding film scaffolds and TCPS.

RT4-D6P2T were first cultured as a monolayer in Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich), supplemented by 5% fetal bovine serum (FBS; Biochrom AG, Germany), 1% l-glutamine (Invitrogen Corp.), and 1% antibiotic and antimycotic formulation [containing penicillin G sodium, streptomycin sulfate, and amphotericine B (Invitrogen Corp.)]. The cells were incubated at 37 °C in a humidified atmosphere containing 5% CO_2, and the culture medium was replaced once every 2 d. Each of the fibrous and the film scaffolds was cut into circular disks (∼7 mm in diameter), and the disk specimens were placed in the wells of a 96-well TCPS (Biokom System, Poland), which were later sterilized in 70% ethanol for 30 min. The specimens were then washed with autoclaved deionized water and subsequently immersed in DMEM overnight. To ensure a complete contact between the specimens and the wells, each specimen was pressed with a stainless steel ring (∼7 mm in diameter). The reference cells from the cultures were trypsinized [0.25% trypsin containing 1 mM ethylenediaminetetraacetic acid (EDTA; Invitrogen Crop.)], counted by a hemacytometer (Hausser Scientific), and seeded at a density of about 1.0×10^4 cells/well on both the fibrous and the film scaffold specimens.

2.4. Cytotoxicity Evaluation. The indirect cytotoxicity evaluation of both the fibrous and the film scaffolds was conducted in adaptation from the ISO10993-5 standard test method. First, the extraction media were prepared by immersing the specimens, cut from both the fibrous and the film scaffolds (∼7 mm in diameter), in wells of a 96-well TCPS in a serum-free medium (SFM; containing DMEM, 1% L-glutamine, 1% lactabumin, and 1% antibiotic and antimycotic formulation) and incubated for 24 h. In the preparation of the reference cells, RT4-D6P2T cells were seeded in the wells of a 96-well TCPS at a density of 1.0×10^4 cells/well and incubated in 5% SFM to allow cell attachment on the plate. After 24 h, the culture medium was removed and the as-prepared extraction media were added to the wells. The cells were incubated further for 24 h, after which time the number of viable cells was quantified with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The viability of the cells that were cultured with fresh SFM was used as the control.

2.5. Cell Attachment and Cell Proliferation. For the cell attachment study, RT4-D6P2T cells were seeded on both the fibrous and the film scaffold specimens (∼7 mm in diameter) in the wells of a 96-well TCPS at a cell density of 1.0×10^4 cells/well and allowed to attach to the scaffold specimens for 2, 8, and 24 h. The viability of the cells in bare wells of the TCPS was used as the control. At each specified seeding time, the viability of the attached cells was quantified by the MTT assay. Each specimen was rinsed with phosphate-buffered saline (PBS; Sigma-Aldrich) to remove unattached cells prior to MTT assay. For the cell proliferation study, the cells were first seeded on both the fibrous and the film scaffold specimens (∼15 mm in diameter) in the wells of a 24-well TCPS at a cell density of 4.0×10^4 cells/well and allowed to attach to the scaffold specimens for 24 h. The proliferation of cells on the specimens was quantified by MTT assay after days 1, 3, and 5 of cell culture. After the attachment period of 24 h, the cells were starved with SFM twice (i.e., the medium was changed with SFM once after the 24 h attachment period and again after 3 d).

2.6. Quantification of Viable Cells by MTT Assay. The MTT assay is the method used to quantify the amount of viable cells on the basis of the reduction of the yellow tetrazolium salt to purple formazan crystals by dehydrogenase enzymes secreted from the mitochondria of metabolically active cells. The amount of purple formazan crystals formed is proportional to the number of viable cells. First, the culture medium of each cultured specimen was removed and replaced with 100 μL/well of MTT solution (Sigma-Aldrich) at 5 mg·mL^{-1} for a 96-well TCPS (or 500 μL/well for a 24-well TCPS), and then the plate was incubated for 1 h. After incubation, the MTT solution was removed. Then, 100 μL/well of dimethyl sulfoxide (DMSO; Riedel-de Haën, Germany) was added to dissolve the formazan crystals (or 500 μL/well for a 24-well TCPS), and the plate was left at room temperature in the dark for 1 h on a rotary shaker. Finally, the absorbance at 570 nm, representing the proportion of viable cells, was recorded by a Thermo Labsystems (Multiscan Ex) spectrophotometer.

