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Novel thin-walled nerve conduit with microgrooved surface patterns for enhanced peripheral nerve repair

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Abstract Randomly aligned nerve cells in vitro on conventional culture substrata do not represent the complex neuronal network in vivo and neurites growing in uncontrolled manner may form neuroma. It is of great importance to mimic the organised growth pattern of nerve cells in the study of peripheral nerve repair. The aim of this work was to modify and optimize the photolithographic technique in creating a reusable template in the form of a silicon wafer that could be used to produce contact guidance on biodegradable polymer surface for the orientated growth of nerve cells. Micro-grooves (approximately 3 μm in depth) were etched into the silicon template using KOH at increased temperature. The originality of this work lies in the low cost and high efficiency method in producing microgrooves on the surface of biodegradable ultra-thin polymer substrates (50–100 μm), which can be readily rolled up to form clinically implantable nerve conduits. The design of a pattern with small ridge width (i.e., 5 μm) and bigger groove width (i.e., 20 μm) favored the alignment of cells along the grooves rather than on the ridges of the patterns, which minimized the effect of cross growing of neurites between adjacent grooves. Effectively, enhanced nerve regeneration could be anticipated from these patterned conduits.

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Abbreviations

PCL	Poly (ε-caprolactone)
PLA	Poly (lactic acid)
PDLLA	Poly (D,L-lactic acid)
ECACC	European Collection of Cell Cultures
SEM	Scanning electron microscope
AFM	Atomic force microscope
DMEM	Dulbecco's Modified Eagle's Medium
GGF-2	Glial growth factor-2
HMDS	Hexamethyldisilazane
DAPI	4′-6-Diamidino-2-phenylindole
FITC	Fluorescein isothiocyanate
HAT	Sodium hypoxanthine, aminopterin and thymidine

1 Introduction

Peripheral nerve injuries are common; annually there are approximately 400,000 patients subject to neurosurgical interventions due to neuro-traumas of the peripheral nervous systems [1]. The function of transected nerves can be restored to some extent since peripheral nerves retain a considerable regeneration potential. Entubulation using synthetic biodegradable nerve conduits is a very promising

alternative approach to the current clinically used autografting method when the injury gap is larger than 3 cm and requires additional material to bridge the defect. Although being the widely-employed gold standard in peripheral nerve repair, nerve autografting is far from an optimal treatment. This is because harvesting of the nerve grafts always results in lost of function at the donor site and the graft is often of wrong diameter. There are further complications in the potential difficulties with isolation and controlled scale-up [2]. Numerous natural and synthetic materials have been tested as the candidate entubulation scaffold or nerve guidance tubes; however, none of them has exceeded the performance of nerve autografts. Recently, we have reported the assessment of ultra-thin poly (ε–caprolactone) (PCL) films and PCL/PLA[poly (lactic acid)] blend films as candidate nerve guidance tubes [3]. It was found that these thin films supported the attachment and growth of both NG108-15 cells and Schwann cells. In vivo experiments showed that regenerating axons had the potential to bridge up a 10 mm gap in rat sciatic nerve 2 weeks post-implantation [4], which has exceeded the performance of all the existing materials of this kind. The advantages of these bio-resorable scaffolds include non-toxic, easy to sterilize, low cost and off-the-shelf availability. The present work was to pattern the surface of the polymer films with microgrooves in the expectation for guided nerve growth and increased regeneration rate.

Neurite growth cones are speculated to detect and respond to guidance cues existing in the surrounding environment which determines stereotyped pathways for their development and regeneration [5]. The direction in which axons grow along is an important issue and aberrant growth of axons result in neuromas [6]. Traditionally applied to semiconductor materials, micro-fabrication techniques have been introduced to transfer micromachined structures from inorganic substrate to organic polymeric materials [7]. Several different approaches have been developed to create micro-structured polymers for biological applications because polymers offer an easy and cheap substrate for micro- and nano-patterning and can easily be tuned to a desired design. The most commonly adopted pattern for nerve repair is regular, repeating microgrooves produced using photolithography followed by reactive ion etching technique or anisotropic etching [8]. For example, compression molding and solvent casting were used to transfer micro-patterns onto poly (D,L-lactic acid) (PDLLA) from photolithographically patterned quartz or silicon wafers to produce micropatterned films [9]. These microgrooves with absorbed laminin were found to cause the Schwann cells to align along the direction of the grooves [10]. Similar results were demonstrated in the work of Hsu et al. [11], whereby a layer of Al was evaporated onto the silicon wafer and etched through in acid solution to generate the microgrooves. In particular, enhanced peripheral nerve regeneration has been demonstrated on these microgrooved poly (D,L-lactide) substrates [12] with 100% success rate in grooved conduits and 67% in smooth control conduits for a 6 week implantation process. Pre-clinical testing in Sprague–Dawley rats also showed that porous PDLLA conduits with micro-patterned inner lumens pre-seeded with Schwann cells significantly enhanced the time of recovery of the sciatic nerve [13]. Additionally, square or v-shaped grooves can be fabricated using electron-beam lithography coupled with wet etching or reactive ion etching [8]; however, these techniques have not been fully explored for the surface patterning of biomaterials due to the high cost and time-consuming problems associated. It is of our interest to produce a readily implantable device that possesses the desirable properties of a biodegradable nerve guide with enhanced performance resulted from the surface contact guidance.