2.7. Statistical Analysis. The data are presented as the mean ± the standard error of the mean ($n = 4$). A one-way ANOVA was used to compare the means of different data sets, and a statistical significance was accepted at a 0.05 confidence level.

2.8. Morphological Observation of Cultured Cells. After removal of the culture medium, the cell-cultured fibrous and film scaffold specimens were rinsed with PBS twice and the cells were fixed with 3% glutaraldehyde solution, which was diluted from 50% glutaraldehyde solution (Electron Microscopy Science) with PBS, at 500 μL/well. After 30 min, they were rinsed again with PBS and kept in PBS at 4 °C. After cell fixation, the specimens were dehydrated in an ethanol solution of varying concentration (i.e., 30%, 50%, 70%, 90%, and 100%) for about 2 min at each concentration. The specimens were then dried in 100% hexamethyldisilazane (HMDS; Sigma-Aldrich) for 5 min and later dried in air after the removal of HMDS. After being completely dried, the specimens were mounted on copper stubs, coated with gold using a JEOL JFC-1100E sputtering device for 3 min, and observed by a JEOL JSM-5200 scanning electron microscope. For comparison, the morphology of the cells that were seeded or cultured on a glass substrate (cover glass slide, 1 mm in diameter, Menzel, Germany) was also investigated.

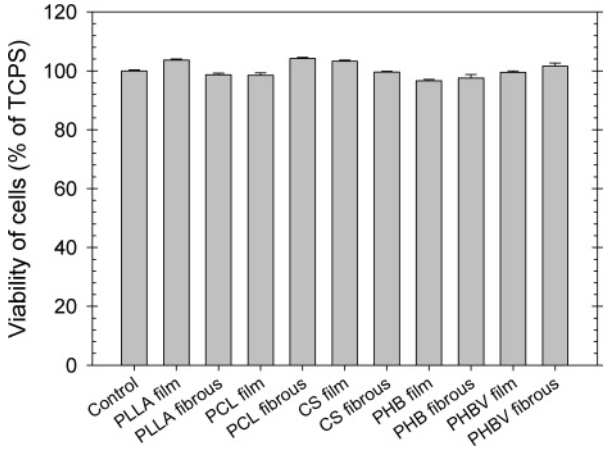


Figure 1. Indirect cytotoxicity evaluation of various electrospun fiber mats and corresponding solution-cast films based on the viability of Schwann cells (RT4-D6P2T) that were cultured with the extraction media from these materials for 24 h. The viability of the cells that were cultured with fresh culture medium (SFM) (i.e., control) was used as the reference to arrive at the viability of the attached cells shown in the figure.

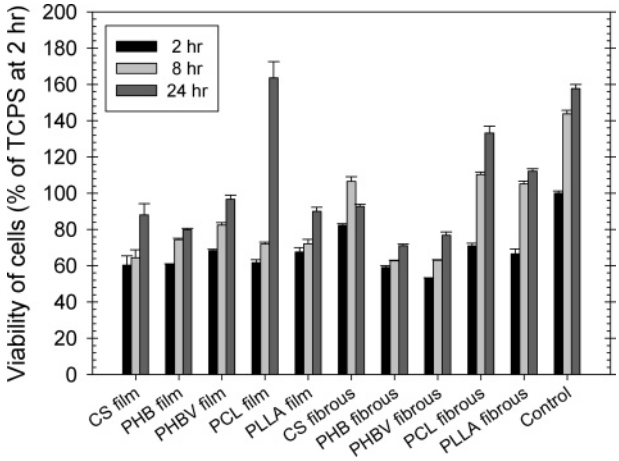


Figure 2. Attachment of Schwann cells (RT4-D6P2T) that were seeded on various electrospun fibrous and corresponding solution-cast film scaffolds and a TCPS (i.e., control) for 2, 8, and 24 h. The viability of the attached cells that were seeded on the TCPS for 2 h was used as the reference to arrive at the viability of the attached cells shown in the figure.