In the solvent casting method, polymers can be simply dissolved and poured onto a master, cured, and removed for further investigation. Many replicates can be fabricated from a single patterned master; as such the cost and processing time can be minimized. By nature of the casting approach, polymer choice is limited to those that once cured can be released from the master. PCL is suitable for this purpose based on our previous study. The aims of this study were to investigate the feasibility of using a modified method based on photolithography to transfer microgrooves from a chrome mask to thin PCL films and to assess the efficiency of the patterned grooves in directing the growth and movement of neurites and in affecting the alignment of Schwann cells. The effect of groove size on the growth and alignment of cells was also examined.

2 Materials and methods

Chrome photo mask was manufactured by Photronics UK Ltd. Poly (ε-caprolactone) (average Mn ~80,000 g/mol) and other chemicals were obtained from Sigma–Aldrich unless otherwise specified. Dichloromethane was obtained from Fisher Scientific. NG108-15 (neuroblastoma × glioma) hybrid cells was purchased from ECACC (Porton Down, UK). Cell culture medium, antibiotics, and HAT supplement (5 mM sodium hypoxanthine, 20 μM aminopterin and 0.8 mM thymidine) were purchased from Invitrogen/Gibco.

2.1 Design of photomask

Mask 1: A chrome photo mask was designed to have 16 patterns with alternating grooves and spacings in varying dimensions (Fig. 1). For example, 5 + 15 represented a

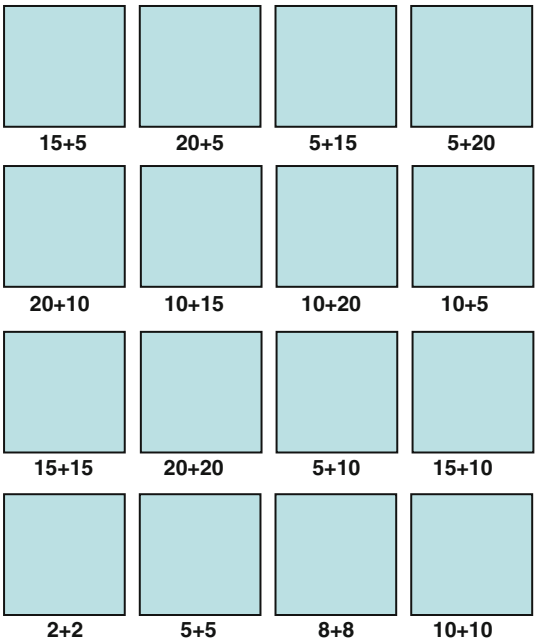

Fig. 1 The design of the photo mask (*Mask 1*) for making patterned silicon wafer template using photolithography. Sixteen 1.3 cm^2 square areas were included to test 16 different combinations of dimensions with alternating grooves and ridges. Dimensions of the pattern were displayed as ridge/spacing width plus groove width in micrometers

1.3 cm^2 square area with grooves 15 μm in width and separated by spacings 5 μm in width.

Mask 2: Based on the results obtained from Mask 1, a new mask with four selected patterns (5 + 5; 10 + 10; 20 + 20, and 5 + 20) and enlarged pattern area (2.7 × 2.7cm^2) was produced for fabricating implantable nerve conduits.

2.2 Photolithography

To make the desired patterns, the n-type wafers (4-inch in diameter with a <100> crystallographic orientation) were firstly cleaned to remove particulate matters on the surface as well as any other traces of impurities. The cleaning procedure used a mixture of sulphuric acid and hydrogen peroxide. Following cleaning, a barrier layer (approximately 500 nm in thickness) of silicon dioxide was thermally deposited onto the surface of the substrate by placing the silicon wafer in an 11–1200°C furnace for 40 min. After the formation of the SiO_2 layer, a thin uniform layer of photoresist (Shipley 1813) was applied onto the wafer by means of spin-coating at 5,000 rpm for 30 s. The photoresist-coated wafer was then "prebaked" to drive off excess solvent at 110°C for 20 min. The photoresist was exposed through the photo mask using standard contact-mode optical photolithography under UV light for 50 s. A brief post-exposure bake was performed before developing. The exposed resist was then washed away in developer solution (Shipley Microposit 351; 60 s) and an exact copy of the pattern remained on the wafer. The resulting wafer was then "hard-baked" at 120°C for 30 min to solidify the remaining photoresist in order to make a more durable protecting layer for further processing.

2.3 Etching of silicon wafer

Masked by photoresist, a solution referred to as buffered HF containing concentrated HF (49%) and a buffering salt (NH_4F) in the ratio of 1:4 was used to etch through the layer of SiO_2. Photoresist was removed by washing in acetone. Silicon wafer was then anisotropically etched using 30% (w/v) potassium hydroxide (KOH). KOH solution was heated up to reach equilibrium at 80° C, which will etch silicon <100> planes at approximately 1.5 μm/min. Etching was conducted for a varying length of time to generate grooves in truncated V-shape at a depth of approximately 3 μm. This is because the etching rate is also dependent on the width of the patterns—the wider the pattern, the faster the etching. Depth of the micro-grooves was monitored using a Form Talysurf-50 Profilometer (Taylor Hobson). SiO_2 was used as a mask since it is etched at a slower rate (1–2 nm/min). A control template was made without KOH etching; therefore, had the same pattern but with much shallower groove depth (approximately 500 nm).

The resulting template was washed thoroughly in dH_2O and then dipped into isopropanol. Nitrogen gun was used to blow dry the specimen. PCL solution (3% w/v) was directly cast onto the surface of the patterned wafer. A thin PCL film with the grooved patterns could be easily peeled off the template ready for further testing. PCL films were treated in 10 N NaOH for 1 h to increase the hydrophilicity of the material and then washed twice in distilled H_2O. UV light was used to sterilize the scaffolds for 45 min prior to cell culturing (Fig. 2).

The surface topography of the PCL films prepared as described above was analyzed using Atomic Force Microscopy (AFM, Veeco CP2) and Philips XL30 Field Emission Gun Scanning Electron Microscopy (SEM). A scanning frequency of approximately 0.4 kHz was used in tapping mode for the AFM imaging. The scanned area was 80 × 80 $μm^2$. Samples were gold coated in a sputter coater (Edwards Ltd.) and mounted onto Aluminium stubs (Agar Scientific Ltd.) prior to SEM imaging and the microscope was operated at 5 kV with a 20 mm working distance.

2.4 Cell culturing and differentiation

NG108-15 cells were maintained in high glucose (4.5 g/ml) DMEM (Dulbecco's Modified Eagle's Medium) medium, containing 5% fetal bovine serum, 1% Penicillin/

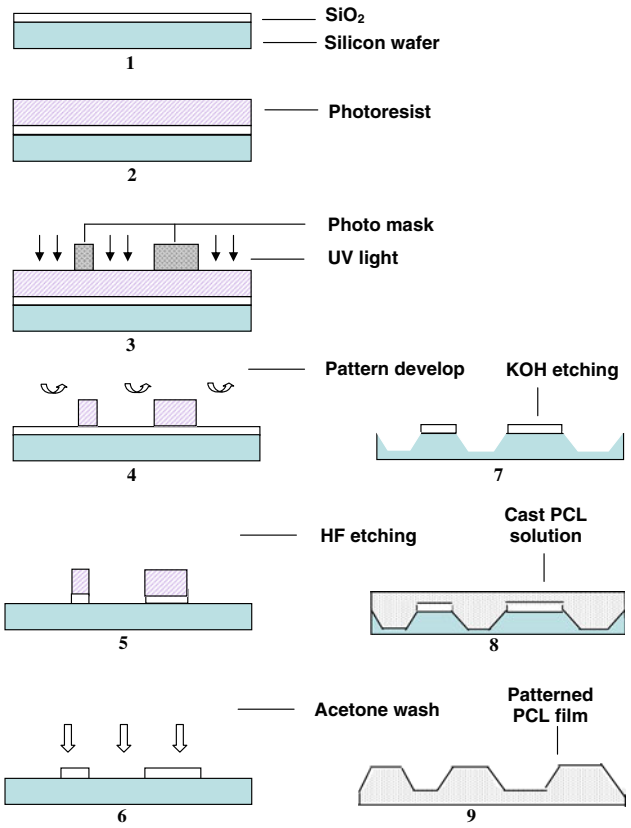

Fig. 2 Fabrication process for patterned PCL films. (*1*) A layer of SiO_2 (500 nm) was grown on top of the silicon wafer; (*2*) a layer of photoresist was spun cast on the surface of a <100> silicon wafer; (*3*) patterned photo mask was applied on top of the photoresist followed by UV exposure; (*4*) pattern formed in developer solution; (*5*) HF etched through SiO_2; (*6*) acetone washed off photoresist; (*7*) KOH etched through silicon wafer; (*8*) PCL solution was cast onto the master template; (*9*) patterned PCL films peeled off the template

Streptomycin, and supplemented with 1× HAT solution at 37°C in a 5% CO_2 humidified atmosphere. To induce differentiation, NG108-15 cells ($1000/cm^2$) were initially cultured onto patterned PCL films in medium containing 10% serum and 1% antibiotics for 2 days. On day 3, cell culture medium was replaced with fresh medium containing 1.5% serum, 1% antibiotics and 1 mM dibutyryl-cAMP. Cells were cultured for another 5 days. Schwann cells were isolated from neonate rats using the method described by Caddick et al. [14] and maintained with 63 ng/ml glial growth factor-2 (GGF-2) and 10 μM forskolin mitogen supplemented media. Schwann cells were cultured at the density of $10^4/cm^2$ for 6 days before SEM and antibody staining were conducted.