3. Results and Discussion

As previously mentioned, a major problem associated with the treatment of peripheral nerve injury is the bridging of a large gap between the cut ends of a transected nerve.[1,2] Various three-dimensional materials have been developed as scaffolds for tissue regeneration in place of traditional direct suture, autograft, and allograft techniques, which pose some disadvantages, such as the limited availability of the donor tissues and morbidity related to their sacrifice.[5−14] The use of artificial nerve materials to create a favorable environment for nerve regeneration has become an alternative strategy for the restoration of major nerve injuries.[5−14] Despite much advancement in this field of research, failure is often encountered for the bridging of large gaps, as previously mentioned. This failure has been postulated to be a result of a combination of a number of factors, e.g., the inability of neurotrophic factors produced by the distal stump to reach the proximal stump and the lack of a matrix bridge as a supporting structure.[1,2,5−14] It is generally accepted that Schwann cells play a crucial role during nerve restoration through the production of growth factors and the excretion of the extracellular matrix.[6−8,31] Recently, there has been interest in the use of biomaterials that are preseeded with Schwann cells to act as a persistent source of neurotrophic factors and an effective substrate for enhancing nerve regeneration.[6−8]

In the present study, electrospinning was used to fabricate fibrous scaffolds from a variety of commercial biocompatible and biodegradable polymers, i.e., PHB, PHBV, PCL, PLLA, and CS, to be used as templates for the culture of Schwann cells. The biocompatibility of these fibrous scaffolds in terms of cytotoxicity, cell attachment, and cell proliferation toward RT4-D6P2T in comparison with that of the corresponding solution-cast film scaffolds and TCPS was carefully evaluated as follows.

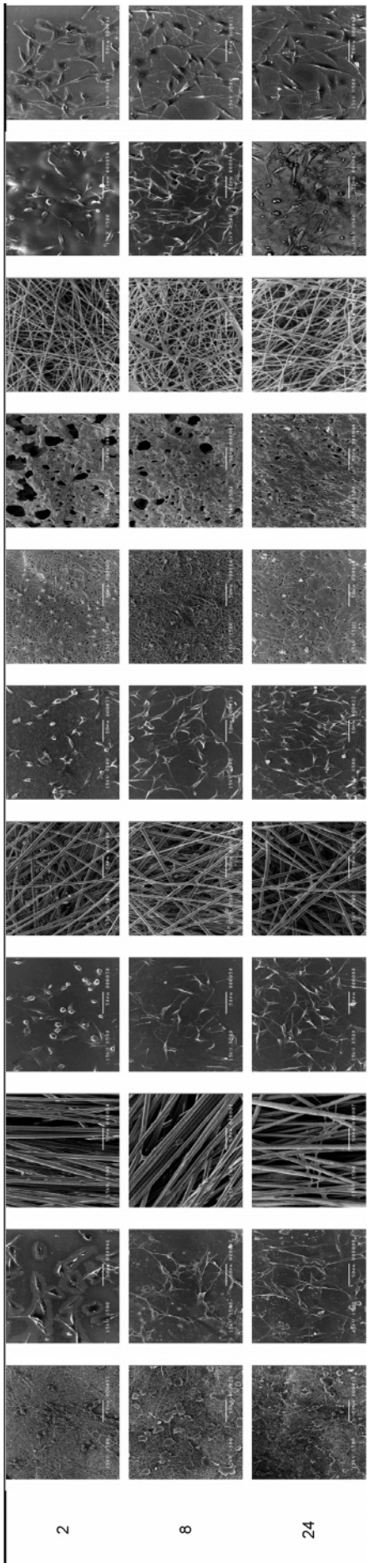
3.1. Cytotoxicity. Cytotoxicity is a basic property of a biomaterial. Figure 1 shows the viability of the cells obtained from MTT assay after the cells had been cultured with extraction media from both the as-spun fiber mat and the as-cast film specimens as compared with that obtained after the cells had been cultured with the fresh SFM. The viability of the cells was reported as the percentage with respect to that of the control. Evidently, the viability of RT4-D6P2T cultured with the extraction media from both the fiber mat and the film specimens was equivalent to that of the cells cultured with fresh SFM, implying the biocompatibility of these materials toward RT4-D6P2T. Previous reports from our group showed that PHB, PHBV, and PCL fiber mats and films were also nontoxic to mouse fibroblasts (L929) and human osteoblasts (SaOS-2).[25,28,29] In the case of CS fiber mats and films, our ongoing research work showed that these materials also showed no threat to L929 (results not shown). Results from this work confirmed that the electrospun fiber mats and the solution-cast films obtained from the studied materials can be used as scaffolds for RT4-D6P2T culture.

3.2. Cell Attachment and Cell Proliferation. The ability to support the attachment and to promote the proliferation of cells is one of the most important aspects of a functional scaffold. To evaluate such characteristics, RT4-D6P2T cells were either seeded or cultured on these scaffolds for various cell seeding or culturing time intervals. The results were compared with those obtained for the corresponding film scaffolds and TCPS (i.e., control).

Figure 2 shows the viability of the attached RT4-D6P2T on various fibrous and film scaffolds in comparison with that on the TCPS (i.e., control) after the cells were seeded on these surfaces for 2, 8, and 24 h. The viability of the attached cells on the TCPS at 2 h after cell seeding was used as the reference value to arrive at the relative viability of the attached cells shown in the figure. For any given substrate, the viability of the attached cells increased with increasing cell seeding time, except for the CS fibrous scaffold, which showed a decrease in the viability of the attached cells between 8 and 24 h after cell seeding. Between 2 and 8 h after cell seeding, the largest increase in the viability of the attached cells was observed on the PCL and the PLLA fibrous scaffolds as well as on the TCPS. Between 8 and 24 h after cell seeding, the largest increase in the viability of the attached cells was observed on the PCL film scaffold.

At any given cell seeding time, the viability of the attached cells on any given type of the fibrous and film scaffolds was inferior to that of the cells on the TCPS, except for the PCL film scaffold at 24 h after cell seeding, which showed a greater viability of the attached cells than the TCPS. Among the various fibrous scaffolds, the viability of the attached cells on the CS fibrous scaffold, at 2 h after cell seeding, was the greatest, followed by that of the cells on the PCL and the PLLA fibrous

Table 4. Selected SEM Images of Schwann Cells (RT4-D6P2T) That Were Seeded on the Surfaces of Electrospun Fibrous and Solution-Cast Film Scaffolds and a Glass Substrate (i.e., Control) for 2, 8, and 24 h at 500× Magnification (Scale Bar 50 μm)



scaffolds, respectively. At 8 h after cell seeding, the viabilities of the attached cells on the CS, PCL, and PLLA fibrous scaffolds appeared to be quite comparable to one another. At 24 h after cell seeding, the viability of the attached cells on the PCL fibrous scaffold was greater than that of the cells on the PLLA and the CS fibrous scaffolds.