2.5 SEM imaging

Cells growing on patterned PCL films were prepared for SEM imaging using hexamethyldisilazane (HMDS) chemical drying method [15]. Briefly, cells were rinsed twice in PBS and then fixed with 1.5% glutaraldehyde (TAAB Laboratories) in phosphate buffer (containing $NaH_2PO_4 \cdot 2H_2O$ 15.6 g and Na_2HPO_4 14.2 g in 1000 ml dH_2O; pH = 7.3) for 30 min at room temperature. After fixation, cells were rinsed twice with phosphate buffer and dehydrated through a series of increasing concentrations of ethanol solutions (50, 70, 90, and 100%) with 10 min in each solution. Films were then dried in HMDS for 2 × 5 min. Finally, HMDS was removed and the films left overnight in fume cupboard for the fully evaporation of HMDS. Prior to SEM imaging, films were mounted onto an aluminium stub and sputter coated with gold.

2.6 Antibody staining

For immunocytochemical analysis, NG108-15 cells or Schwann cells growing on PCL films were washed gently in PBS and fixed with 4% paraformaldehyde for 30 min. Following fixation, cells were washed twice in PBS and then permeabilised with 0.2% Triton X-100 for 20 min. After washing in PBS, a blocking solution of 5% normal goat serum was incubated with the cells for 1 h at room temperature. The blocking solution was removed and mouse monoclonal anti-neurofilament antibody was used as primary antibody for NG108-15 cells (1:500 dilution; Abcam plc) or rabbit polyclonal S100 for Schwann cells

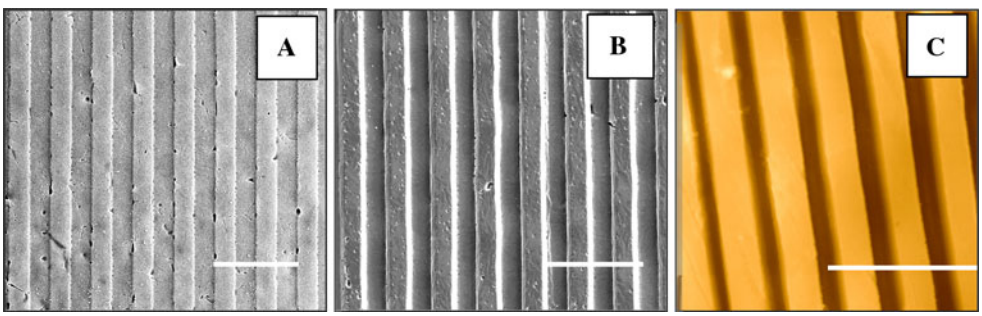

Fig. 3 SEM (**a** and **b**) and AFM (**c**) images of patterned PCL films. The width of spacings and grooves shown in these pictures was 10 μm for **a** and **b** and 8 μm for **c**. (Bar = 40 μm). Depth of grooves was 3 μm for **b** and **c**. Depth of grooves in **a** was 500 nm

(1:500 dilution; Dako cytomation). Incubation was conducted at 4°C overnight. Following thorough washing in PBS (3 × 10 min) to remove non-specifically bound antibodies, goat anti-mouse CY3 secondary antibody for NG108-15 cells (1:200 dilution; Amersham plc) or goat anti-rabbit FITC-conjugated secondary antibody for Schwann cells (1:100 dilution; Vector Labs) was incubated with the cells for 1 h in dark. Films were then washed again (3 × 10 min) before they were mounted onto microscope slides. ProLong® Gold antifade reagent with DAPI (Invitrogen/Gibco) was used to prevent the samples from bleaching and to counter-stain the nuclei of the cells. Pictures were taken using Nikon Eclipse 50*i* fluorescence microscope.

3 Results

3.1 Successful surface patterning

In the present study, except for the 2 μm + 2 μm and 5 μm + 5 μm, all patterns were successfully transferred onto the surface of PCL films; therefore, photolithography and subsequent HF and KOH etching were found to be sufficient approaches to produce the silicon master template for the surface patterning of PCL films (Fig. 3). Depth of these microgrooves was approximately 3 μm and the control material (not etched in KOH) 500 nm. Rather than being upright, the walls of the grooves were truncated V-shape at an angle of 54.7° due to the anisotropic etching fashion of KOH through silicon. Although this method has been described in previous work [16], it is the first time that it is applied to biodegradable material for the purpose of nerve tissue engineering. Importantly, the patterns on these silicon wafers were transferred with high fidelity. Polymers have been demonstrated to be very good material for micro- and nano- patterning and features from millimeter scale down to 10 nm could be achieved using imprint lithography [17].

3.2 Cell morphology and alignment

The definition of cell alignment could account for the different values of percentage alignment on substrates. Miller et al. [9, 10] determined the alignment of Schwann cells by whether the cells oriented in the direction of the grooves without crossing from one groove to another. Many other researchers [11, 18–20] defined the percentage of cells aligned as the fraction within ±10° or ±20° of the patterning direction. In this study, a similar definition (±10°) was adopted to distinguish cell alignment on various substrates.

Although no cell alignment was observed on PCL films with a groove depth of 500 nm, both Schwann cells and NG108-15 cells were found to be successfully aligned along the 3 μm deep grooves. Figure 4 shows the contrast effect of aligned NG108-15 neurites in the patterned area of 10 μm + 10 μm against randomly distributed neurites

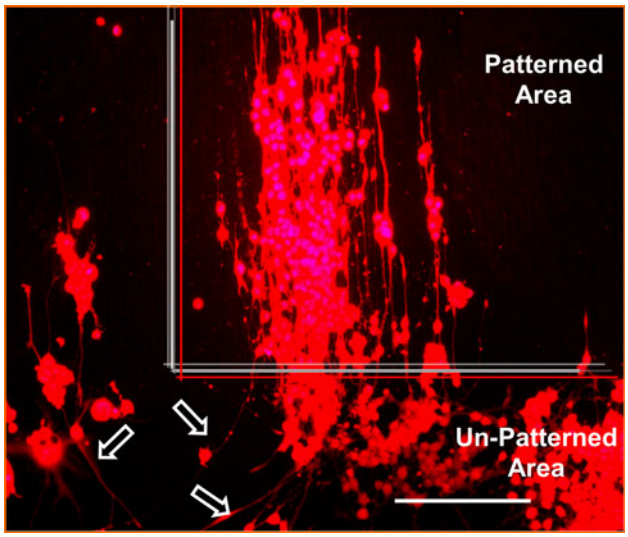

Fig. 4 Anti-neurofilament antibody stained NG108-15 cells on patterned PCL films (10 μm + 10 μm), showing contrast orientation effect of neurites in aligned and un-aligned areas. Cells were differentiated for 5 days. Nuclei were counter-stained with DAPI (*Arrows* are pointing to randomly aligned nerve fibres in the non-patterned area, Bar = 200 μm)

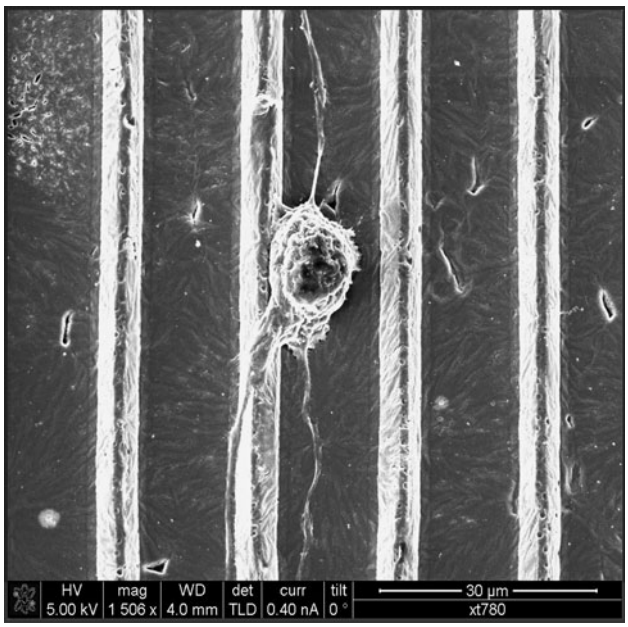

Fig. 5 SEM image of a differentiated NG108-15 cell aligned on patterned PCL film (5 μm + 15 μm for the width of spacings and grooves, respectively), Bar = 30 μm