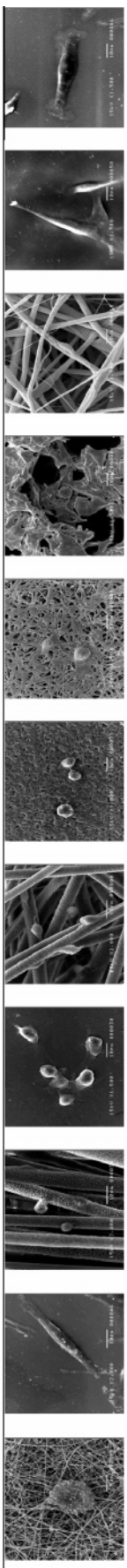
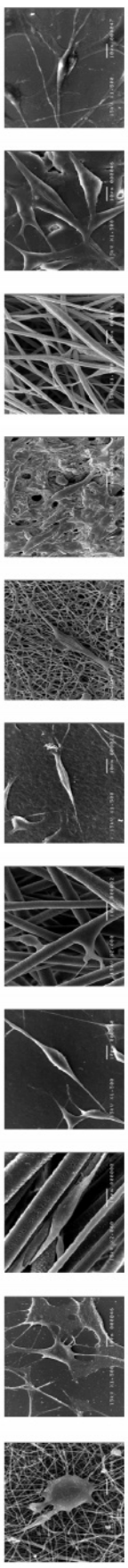
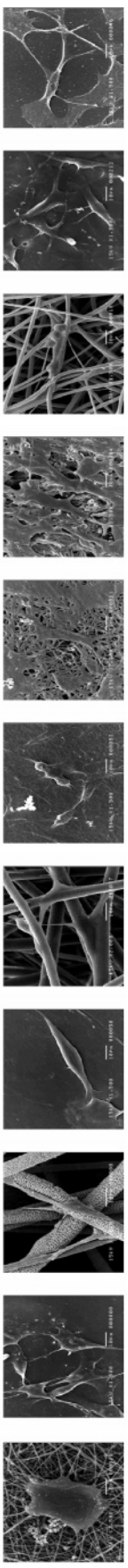
Among the various film scaffolds, the viability of the attached cells on most of the film scaffolds, at 2 h after cell seeding, was slightly less than that of the cells on the PHBV and the PLLA film scaffolds, which showed the greatest relative viability of the attached cells. At 8 h after cell seeding, the viability of the attached cells on the PHBV film scaffold was the greatest, followed by that of the cells on the PHB, PLLA, and PCL film scaffolds, respectively. At 24 h after cell seeding, the viability of the attached cells on the PCL film scaffold preceded that of the cells on the PHBV, PLLA, and CS film scaffolds, respectively. Between the fibrous and the film scaffolds, the PHB and the PHBV film scaffolds showed better support for the attachment of RT4-D6P2T than their corresponding fibrous scaffolds, while the opposite trend was observed for the CS and the PLLA fibrous scaffolds in comparison with their corresponding film scaffolds. For PCL, the fibrous scaffold was a better support for RT4-D6P2T than its corresponding film scaffold at 2 and 8 h after cell seeding, but the film scaffold was better in supporting the cells at 24 h after cell seeding.

Tables 4 and 5 show selected SEM images of RT4-D6P2T that were seeded on the surfaces of the as-spun fibrous and the as-cast film scaffolds as well as the glass substrate (i.e., control) at 2, 8, and 24 h after cell seeding at two different magnifications, i.e., 500× (scale bar 50 μm) and 1500× or 3500× (scale bar 10 or 5 μm, respectively). The purpose of the SEM images with the low magnification was to display the number of cells observed on the surfaces of the scaffolds, while that of the SEM images with the high magnification was to show the morphology of the attached cells.

At any given cell seeding time, most of the RT4-D6P2T cells attached well over the glass surface and their morphology assumed the characteristic spindle shape, which is normally observed when Schwann cells are seeded on a flat surface such as films or glass.[6−8,13,32] For the cells that were seeded on the surfaces of the film scaffolds, at 2 h after cell seeding, the cells attached on the film surfaces rather well, but to a lesser extent when compared with those on the glass surface. Evidently, some of the cells were still in a round shape, suggesting that the cells might not be fully attached on the surfaces. For PCL, due to the roughness of the film surface, the cells attached on its surface most particularly well, but again, most of the cells were still in a round shape. With increasing the cell seeding time to 8 and 24 h, the number of attached cells on the surfaces of the film scaffolds was found to increase rather monotonically, with the number of cells in the spindle-like morphology being found to increase as well. Interestingly, some of the cells on the surfaces of the CS film scaffold and the glass substrate showed the flattened, fibroblast-like morphology, while those on the more hydrophobic surfaces of the PHB, PHBV, PCL, and PLLA film scaffolds showed the characteristic spindle-like morphology.

The difference in the cellular behavior was observed when RT4-D6P2T cells were seeded on the fibrous scaffolds of different polymers which exhibited different surface topographies due to the variation in the diameters of the underlying fibers (see Table 2). At 2 h after cell seeding, most of the attached cells were still in a round shape, indicating that the cells were not yet fully attached on the surfaces, except for those on the surfaces of the PHBV and the PLLA fibrous scaffolds,

Table 5. Selected SEM Images of Schwann Cells (RT4-D6P2T) That Were Seeded on the Surfaces of Electrospun Fibrous and Solution-Cast Film Scaffolds and a Glass Substrate (i.e., Control) for 2, 8, and 24 h at 1500× Magnification (Scale Bar 10 μm), Except Those of the Cells Cultured on the CS Fibrous Scaffold, Which Were Taken at 3500× Magnification (Scale Bar 5 μm)

seeding time (h)	CS		PHB		PHBV		PCL		PLLA		glass (control)
	fibrous	film	fibrous	film	fibrous	film	fibrous	film	fibrous	film	
2											
8											
24											

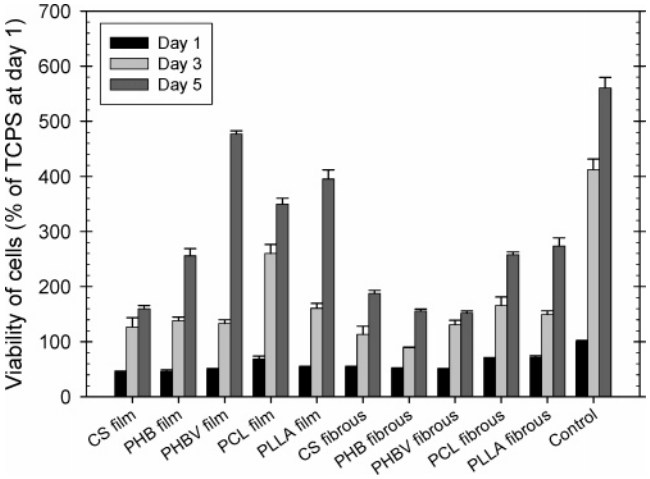


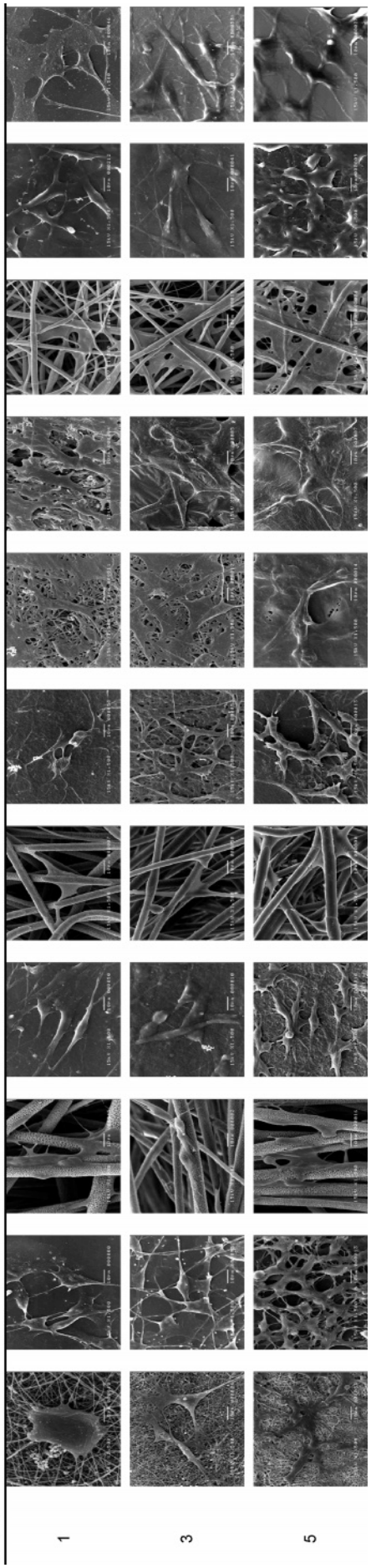
Figure 3. Proliferation of Schwann cells (RT4-D6P2T) that were cultured on various electrospun fibrous and corresponding solution-cast film scaffolds and a TCPS (i.e., control) at days 1, 3, and 5. The viability of the proliferated cells that were cultured on the TCPS at day 1 was used as the reference to arrive at the viability of the proliferated cells shown in the figure.

which attached on the surfaces more particularly well and even started to assume the characteristic spindle shape. At 8 h after cell seeding, the cells on all other surfaces became more stretched, except for those on the surface of the CS fibrous scaffold, which were still in a round shape. At 24 h after cell seeding, the cells on all other surfaces became even more stretched, except, again, for those on the surface of the CS fibrous scaffold, which became more flattened, but still in a round shape.