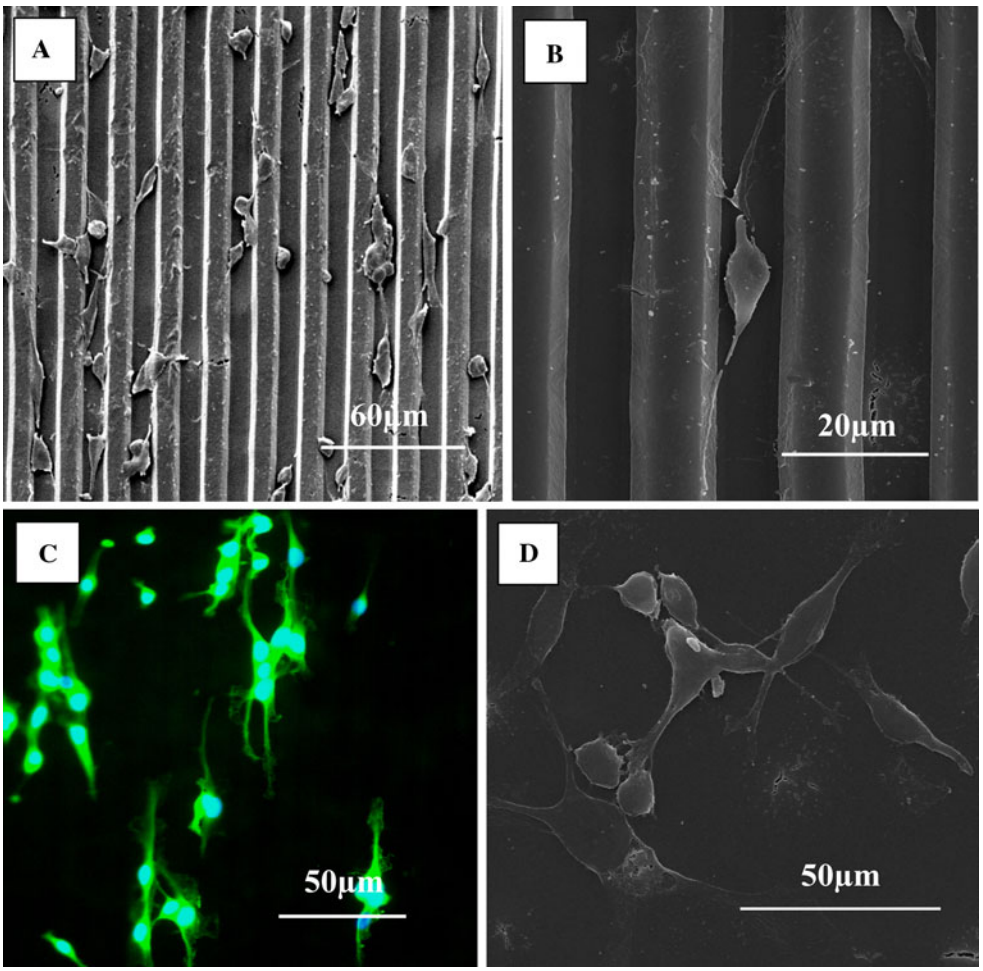

Fig. 6 Schwann cells on patterned PCL films after 5 day of culturing. **a** and **b** were SEM images of Schwann cells on the pattern of 10 μm + 10 μm. **c** was immunocytochemically stained Schwann cells using antibody against S 100 marker protein; nuclei of cells were counter-stained using DAPI. Dimension of pattern was also 10 μm + 10 μm. **d** Showed Schwann cells on unpatterned surface area with random alignment

in the unpatterned area. Figure 5 shows the SEM image of a differentiated NG108-15 cell with axons aligned along the grooves of a 5 μm + 15 μm pattern. Figure 6 shows Schwann cells aligned along the grooves of the patterns with extended processes, which indicated excellent biocompatibility of the material with Schwann cells. Schwann cells were found to attach to the unpatterned, smooth substrate in random directions shown in Fig. 6d. The morphology of both cell types varied on patterns of different dimensions. It was observed that NG108-15 cells tended to have more branched processes and the number of neurites also appeared to be higher on wide grooves and spacings i.e., 15 and 20 μm, than on patterns of smaller dimensions i.e., 5 and 10 μm (Fig. 7). The morphology of Schwann cells was also affected by the groove size. It was observed that the processes of Schwann cells tended to be longer on patterns with smaller groove and spacing sizes i.e., 5 μm + 10 μm and 10 μm + 10 μm; when compared with Schwann cells on patterns with bigger dimension (i.e., 15 μm + 15 μm, 20 μm + 20 μm). Importantly, contamination of fibroblast cells was reduced on smaller dimension, which was speculated to be resulted from the relatively bigger size of fibroblasts (20–30 μm in diameter) than Schwann cells (5–10 μm in diameter). The number of fibroblast cells on patterned areas was between 8.7 and 25.8% of that on unpatterned, with the lowest on 5 μm + 10 μm and the highest on 20 μm + 20 μm. The reduced adhesion of fibroblast cells onto the patterned PCL films might be advantageous for the axonal regeneration and the proliferation of Schwann cells in vivo.

It was observed that both NG108-15 cells and Schwann cells had a tendency to settle in the grooves in all fourteen different patterns, with the average percentage of neural cells in grooves being over 58.2% and Schwann cells 69%