Interestingly, at 8 and, particularly, 24 h after cell seeding, RT4-D6P2T cells that were seeded on the PHB, PHBV, and PLLA macrofibrous scaffolds exhibited the typical characteristics of Schwann cells in which the cells attached along the surfaces of individual fibers, and some even encircled the fibers. On the other hand, the cells on the PCL and the CS nanofibrous scaffolds could only expand and/or elongate along the outer surfaces of the scaffolds. The likely reason for such contradictions in the behavior of RT4-D6P2T on the surfaces of the macro- and the nanofibrous scaffolds should be due to the difference in the sizes of the interfibrous pores. For the macrofibrous scaffolds of PHB, PHBV, and PLLA, the sizes of most pores were much larger than those of the cells, thus allowing the cells to penetrate to the inner side of the scaffolds. On the other hand, due to the much smaller sizes of the pores when compared with those of the cells, the cells could only stay on the surfaces of the PCL and the CS nanofibrous scaffolds.

The biocompatibility of the fibrous and the corresponding film scaffolds in terms of cell proliferation was further investigated by observing the viability of RT4-D6P2T cells that were cultured on both the fibrous and the film scaffolds as well as the TCPS (i.e., control) for 1, 3, and 5 d (see Figure 3). The cells were allowed to attach on the surfaces for 24 h (i.e., the viability of the attached cells at 24 h after cell seeding was taken as that of the proliferated cells at day 1), and the viability of the cells that were cultured on TCPS at day 1 was used as the reference value to obtain the relative viability of the proliferated cells shown in the figure. For any given substrate, the viability of the proliferated cells increased with increasing time in culture. Between days 1 and 3 of cell culture, the largest increase in the viability of the proliferated cells was observed on the TCPS, followed by the PCL and the PLLA film scaffolds as well as

Table 6. Selected SEM Images of Schwann Cells (RT4-D6P2T) That Were Cultured on the Surfaces of Electrospun Fibrous and Solution-Cast Film Scaffolds and a Glass Substrate (i.e., Control) for 1, 3, and 5 d at 1500× Magnification (Scale Bar 10 μm), Except Those of the Cells Cultured on the CS Fibrous Scaffold for 1 d, Which Were Taken at 3500× Magnification (Scale Bar 5 μm)



the PCL fibrous scaffold. Between days 3 and 5 of cell culture, the largest increase in the viability of the proliferated cells was observed on the PHBV film scaffold, followed by the PLLA film scaffold and TCPS.

At any given time in culture, the viability of the proliferated cells on any given fibrous and film scaffold was inferior to that of the cells on the TCPS. Among the fibrous scaffolds, the viability of the proliferated cells on both the PCL and the PLLA fibrous scaffolds, at day 1 of cell culture, was the greatest, followed by that on the CS, PHB, and PHBV fibrous scaffolds, respectively. At day 3 of cell culture, the viability of the proliferated cells on the PCL fibrous scaffold was the greatest, followed by that on the PLLA, PHBV, CS, and PHB fibrous scaffolds, respectively. At day 5 of cell culture, the viability of the proliferated cells on the PLLA fibrous scaffolds was the greatest, followed by that on the PCL, CS, PHB, and PHBV fibrous scaffolds, respectively.

For the film scaffolds, the viabilities of the proliferated cells on most of the film scaffolds, at day 1 of cell culture, appeared to be quite similar, with that on the PCL film scaffold being the greatest, followed by that of the cells on the PLLA film scaffold. At day 3 of cell culture, the viabilities of the proliferated cells on most of the film scaffolds were still quite similar, with that on the PCL film scaffold being the greatest, followed, again, by that of the cells on the PLLA film scaffold. At day 5 of cell culture, the viability of the proliferated cells on the PHBV film scaffold was the greatest, followed by that of the cells on the PLLA, PCL, PHB, and CS film scaffolds, respectively. Between the fibrous and the film scaffolds, most of the film scaffolds provided better support for the proliferation of RT4-D6P2T than their corresponding fibrous scaffolds, except for both the CS film and fibrous scaffolds, which appeared to be similar in their support for the growth of the cells.