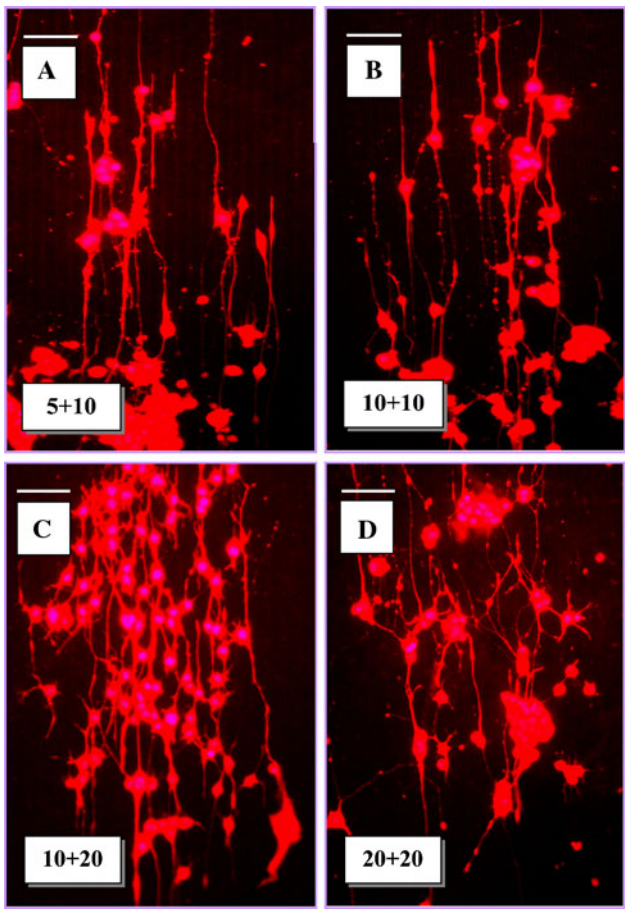

Fig. 7 Dimension of surface patterns affected cell morphology. Differentiated NG108-15 cells growing on patterns with wider grooves and spacings (**c** and **d**) tended to have more branched neurites than on patterns with small groove width (**a** and **b**) and the number of neurites also tended to be higher. Bar = 20 μm

due to the small size of Schwann cells. In the case of NG108-15, when the widths of the grooves were larger, more cells were found in the grooves. This effect was clearly seen in patterns of 5 + 20 μm, 10 + 20 μm, 10 + 15 μm and 5 + 15 μm; however, when the widths of the spacing were also large (e.g., 20 + 20 μm and 20 + 10 μm), this type of cell response diminished (Fig. 8). On the contrary, patterns with small grooves (5 μm) saw more cells attached on the spacing area, particularly when the widths of spacing were large (e.g., 15 + 5 μm and 20 + 5 μm). The design of the pattern with small spacings (5 μm) and large grooves (20 μm) in mask 2 was based on the observation that NG108-15 cells tended to align in the grooves and the chance for the regenerating nerve fibers to grow across the grooves was therefore reduced. This is believed to be unique in our design because other researchers previously adopted the same sizes for both the grooves and the spacings.

3.3 Results on Mask 2

To make implantable conduits, PCL films with enlarged pattern area (2.7 × 2.7 cm^2) were produced from Mask 2. Data obtained from these substrates are shown in Table 1. Differentiated NG108-15 cells were used in this experiment and it was shown that patterns 5 + 20 and 10 μm + 10 μm were comparable with each other in performance and 20 μm + 20 μm was significantly worse ($p < 0.05$) both in terms of the average neurite length and the alignment rate. Statistics was conducted using Student's *T*-test: two-samples assuming equal variance.

4 Discussion

Tissue engineering constructs face many obstacles before successful implementation. One of the greatest challenges in tissue engineering research is to accurately mimic the natural conditions of tissues in vivo. It has been found that not only the soluble, diffusible factors; but also the adhesive, mechanical interactions with physical scaffolds decide the different states and functions of a cell, including gene expression, adhesion, migration, proliferation, and differentiation [21]. Moreover, cells are influenced by spatial domains, structural compositions and mechanical forces at the micro- and nano-scale for the binding interactions with surfaces [22]. Therefore, substrate matrices with controlled and geometrically well-defined structures have superiority over the conventionally used tissue culturing substrates for guiding the regenerating nerve fibers, for the understanding of basic neuron cellular function and for the design of advanced neuronal networks in the long term.

Since directional control of axonal outgrowth is very critical in peripheral nerve repair, approaches such as magnetically oriented collagen [23] and biodegradable microfilament [24] have been explored and shown to provide effective guidance to regenerating axons. Due to the space constricting effects caused by the aligned materials as lumen fillers [25] and the fact that neurites in vitro can extend and grow along the length of microgrooves on flat substrates [26], only surface contact guidance was produced for the in vitro testing in the present work. Additionally, it is believed that neurites have a tendency to extend as a bundle containing hundreds of axons by the process called cofasciculation [27]. Effectively, it has been demonstrated that dissociated dorsal root ganglia cells cultured on micropatterned substrates with laminin and pre-seeded Schwann cells showed excellent directed growth and accelerated elongation when compared with those cultured on smooth, un-patterned substrates [9, 10, 13, 26]. Therefore, contact guidance at the periphery of the

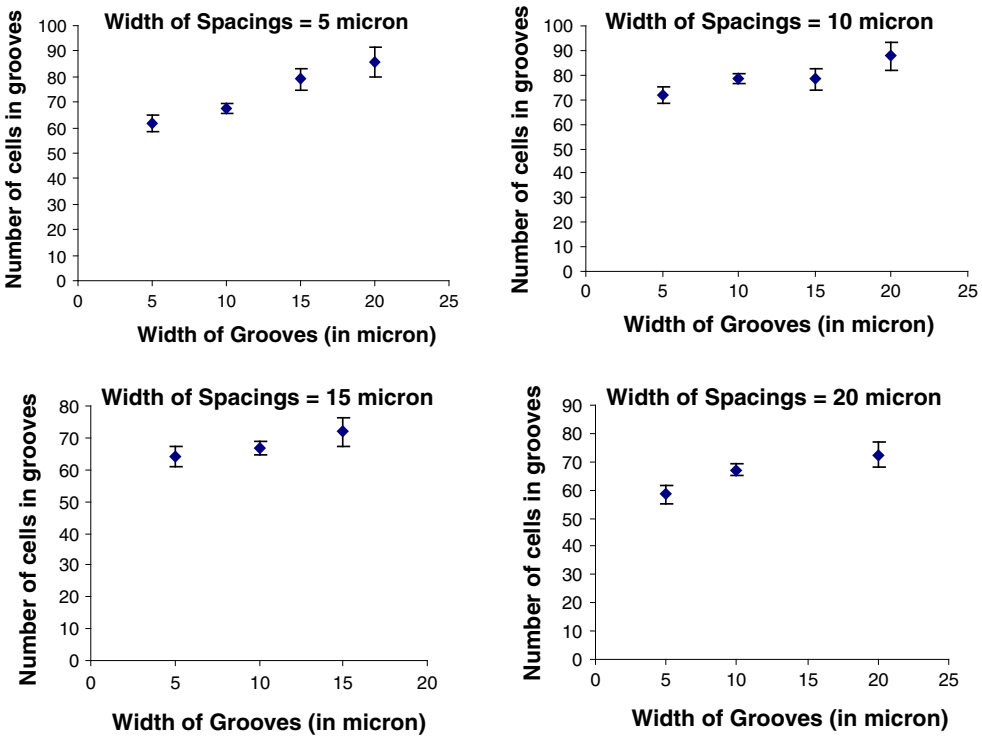

Fig. 8 The tendency of NG108-15 cells growing in the grooves of the patterns. It was shown that when the width of the spacings of the patterns was set, the wider the grooves the more cells aligned in the grooves. However, when the width of the spacings was also big, this effect diminished

Table 1 Average neurite length and neurite alignment rate of differentiated NG108-15 cells on patterned PCL films (Data from Mask 2)

Patterns of microgrooves	5 μm + 20 μm	10 μm + 10 μm	*20 μm + 20 μm
Average neurite length	289.8 μm	279.2 μm	198.7 μm
Neurite alignment rate	86.7%	84.5%	71.9%

* Performance of pattern 20 μm + 20 μm was significantly ($p < 0.05$) worse than that of patterns 5 μm + 20 μm, and 10 μm + 10 μm

nerve conduit is speculated to be able to guide the growth of axons in the inner lumen.

The most commonly used pattern for directing cell growth and for controlling the fashion of cell movement is alternating grooves and spacings/ridges. As far back as in 1945, Weiss reported that both Schwann cells and axons advance along oriented interfaces in the ambient medium and their movement follows the same rules [28]. The dimensions of the aligned pattern are crucial for the design and would make pronounced difference in the results obtained. In keeping with the findings in the present study, groove width was previously found to be a significant factor in promoting Schwann cell alignment, and the grooves with widths and spacings of 10–20 μm were reported to be optimal [9]. Actually, it has been reported that cells seem to exhibit a more in vivo-like morphology on cell culture surfaces with topographic features in similar range to cellular dimensions (10–50 μm) [29]. The similarity of the size of these grooves to that of the cell body was postulated to be the reason for the tendency of the cells to position in between of the pattern.

It has been reported that orientation often increases with increasing depth, but decreases with increasing groove width [7, 30]. The width of ridge was considered to be a more important factor than groove width [30]. Nevertheless, this was proved to be untrue in our work for the differentiated NG108-15 cells, which showed that both the ridge width and the groove width played important roles in guiding and deciding the orientation of regenerating nerve fibers. Conventionally, micro-grooves are designed as alternating groove and spacing with same width. In the present study, small ridge width (5 μm) was combined with big groove width (10, 15 and 20 μm) to investigate the effect of this design on the growth and alignment of differentiated NG108-15 cells. It was observed that the neurites were considerably longer and the cross-growth of nerve fibres across the substrata was reduced on this original design.

The reasons why cells can be aligned along patterned surface are still not known. One hypothesis is that mechanical tensions could re-arrange centromeres through the deformation of the nucleus and cause alignment of cells [31]. Cell guidance also occurs when cells are inhibited from crossing a step. They would follow the edge of the step and become elongated, showing highly polarized phenotype during cell spreading [32, 33]. Another observation was that cells showed reduced dimensions across the grooves and increased dimensions along the grooves with an overall decrease in cell area. It was hypothesized that grooves act by inhibiting marginal expansion across them and that an internal cellular mechanism partially compensates by promoting marginal expansion along the grooves [30]. This effect might have influenced cell alignment on grooved surfaces; however, as mentioned above the exact reasons behind cell alignment are not known.

In conclusion, we have found a simple and efficient approach in producing micro-grooves on the surface of biodegradable ultra-thin polymer films, which can be easily rolled up to form implantable nerve conduits. The original design with big grooves and small ridges would potentially provide enhanced contact guidance for regenerating axons. With the added advantages of the ultra-thin polymer scaffolds, such as excellent handle-ability, low cost and easy availability, these patterned conduits are expected to have improved performance than the unpatterned tubes and other devices of its kind.

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