Table 6 shows selected SEM images of RT4-D6P2T that were cultured on the surfaces of the as-spun fibrous and the as-cast film scaffolds as well as the glass substrate (i.e., control) at 1, 3, and 5 d after cell culture in one magnification of either 1500× or 3500× (scale bar 10 or 5 μm, respectively). The purpose of these SEM images was to reveal the morphology of the proliferated cells. The SEM images with the lower magnification of 500× (scale bar 50 μm), displaying the number of proliferated cells on the surfaces of the scaffolds, were also studied (see the Supporting Information).

On any given substrate, the number of cells was found to increase with increasing time in culture, and interestingly, between the fibrous and the film scaffolds, the number of cells on the film scaffolds was relatively greater than that of cells on the corresponding fibrous scaffolds, which was in accord with the observation from the MTT assay. As shown in Tables 4 and 5, the cells on most of the fibrous and the film scaffolds, at 8 and 24 h after cell seeding, assumed the characteristic spindle shape, except for those on the CS surfaces. A similar trend was also observed after the cells were cultured on the different surfaces of both the film and the fibrous scaffolds in the wells of a 24-well TCPS. At a longer time in culture (i.e., 3 or 5 d), most of the cells on the flat surfaces of all of the film scaffolds and that of the PCL nanofibrous scaffold appeared in their characteristic spindle shape, elongated their cytoplasm along the surfaces, and made cell-to-cell contacts with adjacent cells.

Interestingly, at day 3 after cell culture, the cells on the CS nanofibrous scaffold appeared in their characteristic spindle shape for the first time, and the cells on the surfaces of the PHB, PHBV, and PLLA macrofibrous scaffolds also assumed

their characteristic spindle shape, with the cells being able to penetrate to the inner side of the scaffolds even more. Additionally, some of the cells wrapped around the individual fibers, while others anchored to multiple fibers. All of the results on both the attachment and the proliferation of RT4-D6P2T on the surfaces of these five biocompatible and biodegradable polymers with different surface characteristics emphasized the importance of the surface topography of the scaffolds on the behavior of the particular cell line of Schwann cells, in which the cells showed a greater preference toward flat surfaces over rough ones. The flat surfaces could help the cells to line up and form cell-to-cell contacts with adjacent cells much easier, thus rendering the cells to perform their role during nerve regeneration much more conveniently.[1,2,6] Here, the surfaces are said to be rough when the dimensionality of any discontinuity appearing on the surfaces approaches those of the cells.

4. Conclusions

In the present paper we report the in vitro responses of Schwann cells (RT4-D6P2T) on various types of electrospun fibrous scaffolds that were fabricated from a number of commercially available biocompatible and biodegradable polymers, i.e., PHB, PHBV, PCL, PLLA, and CS. The results were compared with those obtained on corresponding solution-cast film scaffolds as well as on a TCPS, used as the positive control. Indirect cytotoxicity evaluation of the as-spun fiber mats and the as-cast films by the culture of RT4-D6P2T with extraction media from these materials confirmed nontoxicity of these materials toward the cells. At 24 h after cell seeding, the viability of the attached cells on the various substrates could be ranked as follows: PCL film > TCPS > PCL fibrous > PLLA fibrous > PHBV film > CS fibrous ≈ CS film ≈ PLLA film > PHB film > PHBV fibrous > PHB fibrous. At day 3 after cell culture, the viability of the proliferated cells on the various substrates could be ranked as follows: TCPS > PHBV film > PLLA film > PCL film > PLLA fibrous > PHB film ≈ PCL fibrous > CS fibrous > CS film > PHB fibrous > PHBV fibrous. At about 8 h after cell culture, the cells on the flat surfaces of all of the film scaffolds and that of the PCL nanofibrous scaffold appeared in their characteristic spindle shape, while the cells on the surfaces of the PHB, PHBV, and PLLA macrofibrous scaffolds also appeared in their characteristic spindle shape, but with the cells being able to penetrate to the inner side of the scaffolds. Interestingly, at day 3 after cell culture, the cells on the surface of the CS nanofibrous scaffold appeared in their characteristic spindle shape for the first time.

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Supporting Information Available. Selected SEM images of Schwann cells (RT4-D6P2T) that were cultured on the surfaces of electrospun fibrous and solution-cast film scaffolds and glass substrates. This material is available free of charge via the Internet at http://pubs.acs.org.

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